Intra-annual xylem development in seven boreal tree species from eastern Canada

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Abstract

Detailed anatomical investigations of radial growth throughout the growing season are becoming more frequently used in tree-ring studies. Notably missing are studies examining North American boreal tree species, and multiple-species comparisons. Intra-annual xylem development was studied by repeatedly taking microcore stem samples throughout the growing season from seven boreal tree species on the "réserve écologique des Vieux-Arbres", on Lake Duparquet, Québec. Species examined were *Abies balsamea*, *Betula papyrifera*, *Picea glauca*, *Picea mariana*, *Pinus banksiana*, *Pinus resinosa*, and *Thuja occidentalis*. Weekly microcore samples were processed for image analysis, allowing cell and ring characteristics to be measured. Xylem cell division was observed in all species within a week of the start of sampling (May 16, 1999), and completion of the first tangential row of cells was observed 5-17 days thereafter. Latewood initiation dates coincided with a warm, dry period of late summer. Typical sigmoidal curves were characteristic of ring width, number of cells, number of earlywood cells, maximum wall thickness, and maximum wall/lumen ratio, over the growing season. Alternatively, minimum cell length generally approximated a negative linear curve. Growth and development was quickest for *P. glauca* and *T. occidentalis*, while cell production and secondary cell wall thickening of the *Pinus* species continued longest.

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Introduction

Dendroecological studies have used ring width variations in annual tree rings in nine out of ten studies (Schweingruber 1996). Alternatively, tree-ring anatomy, ring density, appearance, chemical composition, and isotopic ratios can be utilized in dendroecology (Schweingruber 1996). In recent years, detailed anatomical investigations conducted throughout the growing season have become more frequent. These studies have aimed at a better understanding of the intra-annual development of tree-rings and the relation to environmental factors (Antonova and Stasova 1993, 1997; Deslauriers *et al*. 2003b; Mäkinen *et al*. 2003; Schmitt *et al*. 2004); it has also been argued that they could be essential to improve assessment of the impact of climate on tree growth (Camarero *et al*. 1998; Gindl *et al*. 2000; Deslauriers *et al*. 2003b; Rossi *et al*. 2003; Schmitt *et al*. 2004). As noted by Belyea *et al*. (1951), within-season growth and development had initially been studied through periodic felling of trees (Hanson and Brenke 1926; Wight 1933). Subsequently, less destructive methods have been utilized: recurrent removal of stem pieces of various sizes (Buckhout 1907; Knudson 1913; Fraser 1952; Bannan 1955; Skene 1969; Ford and Robards 1976), cambial wounding (Wolter 1968; Nobuchi *et al*. 1995; D**h**nisch *et al*. 1999; Schmitt *et al.* 2000, 2004), increment cores (Camarero *et al*. 1998), and more recently, microcores (Antonova and Stasova 1993, 1997; Antonova *et al*. 1995; Bäucker *et al.* 1998; Forster *et al*. 2000; Deslauriers *et al*. 2003a, 2003b; Mäkinen *et al*. 2003; Rossi *et al*. 2003).

Acquiring measurements of tree ring cells from prepared stem samples has also benefited from the recent development of image analysis systems, which enable automatic or

semiautomatic applications (Jagels and Telewski 1990). Images are captured under a microscope from slides created from wood samples, and then analyzed with computer software (Deslauriers *et al*. 2003a, 2003b; Mäkinen *et al*. 2003; Jones *et al*. 2004). Collecting and processing the samples for the observation of seasonal xylem development remains laborious and time consuming, which is a disadvantage.

Alternatively, point and band dendrometers have been used to document seasonal radial growth, and infer the timing of xylem production (Korstian 1921; Pearson 1924; Daubenmire and Deters 1947; Daubenmire 1949, 1950; Belyea *et al*. 1951; Jackson 1952; Ahlgren 1957; Kozlowski and Winget 1964; Tardif *et al*. 2001a). Dendrometers, however, record stem fluctuations only, and do not discriminate between xylem, phloem, or periderm production/development, or water related shrinking and swelling (Belyea *et al*. 1951; Fraser 1952; Ahlgren 1957; Tardif *et al*. 2001a, Mäkinen *et al*. 2003). While dendrometers do not directly measure xylem development or growth rates, they can be used effectively in characterizing seasonal stem fluctuations in relation to climatic conditions (Ahlgren 1957; Kozlowski and Winget 1964; Tardif *et al*. 2001a; Deslauriers *et al*. 2003b). Dendrometer readings can also be calibrated to actual xylem development when used together with anatomical sampling and analysis (Tardif *et al*. 2001a; Mäkinen *et al*. 2003).

Studies of seasonal radial growth and tree-ring development were very limited in Canada approximately 50 years ago (Belyea *et al*. 1951) and remain incomplete today (Tardif *et al.* 2001a; Deslauriers *et al*. 2003a). Few boreal species of North America have been

examined (Belyea 1951; Forster *et al*. 2000; Wang *et al*. 2002; Deslauriers *et al*. 2003a; Rossi *et al*. 2003). Some of the major boreal forest species of North America lack description of their basic phenological/anatomical characteristics (Deslauriers *et al*. 2003a). Most studies of intra-annual development of tree-rings in relation to climatic influences, have been conducted on Eurasia species (Antonova and Stasova 1993, 1997; Antonova *et al*. 1995; Bäucker *et al*. 1998; Camarero *et al*. 1998; Abe and Nakai 1999; Gindl *et al*. 2000; Yasue *et al*. 2000; Oribe *et al*. 2001, 2003; Mäkinen *et al*. 2003; Rossi *et al*. 2003). Notably missing are studies comparing multiple species growing under similar ecological conditions, apart from two-species comparisons (Camarero *et al*. 1998; Rossi *et al*. 2003).

The main objective of this study was to characterize and compare the seasonal xylem development of seven North American boreal tree species—balsam fir (*Abies balsamea* (L.) Mill.), paper birch (*Betula papyrifera* Marsh.), white spruce (*Picea glauca* (Moench) Voss), black spruce (*Picea mariana* (Mill.) BSP), jack pine (*Pinus banksiana* Lamb.), red pine (*Pinus resinosa* Ait.), and eastern white-cedar (*Thuja occidentalis* L.) growing in the same location. It was hypothesized that no differences would be observed among species in relation to i) timing of earlywood and latewood initiation; ii) the rate and duration of radial growth, and iii) the changes in xylem cell dimensions over the course of the growing season. This study provides novel information on intra-annual tree ring formation in boreal tree species.

Materials and Methods

Study area

The study site is on island 45 (48°27’18”N, 79°17’14”W) of the "réserve écologique des Vieux-Arbres", located on Lake Duparquet, Quebec, approximately 700 km northwest of Montreal (Fig. 1). Lake Duparquet covers an area of 25 km^2 and contains about 170 islands of 70 hectares or less in size. This area is in the Northern Clay Belt of Quebec and Ontario, within the Canadian shield (Tardif *et al*. 2001a).

The Lake Duparquet area, more specifically, is part of the boreal shield ecozone (Rowe 1972). Around the lake, the forest cover is dominated by *A. balsamea* mixed with *B. papyrifera,* and with *P. glauca* on mesic sites (Bergeron and Brisson 1990). This region experiences a continental climate: i.e. cold winters and warm summers. The mean annual temperature between 1971 and 2000 was 0.7°C based on the LaSarre meteorological station approximately 42 km to the north (Environment Canada 2004). The mean July temperature was 16.9°C, while it was -18.2°C in January as a result of the dominant cold winter arctic air mass. The average total annual precipitation for the region was 889.8 mm, of which, snowfall comprised 25.2% (Environment Canada 2004).

The study island covers approximately 1.4 hectares (Bergeron and Brisson 1990; Tardif *et al*. 2001a). Its topography is gently undulating, peaking at a height of 14 m above the summer water level of the lake (Bergeron and Brisson 1990). Bergeron and Brisson (1990) noted evidence of five fires on the island since 1799. The last fire dated back to 1914, when approximately half of the island was burned, while the 1799 fire appeared to

