

RESEARCH PAPER

Causes and correlations in cambium phenology: towards an integrated framework of xylogenesis

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Abstract

Although habitually considered as a whole, xylogenesis is a complex process of division and maturation of a pool of cells where the relationship between the phenological phases generating such a growth pattern remains essentially unknown. This study investigated the causal relationships in cambium phenology of black spruce [*Picea mariana* (Mill.) BSP] monitored for 8 years on four sites of the boreal forest of Quebec, Canada. The dependency links connecting the timing of xylem cell differentiation and cell production were defined and the resulting causal model was analysed with d-sep tests and generalized mixed models with repeated measurements, and tested with Fisher's C statistics to determine whether and how causality propagates through the measured variables. The higher correlations were observed between the dates of emergence of the first developing cells and between the ending of the differentiation phases, while the number of cells was significantly correlated with all phenological phases. The model with eight dependency links was statistically valid for explaining the causes and correlations between the dynamics of cambium phenology. Causal modelling suggested that the phenological phases involved in xylogenesis are closely interconnected by complex relationships of cause and effect, with the onset of cell differentiation being the main factor directly or indirectly triggering all successive phases of xylem maturation.

Key words: Causal modelling, cell differentiation, cell production, d-sep test, *Picea mariana*, secondary wall formation, xylogenesis.

Introduction

Xylem is the result of a gradual accumulation of cells that plants produce to renew and increase their transport system, store substances, and ensure mechanical support. At the basis of this process is the cambium, a meristematic tissue that divides incessantly without differentiating and produces new cells, which subsequently develop to generate the transport system (xylem and phloem). Although habitually considered as a whole, xylem is the result of a complex process of division, growth, and maturation of a pool of cells. It is therefore clearly an oversimplification to consider the xylem as a sum of inert elements accumulated in time. Instead, the conducting system of vascular plants is a tissue originating from short-lived and developing cells, which undergo several phases of differentiation before attaining complete maturation (Wodzicki, 1971; Rossi *et al.*, 2006b). During xylogenesis, the phases of

production and differentiation of each cell are separated in space and time according to a partly overlapping delay. The newly produced cells arranged along the radial row gradually mature in the same order as they are produced, respecting the rule of 'first in, first out'. Many phenological phases can therefore be observed from the annual growth pattern, each one corresponding to the onset or ending of a metabolic process. The seasonal dynamics of wood formation have been thoroughly analysed (Deslauriers *et al.*, 2009; Gruber *et al.*, 2010; Moser *et al.*, 2010), but the relationship among the processes generating such a growth pattern remains essentially a puzzling matter. Although the spatial relationships of the phenological phases along the stem have been partially described (Anfodillo *et al.*, 2012), the question of whether xylogenesis is constituted by a succession of independent phases of cell production and

differentiation or by a chain of interconnected phenological events still awaits an answer.

During development, the cambial derivatives (i.e. the cells produced by cambial division) alter both morphologically and physiologically, progressively assuming definite features. In other words, the derivatives differentiate into the specific elements of the stem tissues. In xylem, this process is represented by the phases of cell enlargement and cell wall thickening and lignification, and is associated with changes in composition and organization of both primary and secondary cell walls. Investigations of xylem phenology and climate–growth relationships have focused mainly on the onset of the growth process; that is, the onset of xylem production or differentiation, while the end of growth still remains partly or completely unexplored (Gričar *et al.*, 2007; Rossi *et al.*, 2007; Seo *et al.*, 2008; Turcotte *et al.*, 2009). In the authors' opinion, this is essentially due to a greater number of significant responses being obtained between onset of growth and climate rather than a mere lack of interest in the final phases of the growth process. Thus, results finding significant relationships among growth and climate appear more interesting and are more likely to be reported in papers than those observing no significant relationship (Menzel *et al.*, 2006).

A number of reasons contribute to the need for a better understanding of the mechanisms of xylem growth and development. Within the same stand and with trees experiencing a similar microclimate, cambium phenology has been demonstrated to change with species and to be age and size specific (Rossi *et al.*, 2007, 2008a; Rathgeber *et al.*, 2011). The period in which wood formation occurs is the time window when xylem is differentiating and when environmental factors can act directly on the cells constituting the tree ring (Frankenstein *et al.*, 2005). Plants routinely experience meteorological events that are of very short duration. How and to what extent these events act on production and development of the xylem cells and on duration of wood formation is basically unknown. In dendroecology and dendroclimatology, the growth–climate models combine weather factors with the amount of growth in terms of tree-ring width, but fail to take into account the potential modifications in xylem phenology. Changes in the phenological phases could affect the overall duration of xylogenesis and, as a result, modify the time window of direct influence of the environmental factors on xylem.