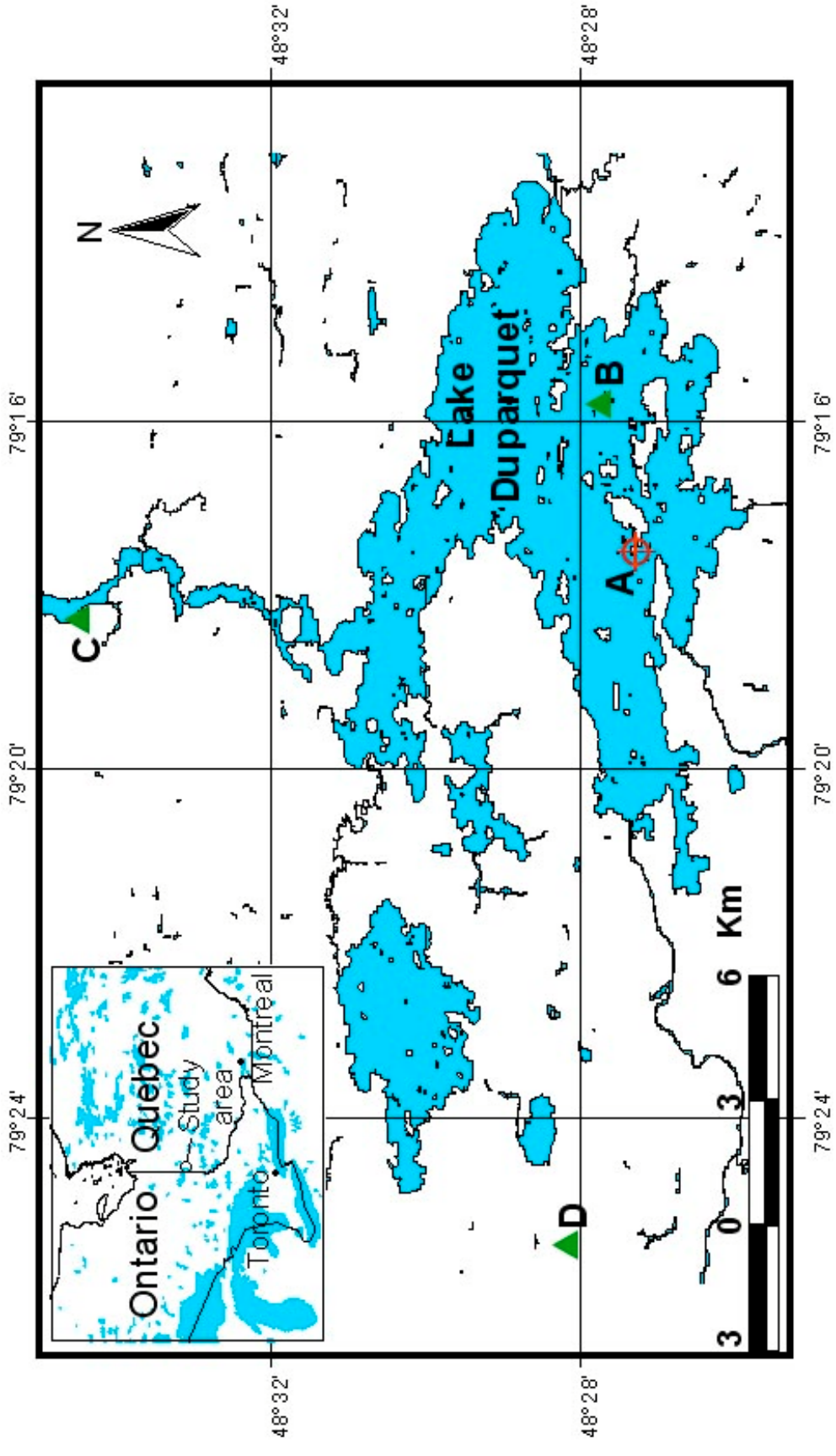


Figure 1. Map of the study area, a preserved island within the "réserve écologique des Vieux-Arbres", on Lake Duparquet. The location of the island and three nearby meteorological stations are represented by letters: A. Study island, B. Heron island station, C. Cetec station, D. Mont Sabrais station.

have burned the entire island (Bergeron and Brisson 1990). Tree species found in the xeric areas of the island—distinguished by the exposed bedrock and thin organic soils—include *P. banksiana*, and *P. resinosa*, as well as a few *T. occidentalis, P. mariana*, and *P. glauca* (Bergeron and Brisson 1990). Alternatively, mesic sites (with an organic layer overtop of till) are typically comprised of *B. papyrifera* and *Populus tremuloides* Michx. (trembling aspen) with *P. resinosa, P. banksiana* intermixed, and *A. balsamea, P. mariana,* and *P. glauca* in the subcanopy (Bergeron and Brisson 1990).

Data collection and site characterization

In 1995, a detailed radial growth study was initiated on the "réserve écologique des Vieux-Arbres" using forty trees from seven species (Tardif *et al*. 2001a, 2001b). Thirty-six of these same trees were used for the current study. General ecological information was gathered for each of the 36 trees sampled, including the date of establishment, height, diameter at 1.3 m, slope, soil thickness, and characteristics of the nearest competitors (Tardif *et al*. 2001a, 2001b) (Table 1). Mean establishment dates of the species ranged from 1891 to 1940, for *P. glauca* and *A. balsamea*, respectively (Table 1). Trees of *P. glauca* were the tallest and exhibited the greatest diameter, while *P. mariana* had the smallest diameter and *T. occidentalis* the shortest mean height. The average slopes on which the trees were situated ranged from 6.4° to 17.4° (*B. papyrifera* and *P. mariana*, respectively). The mean thickness of the LFH layer (organic layer of soil at varying degrees of decomposition) was least for *P. resinosa* (3.5 cm) and thickest under *A. balsamea* (7.3 cm). A distance-weighted size ratio competition index was calculated for each subject tree (Husch *et al*. 2003). Mean competition index values for each species

Table 1. Ecological characteristics and general site conditions (mean and standard deviation) for the seven boreal tree species sampled on the "réserve écologique des Vieux-Arbres" on Lake Duparquet, Quebec. LFH refers to the organic matter (overlying the mineral soil) at varying degrees of decomposition. A - *Abies balsamea*; B - *Betula papyrifera*; C - *Picea glauca*; D - *Picea mariana*; E - *Pinus banksiana*; F - *Pinus resinosa*; G - *Thuja occidentalis*. Data taken from Tardif *et al*. (2001a, 2001b).

	A (n=5)	B (n=5)	C (n=5)	D (n=5)	E (n=6)	F (n=5)	G (n=5)
Establishment date at base	1940 ± 35.2	1900 ± 37.7	1891 ± 28.6	1933 ± 11.2	1920 ± 47.2	1926 ± 36.7	1922 ± 11.3
Diameter at 1.3 m (cm)	15.1 ± 1.4	13.5 ± 3.8	24.1 ± 4.2	12.5 ± 3.5	19.5 ± 10.7	22.1 ± 10.7	16.3 ± 5.9
Height (m)	11.9 ± 1.5	11.9 ± 3.1	16.4 ± 2.1	10.5 ± 1.4	10.3 ± 5.9	11.8 ± 6.2	8.8 ± 2.4
Sapwood width (mm)	34.2 ± 17.7	56.2 ± 10.5	40.2 ± 17.5	14.1 ± 3.0	33.2 ± 21.2	51.1± 21.8	16.2 ± 2.7
1998 ring width at 1.3m (mm)	1.36 ± 0.73	0.71 ± 0.65	1.35 ± 0.84	0.71 ± 0.21	0.53 ± 0.21	0.85 ± 0.24	1.05 ± 0.34
Competition index	1.46 ± 0.73	2.27 ± 1.52	1.20 ± 0.52	2.19 ± 0.90	1.51 ± .94	1.09 ± 0.39	1.78 ± 1.01
LFH thickness (cm)	7.3 ± 7.1	4.6 ± 1.3	5.1 ± 1.7	6.3 ± 1.2	4.2 ± 1.2	3.5 ± 2.1	5.4 ± 1.8
Slope (degrees)	8.6 ± 4.9	6.4 ± 1.7	7.2 ± 0.8	17.4 ± 4.4	13.8 ± 5.1	11.6 ± 4.3	16.6 ± 9.8

indicated that *P. resinosa* and *P. glauca* experienced the least competition, while *B. papyrifera* and *P. mariana* had the most (Table 1). Further information on sampling and procedures can be found in Tardif *et al*. (2001a, 2001b).

Microcore sampling of the 36 trees began in May, 1999. A 2.5 mm diameter microborer was used to extract each microcore sample (5 to 10 mm long) from each tree (Forster *et al*. 2000). Samples were taken at heights between 82 and 146 cm above the ground level. Samples were taken at least one centimetre away from previous sampling locations to minimize trauma (scar) tissue produced by the tree as a result of prior sampling (Forster *et al*. 2000). Sampling took place at six or seven day intervals beginning May 16, and continued for 17 weeks. Three more samples were taken at intervals of 16, 15, and 18 days, respectively, from September 19 to October 22, 1999. A total of 20 samples were extracted from each tree (Table 2). The total number of days spanned by the sampling was 159.

Climatic variables were recorded by means of three soil hydration sensors, one soil temperature sensor, one air temperature sensor, and one photosynthetically active radiation (PAR) sensor. More information on the environmental sensors, as well as the central control and acquisition unit, can be found in Tardif *et al*. (2001a, 2001b). In addition, three automated meteorological stations were in operation nearby, enabling verification of the environmental data collected; Cetec (15 km to the north) and Sabrais (11 km west) were both mainland stations, and the third was on Heron island

Table 2. Calendar and Julian dates, interval, and time elapsed since the start of microcore sampling of seven boreal tree species on the "réserve écologique des Vieux-Arbres" on Lake Duparquet, Quebec.

Week	Date	Interval (days)	Julian day of 1999	Days elapsed
1	16/05/1999	0	136	0
2	22/05/1999	6	142	6
3	28/05/1999	6	148	12
4	04/06/1999	7	155	19
5	11/06/1999	7	162	26
6	18/06/1999	7	169	33
7	25/06/1999	7	176	40
8	02/07/1999	7	183	47
9	09/07/1999	7	190	54
10	16/07/1999	7	197	61
11	23/07/1999	7	204	68
12	30/07/1999	7	211	75
13	06/08/1999	7	218	82
14	13/08/1999	7	225	89
15	20/08/1999	7	232	96
16	27/08/1999	7	239	103
17	03/09/1999	7	246	110
18	19/09/1999	16	262	126
19	04/10/1999	15	277	141
20	22/10/1999	18	295	159

approximately 2 km north-east (Fig. 1). The combined data from the nearby meteorological stations extended from April 2 to October 28, 1999. Meteorological data was recorded on site from May 20 to October 26, 1999. Data was unavailable from June 11-18, 1999, for air and soil temperature at the site, whereas soil moisture (hydration) data was missing from June 12-17.

Laboratory processing techniques

All microcore samples were glued to wooden molding for support and to strengthen the core. Waterproof epoxy was used because the samples were soaked for 30 minutes before sectioning. The microcores were sectioned with a rotary microtome to between 12 and 20 microns and stained in a Safranin 5% bath to aid in cell wall differentiation. They were then mounted on microscope slides using Permount®. Preparation of slides was completed by Université du Québec à Montréal before being sent to University of Winnipeg. Not all slides were useable for image analysis. This was either due to inadequate cell orientation (too greatly departed from the transverse section), or as a result of poor slide quality (e.g. microtome samples were folded over, missing xylem, missing cambium/phloem, compression, or because the samples were severely distorted). For these reasons, the entire 20 slides of any given tree were not represented in the analysis. Out of a possible 720 slides (36 trees, 20 slides each) a total of 594 slides (83%) were kept for the study. Most of the problem slides were encountered in samples taken after mid-late August 1999 (Fig. 2). From the 36 trees, each species sampled had five representatives, except *P. banksiana*, which had six.

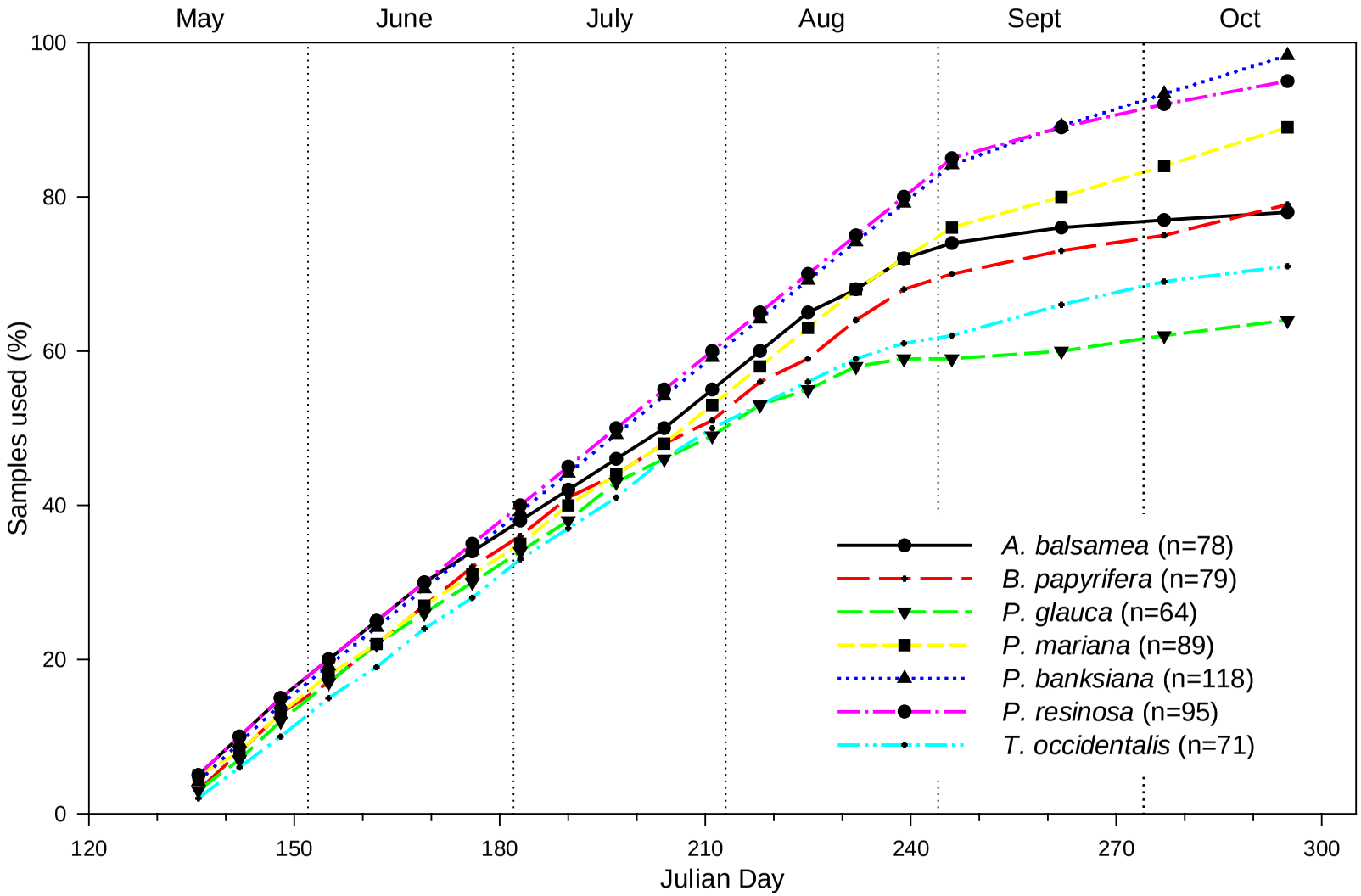
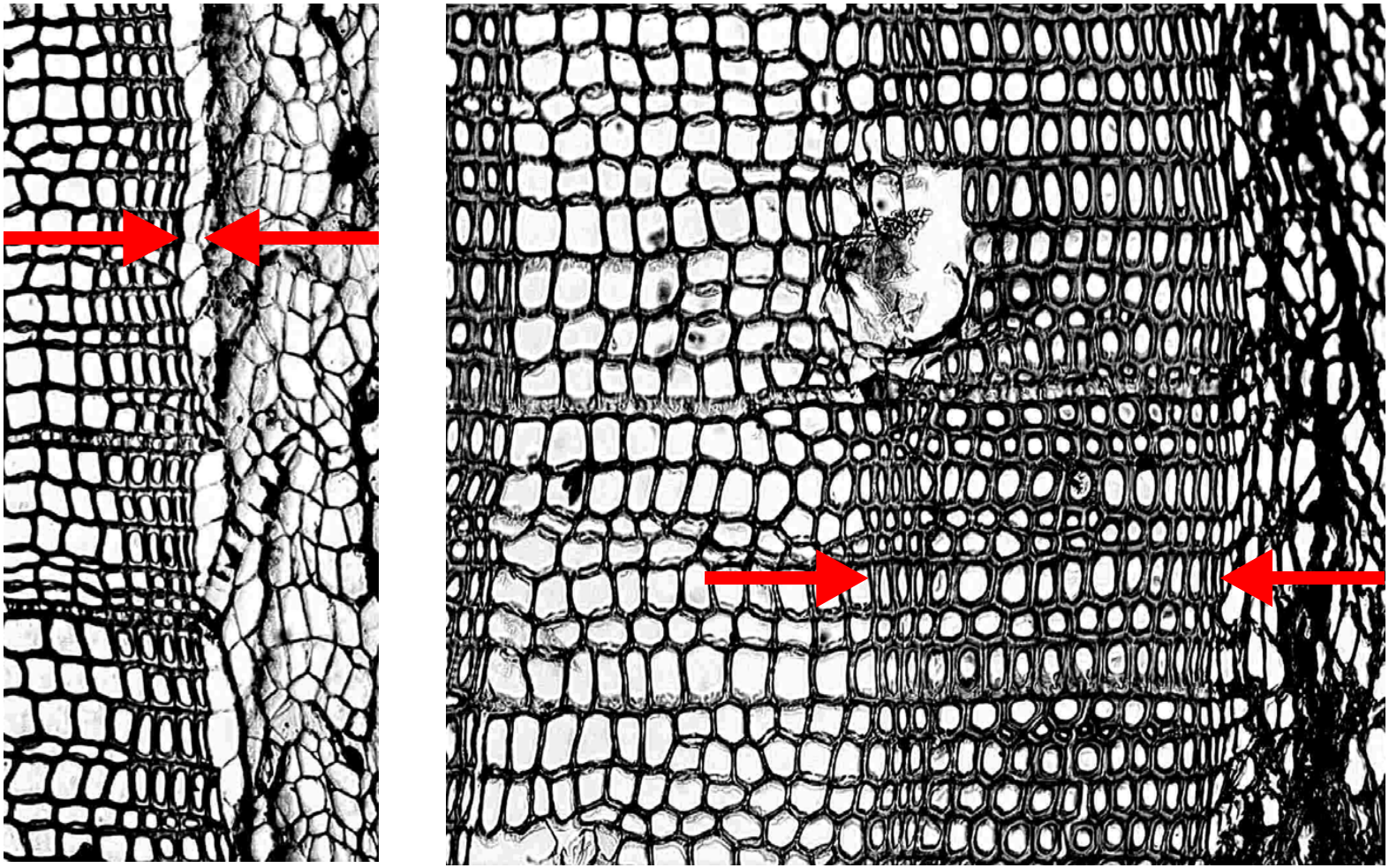


Figure 2. Cumulative percent of slide samples used per sampling event/species. Percents calculated out of the total number of potential samples (5 trees x 20 samples = 100 for each species except *P. banksiana* which had 6 trees x 20 samples = 120). The numbers appearing in the legend refer to the number of samples used for each species.