Recent investigations have demonstrated that cell production is closely related to xylem phenology. According to Lupi *et al.* (2010), the date of onset of xylogenesis would affect the number of cells produced by the cambium which, in turn, would influence the ending of cell differentiation. As a result, earlier cambial resummptions lengthen the period available for cell division in the secondary meristem, increasing the growth potential during the year (Gričar *et al.*, 2005; Rossi *et al.*, 2007, 2008a; Deslauriers *et al.*, 2008). Also, in conifers, the larger amount of cells produced by cambium leads to greater accumulations of cells in the developing xylem, increasing the time spent in the differentiation and maturation of the tracheids and delaying the

ending of wood formation (Lupi *et al.*, 2010; Rossi *et al.*, 2011b). Thus, onset and ending of cell formation could be indirectly connected, and changes in the timing of a phenological phase could modify the occurrence of a successive phase. This said, it may be assumed that whatever endogenous or environmental factor affects the resumption of growth could directly or indirectly influence the production and temporal dynamics of cell differentiation in wood by affecting the successive phenological phases of xylem. This hypothesis, if valid, would provide valuable cues for identifying the relative importance of the factors affecting the timing and dynamics of xylogenesis.

The aim of this study was to explore the causal relationships, if any, in the phenological phases of cambium in black spruce [*Picea mariana* (Mill.) BSP] by investigating the causes and correlations between cambium phenology and cell production monitored for 8 years on four sites of the boreal forest of Quebec, Canada. The four study sites were selected along an alti-latitudinal gradient in order to analyse as wide a range of cambial phenology as possible. Using six observed variables (dates of emergence of the first enlarging, wall-thickening, and mature cell, dates of ending of cell enlargement and cell wall lignification, and final number of cells), all potential causal relationships were identified and the resulting model was statistically tested to determine whether and how causality propagates through the measured variables. The procedure involved two different steps: (i) to verify the hypothesis of independence of the variables (i.e. that no causal relationship existed among variables); and (ii) to test statistically the resulting causal model, if any. Model definition assumed a temporal rule in which effects might propagate only from preceding to subsequent phenological phases.

Materials and methods

Study area and tree selection

The study was conducted on black spruce in the Saguenay–Lac-Saint-Jean area, in the boreal forest of Quebec (Canada). The region has a gently rolling topography with hills reaching 500–700 m a.s.l. on thick and undifferentiated glacial till deposits. Four permanent sites (Simoncouche, Bernatchez, Mistassibi, and Camp Daniel) were selected at different altitudes and latitudes in mature even-aged black spruce stands (Table 1). In each site, the dominant 120- to 140-year-old trees with upright stems were chosen from a pre-selected group of trees with similar growth rates. Trees with polycormic stems, partially dead crowns, reaction wood, or evident damage due to parasites were avoided.

Xylem sampling and preparation

In each site, tree-ring formation was studied from April to October during 2002–2009 in five (2002–2005) and 10 (2006–2009) trees. Wood microcores were collected weekly following a spiral trajectory on the stem from 30 cm below to 30 cm above breast height (1.3 m) using surgical bone sampling needles in 2002–2006 and Trephor in 2007–2009 (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 (Forster *et al.*, 2000). Samples usually contained the previous four or five tree rings

and the developing annual layer with the cambial zone and adjacent phloem. Samplings were performed on the same tree for not more than 4–5 years and wood 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 stereo-microscope at magnifications of $\times 10$ – 20 , 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 min under visible and polarized light at magnifications of $\times 400$ – 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 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 3–5 closely spaced cells (Fig. 1A). 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 (Fig. 1B). 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 (Fig. 1C). Deformed rows of tracheids were frequently observed in this phase, due to the enlargement process occurring despite strong compression between xylem tissues

Table 1. Location and climatic characteristics of the four study sites in the boreal forest of Quebec (Canada) listed at increasing latitudes

Site	Latitude	Longitude	Altitude (m a.s.l.)	Mean air temperature		Absolute annual air temperature	
				Annual (°C)	May–September (°C)	Maximum (°C)	Minimum (°C)
Simoncouche	48°13'	71°15'	338	1.9	13.3	32.3	–34.8
Bernatchez	48°51'	70°20'	611	0.2	11.4	30.3	–36.2
Mistassibi	49°43'	71°56'	342	0.7	12.8	31.6	–36.0
Camp Daniel	50°41'	72°11'	487	–1.2	11.0	31.6	–41.5