At the University of Winnipeg, images of each of the 594 microcore slides were acquired by means of a Polaroid digital microscope camera with a Nikon TV C-0.6x lens attached to a Nikon Eclipse E200 microscope (Jones *et al*. 2004, Sutton and Tardif 2005). The images were captured in .tiff format, in black and white, at a resolution of 1200 x 1600, using either 100, 200, or 400x power. The 200x power was used for all slides except those taken of *B. papyrifera* (400x power), due to the smaller cell sizes. The 100x power was used when images were not of sufficient quality to analyse completely, but still good enough to determine the number of cells and overall ring width. A green filter was used to maximize contrast between cell walls and lumen. Images were taken with the previous years latewood (and the centre of the tree) on the left side, resulting in the radial files of xylem cells progressing from left to right toward the cambium, phloem, and bark on the right (Fig. 3). Adobe Photoshop 5.0 Limited Edition (Adobe Systems Inc. 1998) was used to merge any images when a given sample required more than one image to cover the ring width at the given power.

Establishment of the timing of xylem cell production, completion of the first tangential row of cells, and of the start of latewood production was done visually from the slides. Timing of xylem cell production was typically characterized by a few scattered cells being present, and a partial tangential file of cells at best. Completion of the first tangential row of cells was identified as the date when a complete file of cells between the cambium and the previous years latewood could be found (Fig. 3). This was a conservative approach and ensured that there was no doubt that cambial activity and division was underway. Determination of latewood initiation was based on a visual



A B

Figure 3. Completion of the first tangential row of cells (A) and of latewood (B) in *Pinus banksiana*. Image A and B correspond to May 28 and October 4, respectively. In A) the arrows indicate the first complete tangential row of cells produced for that tree. In B) the arrows indicate the latewood portion of the current growth ring. The phloem and bark is to the right of each image, while the centre of the tree is to left. The newest cells in B are on the right of the growth ring, and cells toward the left are progressively older.

estimate of the latewood definition by Mork (1928), where a latewood cell is described as one where the width of a double wall (common between two cells) multiplied by two is equal to or greater than the length of the lumen (Fig. 3). Latewood initiation was considered as the first sampling date when a consistent tangential file or band of cells fitting the latewood description appeared. Time elapsed from the completion of the first tangential row of cells to the start of latewood production was also calculated from the previous measurements.

Images were analyzed using the program WinCELL Pro 2001 (Régent Instruments, Inc. 2001) which specializes in measuring dimensions of wood cells (Fig. 4). Each image was equally divided (horizontally) into thirds and had one radial file of xylem cells randomly located in each portion. To ensure appropriate recognition of cell walls versus lumen space (by the software), any debris was removed from the lumen space, and cell walls were filled in (using the software) where sufficient contrast was uncertain. Initially, each cell (if required) of each radial file had debris removed to allow for analysis; however, this proved unnecessarily time consuming and it was decided to prepare only one file per image for complete data collection (Fig. 4). If the radial file randomly selected encountered resin canals or traumatic tissue, then the radial file above was considered, followed by the file below, until an adequate radial file was found. For *B. papyrifera*, vessel cells were avoided—radial files of fibre cells were used. Three radial files from each image had ring width and the number of cells recorded; this was all that was recorded for some images that were of intermediate quality. The one prepared radial file also had lumen area, lumen length, cell wall length, cell length, average cell wall-to-

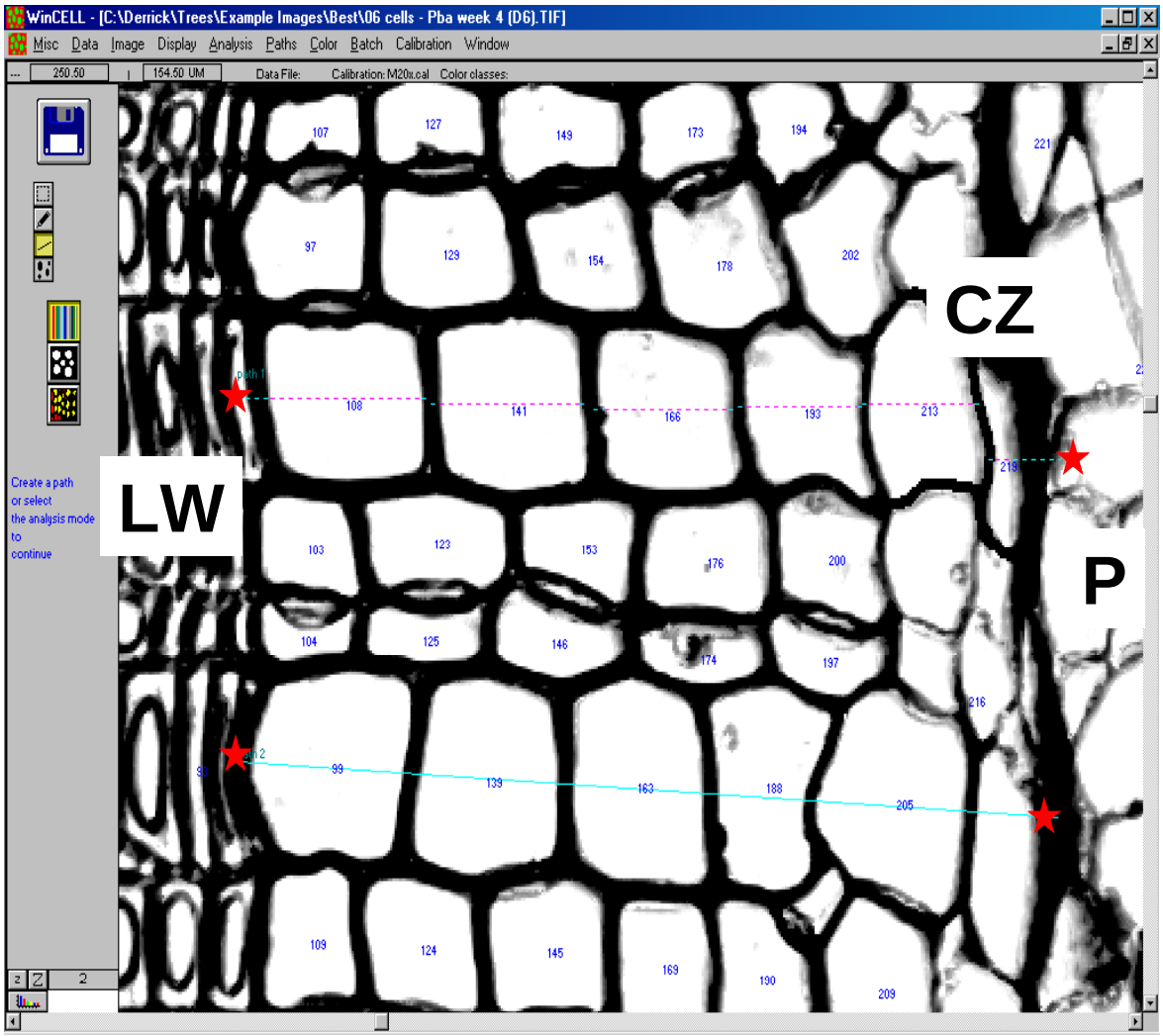


Figure 4. Captured image of a slide representing xylem development in a *Pinus banksiana* tree on June 4 using WinCell Pro 2001. Left is the latewood (LW) of the previous year, and right is the inner-most phloem (P),while the dark band on the right side represents the cambial zone (CZ). Two radial files of xylem cells are highlighted; between the upper pair of stars is an automatic path where multiple measurements were recorded by the software for each of the six cells encountered, while between the lower pair of stars, a manual path measured only the distance of the line shown. For the manual paths, the number of cells were counted and recorded by the operator.

lumen ratio, and the cell type (earlywood or latewood) measured and recorded. Measurement of these cell parameters along the prepared radial file was made automatically by WinCELL Pro 2001, while manual measurements were made along the other two radial files. This resulted in six anatomical variables describing the seasons ring and cell development: ring width (mean of three radii/sample), number of cells (mean of three radii/sample), number of earlywood cells (one radius/sample), maximum cell wall thickness (one cell/sample), maximum cell wall-to-lumen ratio (one cell/sample), and minimum cell length (one cell/sample). Maximum values of cell wall thickness and maximum cell wall-to-lumen ratio were used because they were thought to better represent the increase in cell wall thickness and cell wall-to-lumen ratio as latewood was produced over the course of the growing season. The cell wall-to-lumen ratio was also used by the software to classify cells as earlywood or latewood (a ratio equal or greater than 0.25 was considered latewood). The minimum cell length variable best described the decreasing radial dimension of the xylem cells over the growing season.

Data analysis: phenological and anatomical variables

The dates for timing of xylem cell production, completion of the first tangential row of cells, the start of latewood production, and elapsed time from completion of the first tangential row of cells to start of latewood production were each compared among species using the non-parametric Kruskal-Wallis one-way analysis of variance by ranks test (Sheskin 1997). If a significant difference was found, this was followed by the Mann-Whitney U test for pairwise comparisons (Sheskin 1997; Zar 1974).

The six anatomical/cellular variables measured using WinCELL Pro 2001 were modelled using a sigmoidal function, specifically the Gompertz function (Gompertz 1825). Preliminary analyses were performed, and data from each individual tree was screened to identify and remove outliers. Three of the six anatomical variables (ring width, number of cells, and number of earlywood cells) were also standardized before fitting the Gompertz function. This was done by dividing each raw value by the maximum value of that variable for that individual tree. This helped to minimize variations related to age and size, and increased adjusted r^2 values of the model, thus allowing better comparison among species (Fig. 5, Appendices A-C). The remaining three variables (maximum cell wall thickness, maximum cell wall-to-lumen ratio, and minimum cell length) were not standardized, as cell dimensions in trees develop independently from individual tree growth rate (Antonova and Stasova 1993, 1997; Mäkinen *et al*. 2003; Deslauriers *et al*. 2003a).

The three parameter Gompertz function is a suitable model for biological growth-curves (Winsor 1932) and has been used to study intra annual tree ring development (Camarero *et al*. 1998; Deslauriers *et al*. 2003a, 2003b; Mäkinen *et al*. 2003; Rossi *et al*. 2003). SigmaPlot 8.0.2 provides a form of the Gompertz function:

(1) $$y = ae^{-e^{-\left(\frac{x-x_0}{b}\right)}}$$

where y is the modelled value at time t of the variable under consideration, a is the upper asymptote, x_0 is the x-axis placement parameter, and b is the rate of change parameter (Vantornhout *et al* 2004) (Fig. 5D), or more specifically, the ‘rate constant’ described by

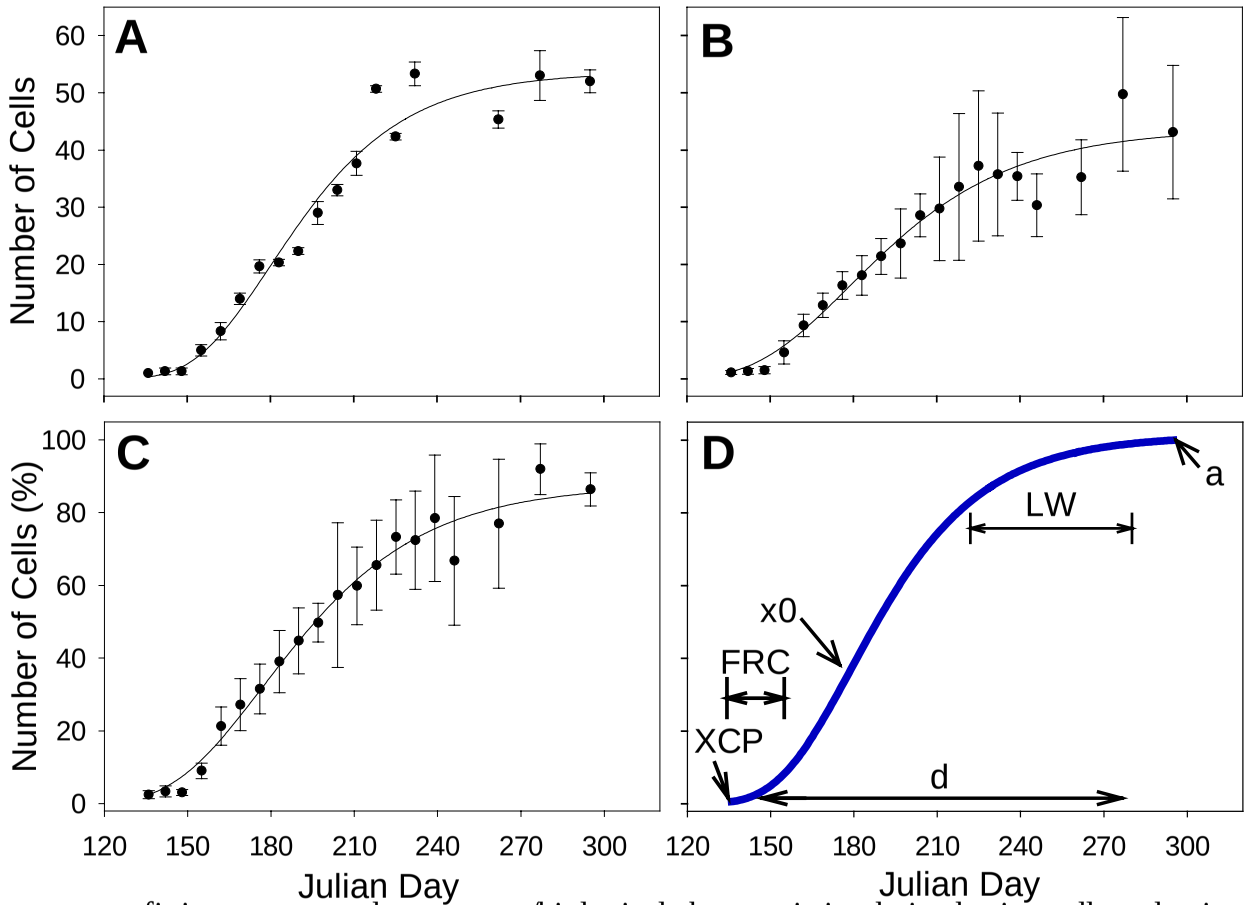


Figure 5. Gompertz curve fitting process and parameters/biological characteristics derived using cell production in *Picea mariana*. A: Gompertz function fitted to untransformed number of cells produced in one *P. mariana* tree. Standard deviation is indicated by vertical bars in A, B, and C. B: Gompertz curve fit to untransformed number of cells produced by all five *P. mariana* trees. C: Gompertz curve fit to standardized number of cells produced by all five *P. mariana* trees. D: Gompertz curve shown in C with the asymptote forced to 100%. Parameters examined: XCP - mean date of xylem cell production (scattered cell production); FRC - completion of the first tangential row of cells; LW - start of latewood production; x0 - date of greatest cell production; a - maximum cell production; d - duration of majority of cell production.

Richards (1959) that controls the curve's distribution over time. Equation 1 is equivalent to equation 2, which has been the popular form in the literature noted, and well described by Cheng and Gordon (2000):

(2) $y = a\exp(-e^{(\beta-\kappa t)})$

where parameters *y* and *a* do not differ from above, **6** is the rate of change parameter or 'rate constant' (where **6** = 1/*b*), and **$** is the x-axis placement parameter. Parameter x_0 (eq. 1) also marks the date of inflection when rate of change in *y* is greatest; for the Gompertz function, this is at approximately 37% of *y* (Winsor 1932) (Fig. 5D), however, **$** does not directly provide this information. This can be extracted as follows: x_0=**$***b*. In addition to the biologically useful parameters *a* and x_0 (asymptote or maximum *y*, and date of inflection point), Richards (1959) provides two more parameters that can simply be calculated from the fitted statistics. The weighted mean absolute rate of change in *y* is *r* (eq. 3), and the duration or time required for the major portion of *y*max-*y*min to occur is *d* (eq. 4, Fig. 5D) (Cheng and Gordon 2000). For eq. 3 and 4, parameter *v* was set at 0.0001 because the Gompertz function is a special case of the Richards function when *v*–0 (Cheng and Gordon 2000; Deslauriers 2003a).

(3) $$r = \frac{a\kappa}{2(v+2)}$$

(4) $$d = \frac{2(v+2)}{\kappa}$$

After calculating the Gompertz function for each species and variable, each Gompertz model was recalibrated by forcing the asymptote (or beginning for the negative linear shaped minimum cell length curves) to 100%, allowing better visual comparison among

the species curves (Fig. 5D). The parameters a and x_0 of each curve were also compared among species where appropriate using an unpaired t-test (eq. 6) (Motulsky and Christopoulos 2004).

(6) $$t = \frac{B_1 - B_2}{\sqrt{SE_1^2 + SE_2^2}}$$

This equation compares two best-fit values (B_1 and B_2, which would be values of parameter a or x_0 of two species) from one experiment, using the species' standard error (SE) values.