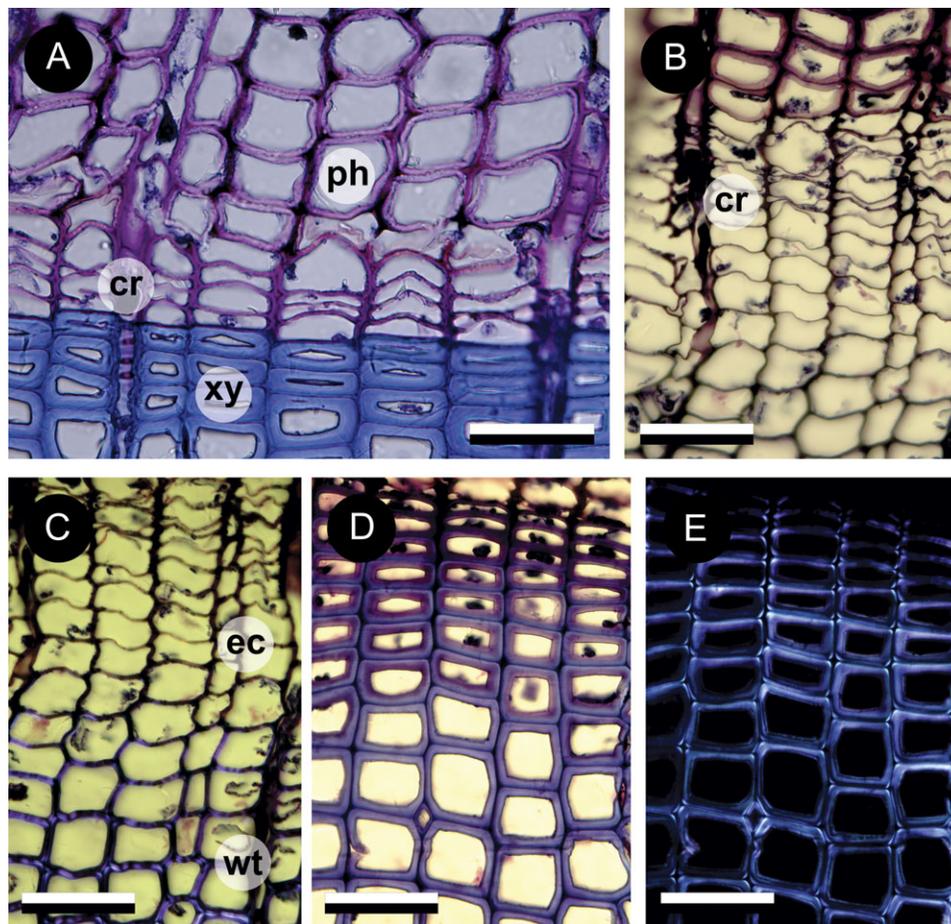


Fig. 1. Cross-sections of the outer part of the stem in black spruce. The cambial region (cr) during dormancy (A) and cell division (B) located between phloem cells (ph) and mature xylem (xy). The xylem at different development stages (C, D) with enlarging (ec) and wall-thickening (wt) cells. Wood sections were observed under visible (D) and polarized (E) light showing both partially (upper side) and completely (lower side) lignified cells. Scale bars=40 μm . Photographs by J. Boulouf.

and bark. Observations under polarized light discriminated between enlarging and cell wall-thickening tracheids (Fig. 1D, E). 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 just 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 three rows was averaged for each tree and used to assess onset and ending of each phase of cell differentiation. In spring, when at least one horizontal row of differentiating or mature cells was observed, differentiation or maturation was considered to be occurring or to have begun, respectively. In late summer, when no further cell was observed undergoing wall thickening and lignification, xylem formation was considered complete. The dates of cambium phenology [the first observed enlarging (FE), wall-thickening (FW), and mature (FM) cell, ending of cell enlargement (EE) and cell wall lignification (EL) computed on the day of the year (DOY)] and final number of cells (NC) were calculated for each tree, site, and year, obtaining a matrix with six variables and 252 observations that was used for exploring and testing causal models.

Exploring causal models

The relationships between pairs of variables were initially assessed by Pearson correlation coefficients. The matrix as a whole was then used to search for path models that were consistent with the observations at a specified probability level. The analysis explored potential causal relationships employing the SGS algorithm as implemented in the EPA2 program (Shiple, 1997), which were set at significance values of between 0.05 and 0.9. Firstly, the algorithm explores all possible dependency links between pairs of variables when all possible subsets of other variables in the model are mathematically fixed. Secondly, the algorithm attempts to orient the undirected dependency links between each pair of related variables. In other words, either the link or independency between each variable and every other variable was analysed, and the resulting links between variables were converted into causal relationships. The complete analytical description of the algorithm was reported in detail by Shipley (2000) with the mathematical proof given by Spirtes *et al.* (1993).