Results

Phenological Activity

Xylem cell production—defined in this case by the presence of at least one new xylem cell—had begun by the start of sampling (May 16, 1999) for many trees (Table 3). The mean date of xylem cell production ranged from day 136 to 142. In both *P. mariana and P. resinosa*, cell production was observed significantly earlier that in *T. occidentalis*. Using the more conservative approach, the mean Julian dates for completion of the first tangential row of cells ranged from day 142 to 159. *Abies balsamea* and *P. resinosa* were the first species to produce a complete tangential row of xylem cells (late May), *P. glauca*, *P. mariana*, *T. occidentalis*, and then *P. banksiana* followed, while *B. papyrifera* was the latest (early June). There were no significant differences among species in the start of latewood production, which ranged from Julian day 236 to 251 (Table 3). There were also no significant differences among species in the time elapsed between completion of the first tangential row of cells and the start of latewood production, which ranged from 78 to 103 days. Generally, xylem cell production started while mean air and soil temperatures were still increasing during the second half of May, and into June (Fig. 6). More specifically, the start of xylem cell production (scattered cells) appeared to commence within about two weeks of mean air temperatures exceeding 15 °C, and coincided with mean soil temperatures of about 10 °C (Fig. 6). Latewood initiation coincided with a warm period with low water availability and reduced precipitation ranging from mid-late August until early September (Fig. 6).

Table 3. Timing of xylem cell production, completion of the first tangential row of xylem cells, the start of latewood production, and time elapsed between completion of the first tangential row of cells and the start of latewood production, in Julian days (mean and standard deviation) for seven boreal tree species.

	Date of xylem cell production	Date of first tangential row of cells	Start date of latewood production	Time elapsed between date of first tangential row of cells and start of latewood production
Abies balsamea (n=5)	137ab ± 2.7	142a ± 0.0	242 ± 21.4	100 ± 21.4
Betula papyrifera (n=5)	142ab ± 7.8	159c ± 3.8	238 ± 5.9	78 ± 5.9
Picea glauca (n=5)	141ab ± 5.0	147b ± 2.7	242 ± 12.5	95 ± 12.4
Picea mariana (n=5)	136a ± 0.0	147ab ± 8.3	251 ± 29.3	103 ± 35.0
Pinus banksiana (n=6)	138ab ± 3.1	152b ± 5.5	241 ± 11.9	89 ± 11.0
Pinus resinosa (n=5)	136a ± 0.0	142a ± 0.0	236 ± 3.8	94 ± 3.8
Thuja occidentalis (n=5)	142b ± 4.2	147ab ± 7.1	236 ± 6.3	89 ± 7.3
P value[1]	0.047	0.003	0.982	0.212

1: A non-parametric Kruskal-Wallis test was run to test the null hypothesis of equality of mean among species. If the null hypothesis was rejected ($P < 0.05$), a two-independent-samples test (Mann-Whitney U test) was run between each pair. Means with different letters are significantly different at $P < 0.05$.

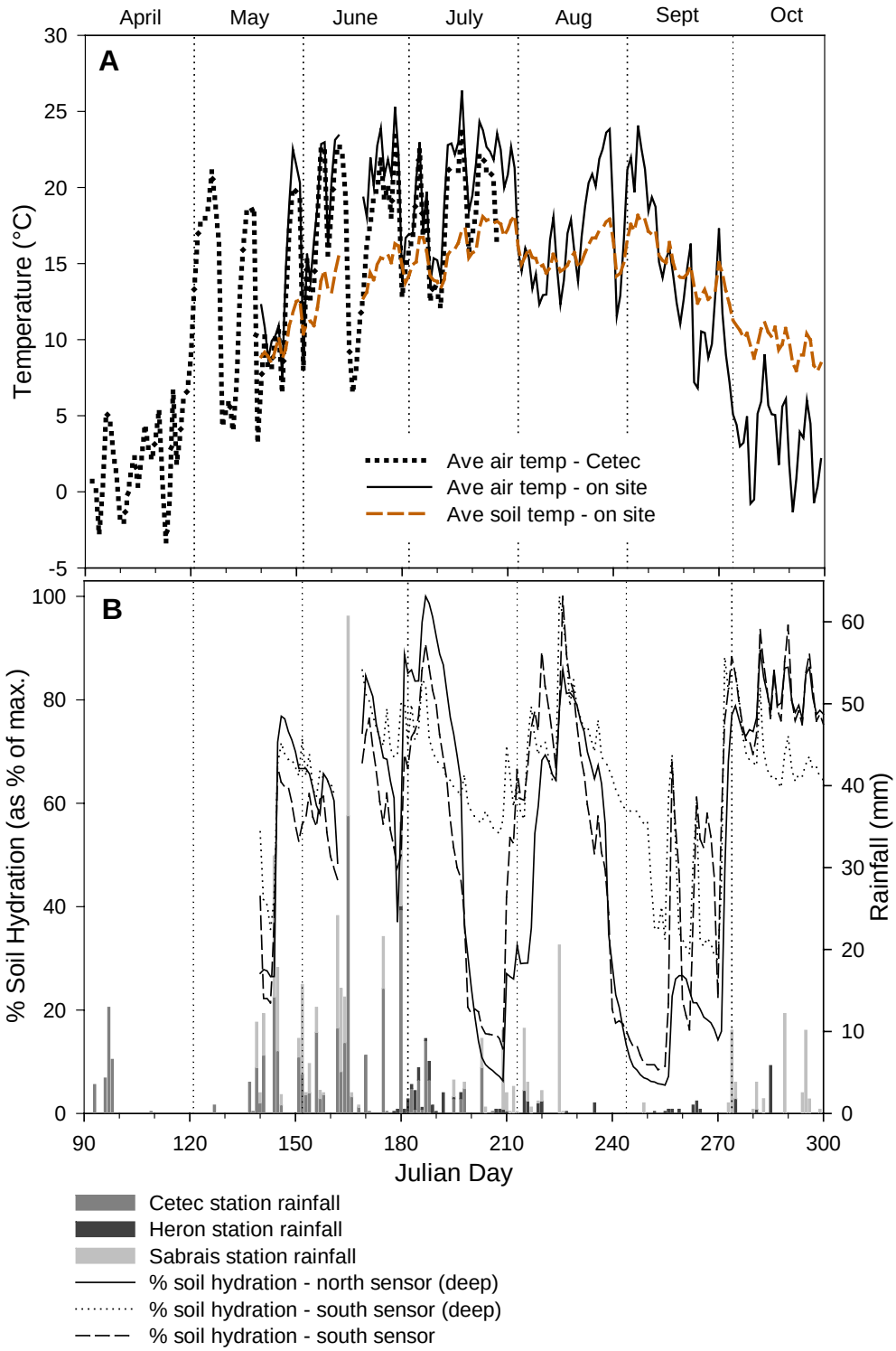


Figure 6. Meteorological variables recorded on site or nearby. A: Air and soil temperatures recorded on site and air temperature recorded at a nearby meteorological station (Cetec). B: Average soil hydration values transformed to 100% of maximum recorded on site, and rainfall amounts from nearby meteorological stations (Cetec, Heron, and Sabrais stations).

Ring width and cell production

Ring width, number of cells, and number of earlywood cells for each species displayed a typical sigmoidal curve (Fig. 7 and 8, Appendices A-C). The Gompertz models were all significant, with an adjusted r^2 ranging from 0.61 to 0.92 ($P < 0.0001$) (Fig. 8). Little differences were observed between the Gompertz model fitted to ring width and number of cells produced and unless otherwise indicated, only the later will be presented (Fig. 8).

Results indicated that *P. glauca* followed by *T. occidentalis* reached the date of their maximum xylem cell production significantly earlier than the other species and that they experienced faster cell production rates and the shortest duration of cell production (Fig. 7, Table 4). These results indicate a shorter growing season for both *P. glauca* and *T. occidentalis*. Conversely, *B. papyrifera, A. balsamea* and *P. resinosa* reached the date of their maximum xylem cell production (x_0) the latest. *Abies balsamea*, *B. papyrifera*, *P. mariana* and *P. resinosa* all responded similarly. They had the lowest mean rates and the longest duration of increment growth (excluding *P. resinosa*) and cell production (Table 4). These four species had a longer growing season, while *P. banksiana* was more intermediate in this respect. The dates of greatest cell production for all of the species appeared to occur after mean soil temperatures reached or exceeded 14°C, and mean air temperatures were around 20°C (Fig. 6, Table 4).