Testing causal models

Based on the exploratory analysis, which identified all possible causality links between the dates of cambium phenology and cell production, the causal models produced by the SGS algorithm were tested according to d-sep tests (Shipley, 2000). All constraints in the form of independence statements were identified. The number of constraints was associated with the amount and arrangement of the causal links of each model, where arrows (\rightarrow) describe the causal links between variables. Let α , β , γ be three variables with $\alpha \rightarrow \beta$ and no causal link between β and γ ; the notation $\beta \perp\!\!\!\perp \gamma \mid \alpha$ means that β is independent of γ upon conditioning on the complete set of parents of β and γ , here represented by α . Each constraint was tested by generalized mixed models [MIXED procedure in SAS 9.2 (SAS Institute Inc., Cary, NC, USA)] according to Shipley (2009). Repeated measurements were applied because measurements were taken on the same experimental unit, the tree, for several consecutive years. Each mixed model included the variable site as the fixed effect and measured the connection between the independent and dependent variables (e.g. β and γ , respectively), controlling for the effect of a third (e.g. α), by obtaining the probability that the partial regression slope of the dependent variable was zero. The exact probability level of the j -independence relationship being p_j ,

Shipley (2009) illustrates the procedure to verify each model as a whole according to the Fisher's C statistic

$$C = -2 \sum_{j=1}^k \ln(p_j)$$

where k is the number of independence relationships listed for the causal model. The statistical significance of the Fisher's C is verified by the χ^2 probability function with $2k$ degrees of freedom (df).

Results

Phenological phases and cell production

Overall, the first enlarging cells, corresponding to the onset of xylem differentiation, were observed between mid-May and mid-June (DOY 138–177) (Fig. 2). On average, the first wall-thickening cells were detected 14 d after the onset of the enlargement phase, between the beginning of June and mid-July (DOY 151–193). More than 60% of observations occurred before the end of June (DOY 175). The first mature cells appeared 14 d after the onset of cell wall thickening, between DOY 161 and 209, which corresponded to the period between mid-June and the end of July (Fig. 2). The earliest onsets of each phenological phase were always observed in the southern site (Simoncouche), while cell differentiations began later in Camp Daniel and Bernatchez, the sites located at the higher latitude and altitude, respectively (data not shown).

In general, the ending of cell enlargement occurred between mid-July and the beginning of September (DOY 189–250), although the enlargement phase only occasionally extended after mid-August (in 5.1% of cases) (Fig. 2). On average, the period when cell enlargement was observed lasted 59 d. The last cells in cell wall lignification, which corresponded to the ending of both cell wall thickening and lignification and xylem differentiation, were observed between Mid-August and mid-October (DOY 228–288). However, 46% of the ending of lignification occurred during the middle 15 d of September (Fig. 2). As a result, secondary wall differentiation and xylem formation lasted on average 90 d and 104 d, respectively. The earliest endings of cell wall lignification were observed in Bernatchez and Camp Daniel, while longer durations of xylem formation occurred in Simoncouche (data not shown). The ranges of variation of each phenological phase gradually increased from the onset of cell enlargement (39 d) to the endings of cell enlargement and cell wall lignification (61 d and 60 d, respectively).

A high variability in cell production was observed in the four sites and during the studied years (Fig. 2). The number of cells varied between 5 and 58, most frequently (72%) between 10 and 25. Data distribution was slightly right skewed, with 3.7% of individuals producing >45 cells. In general, the highest numbers of cells were in Simoncouche, with an average of 32 cells observed during the 8 years of study, while fewer were produced in Mistassibi and Camp Daniel, which exhibited ranges of 6–38 and 5–38 cells, respectively.

Exploring causal models

Strong and significant correlations were observed between the dates of emergence of the first enlarging, wall-thickening,