In all species, except *A. balsamea*, the earlywood cell production curves plateaued earlier than the number of cells curves, and the difference between the two curves reflects the proportion of latewood cells, in theory (Fig. 7, Appendix B-C). *Picea* and *Pinus* all had

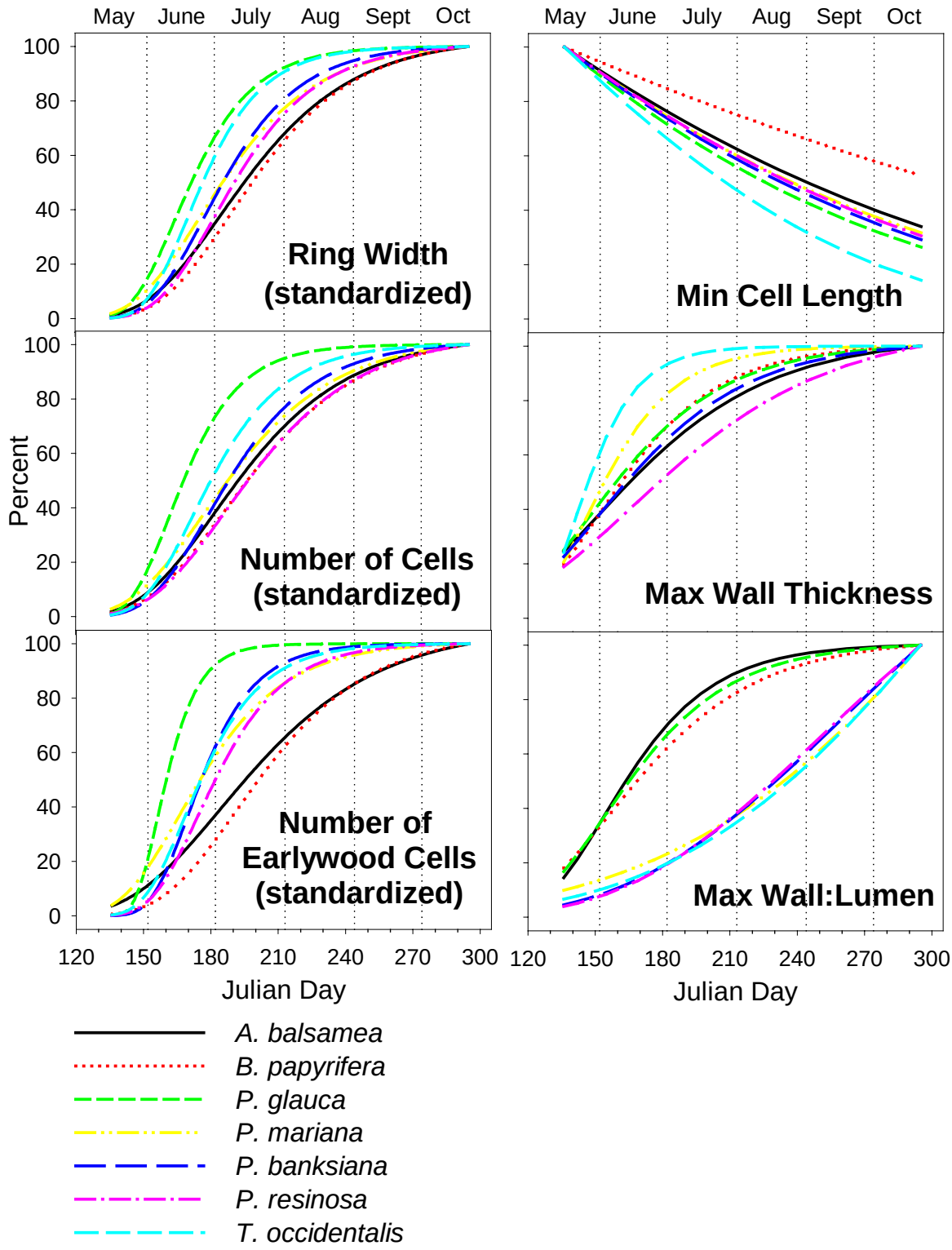


Figure 7. Gompertz curves for seven species and six variables. All curves were forced to 100%. Ring Width, Number of Cells, and Number of Earlywood Cells curves were first standardized.

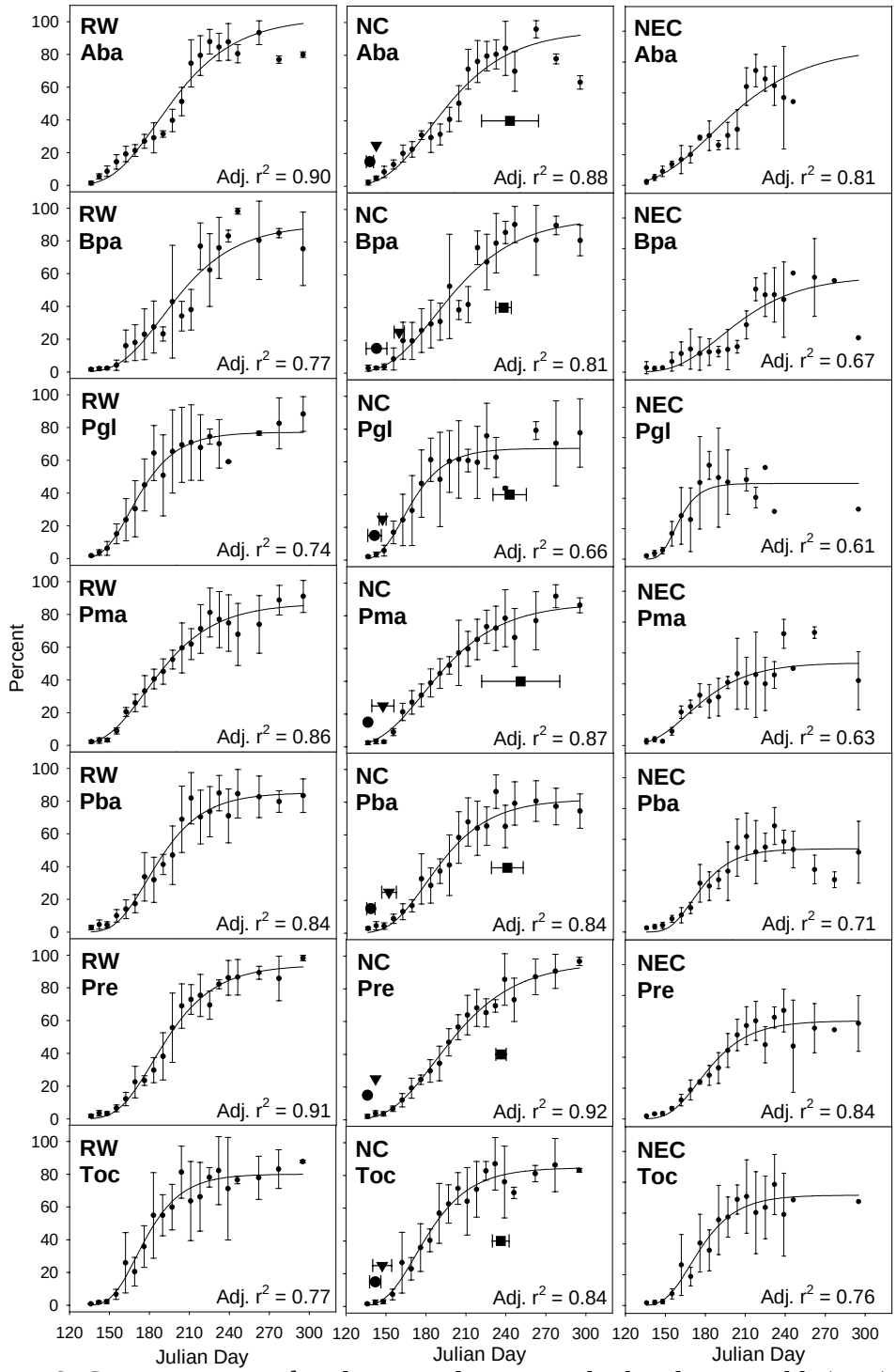


Figure 8. Gompertz curve fitted to cumulative standardized ring width (RW), standardized number of cells (NC) and standardized number of earlywood cells (NEC). Vertical bars are standard deviation. NC sub-figures show date of xylem cell production (circle), completion of the first tangential row of cells (triangle), start of latewood production (square), and standard deviation (horizontal bars). Aba: *Abies balsamea* (n=5) Bpa: *Betula papyrifera* (n=5) Pgl: *Picea glauca* (n=5) Pma: *Picea mariana* (n=5) Pba: *Pinus banksiana* (n=6) Pre: *Pinus resinosa* (n=5) Toc: *Thuja occidentalis* (n=5).

Table 4. Parameters for the Gompertz function fitted to the data of seven boreal tree species. Three growth variables are represented (ring width, number of cells, and number of earlywood cells), and three cell level variables are represented (minimum cell length, maximum cell wall thickness, and maximum cell wall-to-lumen ratio).