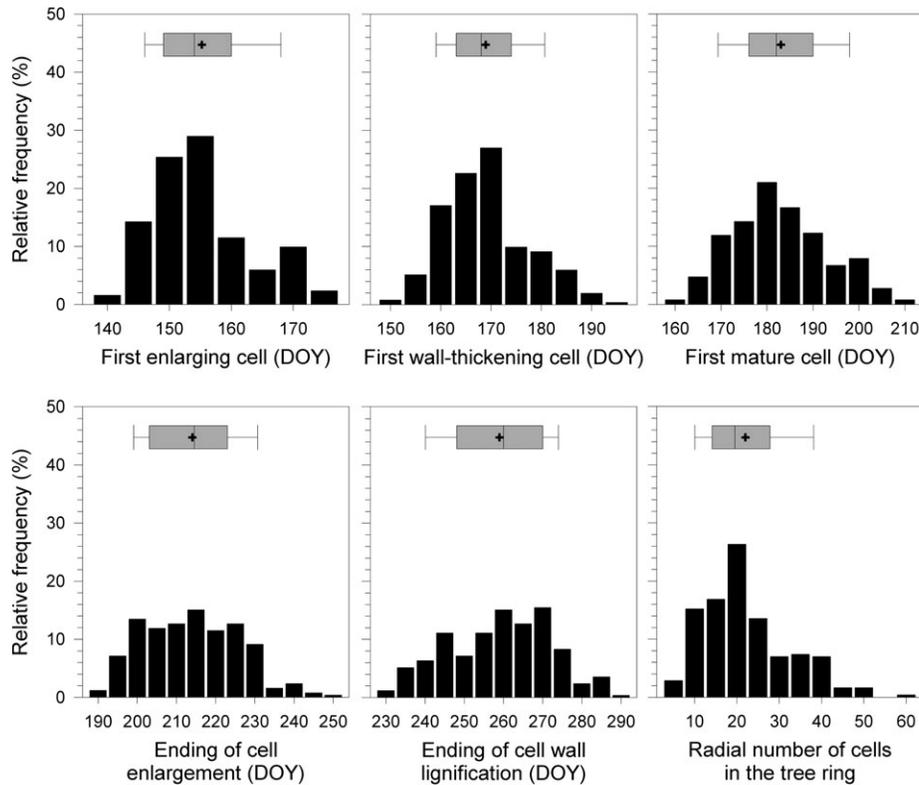


Fig. 2. Frequency distributions of the dates of cambium phenology (first enlarging, wall-thickening, and mature cell, ending of cell enlargement, and cell wall lignification) and the radial number of cells produced annually by black spruce during 2002–2009. Horizontal boxes represent upper and lower quartiles, whiskers achieve the 10th and 90th percentiles, and the mean and median are drawn as a cross and vertical solid lines, respectively.

and mature cells, with Pearson correlations calculating positive coefficients r of between 0.64 and 0.74, and $P < 0.001$ (Fig. 3). Accordingly, late onsets of cell enlargement led to delayed onsets of secondary cell wall formation and late emergences of mature cells in the xylem. In contrast, weak correlations were observed between the phases of onset and ending of differentiation, with r negative and lower than 0.24. The ending of cell enlargement was positively correlated with the ending of cell wall lignification, with $r=0.50$ and $P < 0.001$. The number of cells was significantly correlated with all phenological phases, with the greater coefficients resulting from the correlations with the endings of cell enlargement and cell wall lignification ($r=0.51$ and 0.54, respectively). Later dates of first observed enlarging, wall-thickening, and mature cells corresponded to lower cell productions, although the relationships appeared not to be perfectly linear at the higher numbers of cells. In contrast, greater numbers of cells were observed when cell enlargement and cell wall lignification occurred late in the season (Fig. 3).

According to the combinatorial computation, the number of potential causal models that could be defined using the six measured variables were >1 billion, more precisely 1 073 741 824. Among them, the EPA2 program found eight possible dependency links between pairs of variables resulting in 12 sets of equivalent models that were not rejected by the SGS algorithm and that could be valid. The potential causal model showing all possible dependency links consisted

of an undirected dependency graph where the lines connected the two variables probabilistically dependent conditional on every subset of other variables in the graph (Fig. 4, left). In its present form, the relationships identified by the lines express only a symmetrical association between pairs of variables and not an asymmetrical causality.

Testing causal models

The symmetrical association between variables of the undirected causal graph were successively converted into asymmetrical causal relationships as illustrated by the directed causal graph in Fig. 4 on the right, where arrows describe the causal links between variables (e.g. $\alpha \rightarrow \beta$ indicates that α affects β) and specify how causal effects are hypothesized to propagate through the measured variables. For the identification of the causal relationships, the temporal rule in which effects might propagate only from preceding to subsequent phenological phases was assumed. In the resulting directed causal graph, causal links existed between FE, FW, and FM, and between NC, EE, and EL, with FE also affecting NC. FE and NC affected FM and EL, respectively, while causality also connected FM with NC (Fig. 4). The causal relationships entailed six independence constraints that are listed in Table 2. The slopes of partial regressions produced F -values ranging between 0.03 and 3.72, with only one constraint being statistically significant

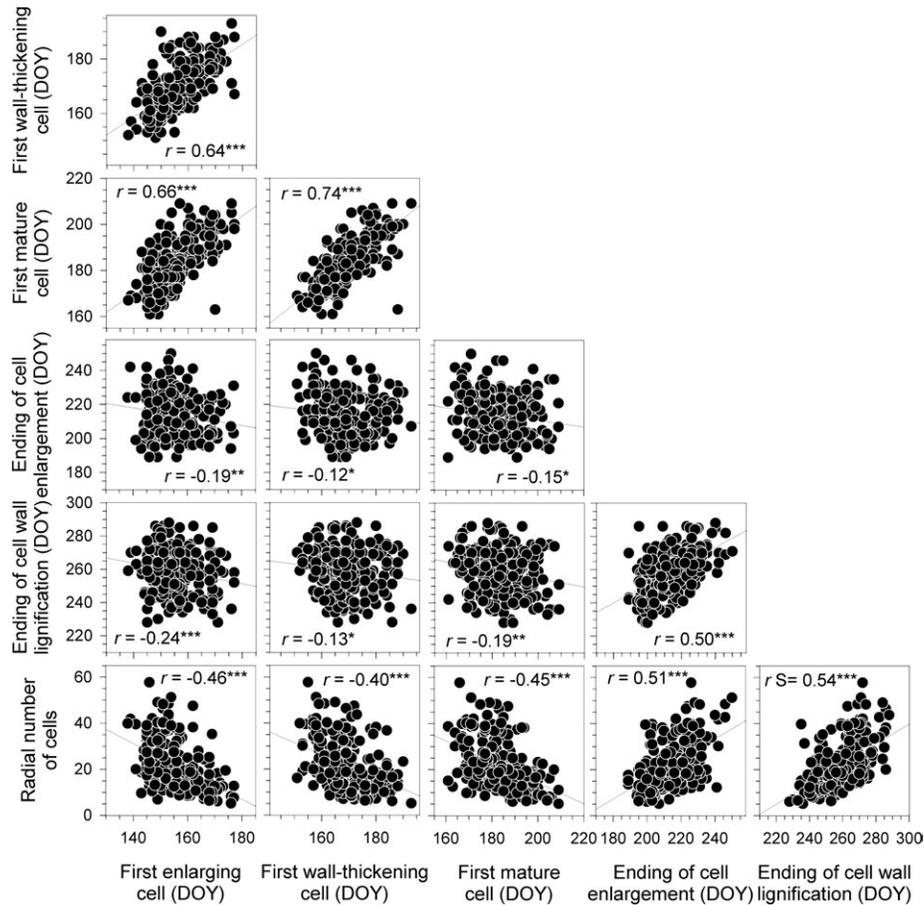


Fig. 3. Correlation matrix between the dates of cambium phenology (first enlarging, wall-thickening, and mature cell, ending of cell enlargement, and cell wall lignification) and the radial number of cells produced annually by black spruce during 2002–2009. One, two, and three asterisks correspond to a probability lower than 0.05, 0.01, and 0.001, respectively.

at $P < 0.05$ (Table 2). The Fisher's C statistic calculated on these constraints was 13.23. The χ^2 probability function with 14 df corresponded to a $P=0.50$. Consequently, the non-significant d-sep test was not able to reject the proposed model and the directed causal graph described in Fig. 4 should be considered statistically valid.

Discussion

The approach applied herein consisted of translating an *a priori* hypothesis concerning the causal links between the timing of cell production and differentiation into a statistical model capable of falsification. Although the effects of the variables were not experimentally disentangled, a conceptually straightforward model with causal links in the form of partial regressions performed among the phenological phases and cell production was tested on an extensive data set, which represented 8 years of monitoring black spruce growth at high temporal resolution on four permanent plots in the boreal forest of Quebec, Canada. This investigation was performed to infer the alternative patterns of dependence among the variables and obtain a mathematical and comprehensive description of the phenomenon of xylogenesis.

The explorative analysis found that the measured variables were significantly correlated, although with different

degrees of intensity, and identified eight possible dependency links connecting pairs of variables probabilistically dependent conditional on every subset of other variables. This allowed the hypothesis of independence of the variables to be rejected and provided evidence of the importance of alternative procedures in studies concerning phenology. Attempts to manipulate xylem formation with environmental forcing looked at only the onset of cambial resumption and were applied locally on stem or roots, thus hardly representing a natural condition for trees, and rarely produced substantial and stable results (Oribe and Kubo, 1997; Begum et al., 2010; Lupi et al., 2012). Unless working in controlled environments with seedlings, which, however, have a reduced sensitivity and respond differently from adult individuals (Rossi et al., 2008a, 2009), modifications of the growth conditions in trees are physically impossible or prohibitively expensive. The proposed indirect experimentation in search of consequences of phenological events can be a suitable tool when investigating complex systems founded on several components of causation, which cannot be directly manipulated in terms of actual knowledge (Pearl, 2010).

The resulting causal model produced a P -value of 0.50, which indicated that the model was statistically appropriate for explaining how causality propagates through the variables. The applied statistical approach was unable to evaluate

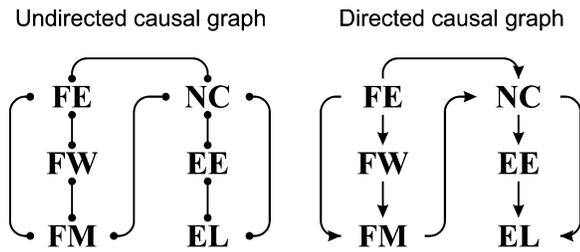


Fig. 4. Undirected and directed causal graph involving the dates of cambium phenology [the first observed enlarging (FE), wall-thickening (FW), and mature (FM) cell, ending of cell enlargement (EE), and cell wall lignification (EL)] and final number of cells (NC). Dots represent a symmetrical association between variables, while arrows describe the causal links between variables, where $\alpha \rightarrow \beta$ indicates that α affects β .

Table 2. Independence constraints and relative statistical verification in terms of significance of the partial regression slope assessed using generalized mixed models with repeated measurements (F-value), degrees of freedom (df), and Fisher's C statistic for the directed causal graph involving the dates of cambium phenology [the first observed enlarging (FE), wall-thickening (FW), and mature (FM) cell, ending of cell enlargement (EE), and cell wall lignification (EL)] and final number of cells (NC)

The notation $\beta \perp\!\!\!\perp \gamma \mid \alpha$ means that β is independent of γ upon conditioning on the complete set of parents of β and γ , here represented by α . One asterisk corresponds to a probability < 0.05 .

Constraints of the directed causal model	Statistical verification of the constraints
$FE \perp\!\!\!\perp EE \mid NC$	$F_{FE,EE \mid NC} = 0.12$
$FE \perp\!\!\!\perp EL \mid (NC, EE)$	$F_{FE,EL \mid (NC, EE)} = 0.03$
$FW \perp\!\!\!\perp NC \mid (FE, FM)$	$F_{FW,NC \mid (FE, FM)} = 0.27$
$FW \perp\!\!\!\perp EE \mid (FE, NC)$	$F_{FW,EE \mid (FE, NC)} = 3.72^*$
$FW \perp\!\!\!\perp EL \mid (FE, NC, EE)$	$F_{FW,EL \mid (FE, NC, EE)} = 1.58$
$FM \perp\!\!\!\perp EE \mid (FE, FW, NC)$	$F_{FM,EE \mid (FE, FW, NC)} = 0.42$
$FM \perp\!\!\!\perp EL \mid (FE, FW, NC, EE)$	$F_{FM,EL \mid (FE, FW, NC, EE)} = 0.36$
	df = 14
	Fisher's C = 13.23

the strength of causal relationship between each pair of variables, but a global evaluation was produced of the reliability of the model, and assumptions about the structure of the causal relationships could be harnessed. This revealed that xylogenesis is not constituted by episodic phenological phases occurring independently of one another. Genetic studies reveal that during xylem development, a regulatory network in different xylem developmental stages exists, which is mainly composed of transcription factors and plant hormones (see the review by Zhang *et al.*, 2011). The statistical approach used here demonstrated for the first time that the successive phenological phases of cell production and differentiation involved in xylem formation are closely interconnected by complex relationships of cause and effect, suggesting that xylogenesis could have a precise and recognizable framework. This could imply that, along the radial

row, the maturation of one cell is not independent of that of the others and that changes in the timing of differentiation of earlywood can induce changes in the timing of maturation of latewood. Consequently, environmental events able to modify the dates of emergence or formation of the first cells could indirectly affect all successive timing of cell development of xylem. Accordingly, the effects of spring snowmelts on the long-term amount and duration of xylem cell production, which are related to processes concluding their activities in summer and early autumn, respectively (Rossi *et al.*, 2011a), can be explained by the causal relationships between onset and ending of xylogenesis.

The presence of several causal relationships in the model indicated that the simple temporal succession of phenological phases is not sufficient to explain a linear causal link between variables (e.g. the date of the first mature cell is affected by the first wall-thickening cell, which emerges before, but also by the first enlarging cell). Consequently, there is no evidence of a mechanism in which a phenological phase necessarily affects the successive one just because it occurs first. Accordingly, no direct relationship exists between the phases of onset (variables FW and FM) and ending (variables EE and EL) of cell differentiation, as shown in the oriented causal graph in Fig. 4. Nevertheless, these phenological phases are influenced directly by the variable FE, or indirectly via the number of cells. This could be explained by the fact that onset and ending of cell differentiation are related to different pools of cells, either the first or last cells in development, respectively.

The onset of xylem differentiation (represented in the tested model by the onset of cell enlargement) is the phenological phase driving all processes of tree-ring formation by triggering the successive timings of cell production and maturation. In cold climates, trees concentrate cambial activity in the first part of the growing season, concluding the production of the new xylem cells early, and so independently of the high temperatures in mid and late summer (Rossi *et al.*, 2006c, 2008b; Heinrichs *et al.*, 2007). Most of the tree-ring formation has taken place in summer to prevent shifts of tracheid production, leaving enough time to complete cell wall formation and lignification before winter. As a consequence, it could be deduced that earlier growth resumptions in spring can provide more time for increasing cell production in trees. An attempt to predict wood formation under future warming scenarios was represented by a model using thermal thresholds, which estimated longer durations of xylogenesis in black spruce at increasing air temperatures (Rossi *et al.*, 2011b). Although such a model identified the presence or absence of xylogenesis with a sufficient approximation, the physiological explanation of a change in the growing season was missing. Warmer temperatures in spring and early summer lead trees to achieve earlier the thermal thresholds required for cambial resumption and allow a longer period for cell division. According to the results of the causal model, higher accumulations of derivative cells in cambium need more time to undergo differentiation, delaying the ending of cell enlargement and wall thickening and lignification in late summer and extending the length of xylogenesis.

According to the matrix of correlation, only 21% of the variability in number of cells was taken into account by the relationship with the date of the first enlarging cell (Fig. 3). It is likely that other unknown or unmeasured factors, such as the growth rate, reasonably contribute to defining cell production of xylem. Thus, on the one hand, the low temperatures of April–May could severely limit the rate of cell division being achieved with an unusually early cambial resumption (Deslauriers *et al.*, 2003). On the other hand, cambial activity lasts at least 2 months at the latitudes where this study has been carried out, and high rates of cambial activity could still occur during warm and favourable days between late June and mid-July, leading to increased production of cells to be differentiated in summer (Kujansuu *et al.*, 2007; Rossi *et al.*, 2008b; Lupi *et al.*, 2010). Accordingly, a number of studies have revealed positive effects of June–July temperature on tree-ring width of conifers in cold climates (Urbinati *et al.*, 1998; Carrer and Urbinati, 2004; Oberhuber, 2004). Nevertheless, the environmental conditions occurring during the previous winter can modify autumnal carbon accumulation, mycorrhizal root growth, or maturation of needles, shoots, and buds, thus appreciably affecting radial growth of conifers in the following year (Oberhuber, 2004).

Cooling treatment of the stems affected xylem development by reducing the width of tree rings and shortening the period of cambial activity (Gričar *et al.*, 2007). Thibeault-Martel *et al.* (2008) observed larger tree rings in roots than in the stem, and, consequently, later endings of cell wall lignification. Similarly, the earlier and higher xylem production observed in younger trees delays the period required to complete the maturation of xylem, so extending the time window involved in wood formation (Rossi *et al.*, 2008a). The relationship between the timing of the onset and the ending of xylogenesis mediated by cell production definitively explains the correlations observed previously, whose causal links were still unresolved. The well-known reductions in the period and amount of xylem production observed along latitudinal and altitudinal gradients is associated with both later resumptions of growth in spring and earlier conclusions of xylem differentiation in autumn (Rossi *et al.*, 2008b, 2010; Moser *et al.*, 2010). The resulting directed causal graph (Fig. 4) suggests that the greater amount of xylem produced by an earlier onset of growth requires more time to complete maturation, delaying the ending of cell enlargement and cell wall lignification. A higher cell production leads to larger accumulations of cells in the developing xylem. Accordingly, even with greater cell production, xylem differentiation is not able to occur faster, as demonstrated by the longer period required to complete cell maturation. Consequently, it seems that the tree resources allocated to xylogenesis do not increase even if cell division is more vigorous and cambium provides greater amounts of cells to differentiate. The process of secondary wall formation is a long-lasting and expensive deposition of cellulose and lignin, which requires up to 40 d for a latewood cell (Rossi *et al.*, 2006b). These findings confirm the hypothesis of constraints to sink activity for secondary growth, namely cell differentiation, in xylem and the low priority of cambium

among the sinks of a plant (Oribe *et al.*, 2003; Polák *et al.*, 2006; Lupi *et al.*, 2010).

The period when xylem is developing corresponds to the time window during which trees and their wood cells are open to receive environmental signals directly (Frankenstein *et al.*, 2005), resulting in tree rings being a noteworthy and profitable archive of long-term meteorological proxy data (Alley, 2001; Bräker, 2002). Timing of the onset and ending of growth are the two most important factors because they definitively identify the limits of xylogenesis, which in temperate and boreal regions is concentrated during the thermally favourable period between spring and autumn. This study demonstrates the complex relationships which exist among the biological processes of xylogenesis, how causal effects propagate through the phenological phases of cambium, and the role of cell production in defining the timing and dynamics of xylogenesis. In dendrochronology, it is commonly assumed that during the most favourable years, trees can maintain xylogenesis active for a long time, thus allowing cambium to produce more cells. On the contrary, the duration of xylogenesis is the effect, and not the cause, of cell production, because the amount of xylem cells controls the ending of xylem maturation. According to the model presented here, the climatic variables affecting the onset of xylem differentiation in spring constitute key factors that could indirectly influence the formation of the tree ring during the year. Although this study did not arise from a direct manipulation, the analytical model investigated probabilities of causes among variables and was based on an extensive data set represented by an 8 year long monitoring of xylem phenology in four sites of the boreal forest of Quebec, Canada. The findings demonstrated that the successive phenological phases of cell production and differentiation involved in xylem formation are closely interconnected by complex relationships of cause and effect, suggesting that xylogenesis could have a precise and recognizable framework. This knowledge can clarify the relative importance of endogenous and environmental factors occurring throughout the growing season that affect the final phases of xylogenesis.

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