Variables	***A.balsamea***	***B.papyrifera***	***P.glauca***	***P.mariana***	***P.banksiana***	***P.resinosa***	***T.occidentalis***
Ring Width-							
x0: date of greatest growth	184.9de	189.0e	164.8a	175.8c	177.6c	182.1d	170.1b
r: increment growth rate (%RW/day)	0.78	0.73	1.00	0.75	0.93	0.92	1.08
d: duration of growth (days)	130.8	123.7	77.2	115.4	91.9	102.2	74.3
Number of Cells-							
X0: date of greatest cell production	181.9cd	186.0d	161.9a	177.2c	179.1c	186.4d	172.2b
R: cell production rate (%cells/day)	0.72	0.69	0.98	0.68	0.78	0.72	0.95
d: duration of cell production (days)	132.1	136.8	69.3	129.5	105.5	133.2	89.6
Number of EW Cells							
x0: date of greatest EW cell production	184.2bc	191.3c	156.5a	166.1ab	169.8b	174.5bc	168.8b
r: EW cell production rate (%cells/day)	0.56	0.47	1.23	0.52	0.82	0.76	0.95
d: duration of EW cell production (days)	158.6	132.1	40.5	103.1	65.8	83.8	75.0
Minimum Cell Length-							
r: cell length decrease rate (microns/day)	-0.12	-0.03	-0.19	-0.17	-0.15	-0.14	-0.11
Maximum Cell Wall Thickness-							
a: max. wall thickness (microns)	6.2cd	4.4b	4.9bc	4.4b	4.6b	8.1d	3.3a
x0: date of greatest wall increase	151.7abc	150.9bc	147.4abc	146.0ab	151.4bc	165.2c	141.3a
r: wall thickening rate (microns/day)	0.04	0.04	0.04	0.05	0.03	0.04	0.05
d: duration of wall thickening (days)	171.9	121.5	136.2	87.8	149.4	208.8	60.7
Maximum Cell Wall-to-Lumen Ratio							
a: max. wall/lumen ratio	0.5a	1.4b	0.5a	9.6ab	1.9ab	2.3ab	5.8ab
x0: date of greatest ratio increase	153.8a	156.3a	154.2a	457.2a	262.0a	251.6a	356.5a
r: rate of ratio increase (ratio/day)	0.005	0.01	0.004	0.01	0.01	0.01	0.01
d: duration of ratio increase (days)	108.0	147.5	124.9	863.1	377.9	344.0	613.8

Note: Data was standardized as a percent of the maximum for ring width, number of cells and number of earlywood cells. Values in rows with different letters are significantly different ($P<0.05$) using an unpaired t-test.

larger portions of latewood by this standard, while both *A. balsamea* and *T. occidentalis* had much less, being comprised mostly of earlywood (Fig. 7). The earlywood cell production curve plateaued very rapidly for *P. glauca,* which had the shortest duration, followed by *P. banksiana, T. occidentalis, P. resinosa,* and *P. mariana* (Fig. 7, Table 4). The longest duration of earlywood cell production (apart from *A. balsamea*) was observed for *B. papyrifera* (Table 4). Both *A. balsamea* and *B. papyrifera* earlywood cell production curves were more linear than the other species. *Abies balsamea* may have produced the most earlywood, however, less data (*n*) was used in the earlywood curve determination (compared to total number of cells). This likely accounted for the irregular curve and late date (x_0) of greatest earlywood cell production (Fig. 7, Table 4). Furthermore, the observation of the start of latewood production, displayed on Figure 8, contradicts the amount of latewood that was indicated by comparison of the earlywood and total cell production curves for *A. balsamea,* as well as *B. papyrifera*. The date (x_0) of greatest earlywood cell production was earliest for *P. glauca* and differed from all but *P. mariana* which was most similar; *B. papyrifera* was the latest and differed from all but *A. balsamea* and *P. resinosa,* which were the next latest (Table 4).

Cell level characteristics

Changes in minimum cell length over the growing season demonstrated the shift from radially enlarged tracheids typical of the earlywood portion of a ring, to tracheids with reduced radial enlargement typical of the latter portion of the ring (Fig. 7 and 9). The adjusted r^2 values ranged from 0.22 to 0.48, which were the lowest among the variables examined (P-value range of 0.004 to < 0.0001). The curves of this variable demonstrated

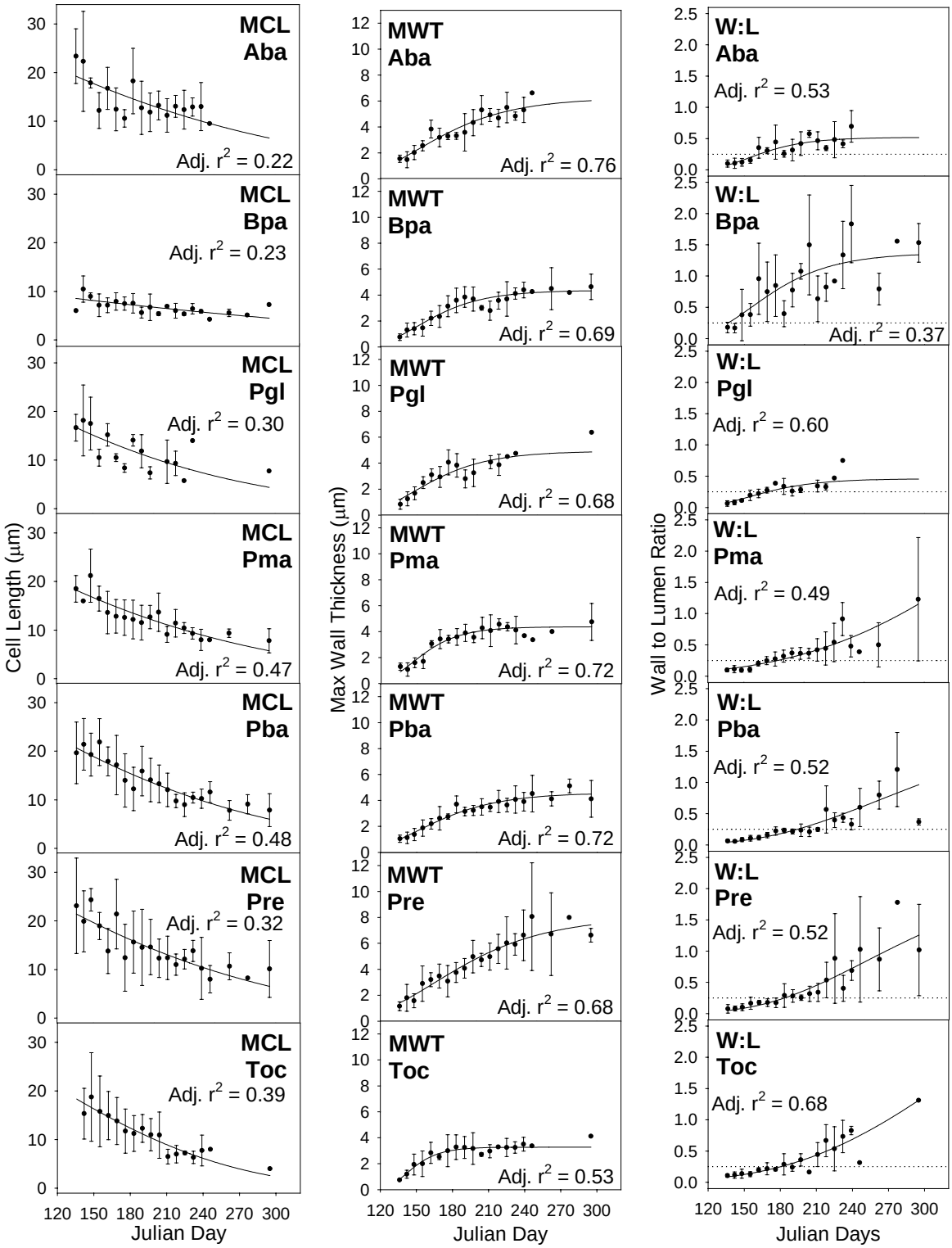


Figure 9. Gompertz curve fitted to minimum cell length (MCL), maximum wall thickness (MWT) and maximum wall-to-lumen ratio (W:L, dotted line indicates the ratio of 0.25 used to identify latewood). Vertical bars are standard deviation. Aba: *Abies balsamea* (n=5) Bpa: *Betula papyrifera* (n=5) Pgl: *Picea glauca* (n=5) Pma: *Picea mariana* (n=5) Pba: *Pinus banksiana* (n=6) Pre: *Pinus resinosa* (n=5) Toc: *Thuja occidentalis* (n=5).

a near linear decrease for all species. The *Picea* species demonstrated the most rapid decrease for minimum cell lengths (Table 4). *Thuja occidentalis* had the greatest overall decrease, as the minimum cell length was approximately 15% of what it started at (Fig. 7). The minimum cell length curve for *B. papyrifera* showed the least decrease of all the species, indicating little change in fiber cell length from the start to the end of the growing season (Fig. 7 and 9, Table 4). Minimum cell length of *B. papyrifera* decreased to about 50% of where it started (Fig. 7). *Abies balsamea* was the most similar conifer, indicative of slightly more uniform cell sizes across the growth ring compared to the other conifers (Fig. 7). By the time latewood initiation was visually identified, most of these curves had decreased by more than two-thirds of what they would by the end of the growing season. Cell lengths continued to decrease until the end of the growing season, while thickening of the cell walls had begun to level off around the start of latewood production (Fig. 7).

Maximum wall thickness curves were mostly similar in shape among the seven species (Fig. 7 and 9). Both *T. occidentalis* and *P. mariana* reached their maximum wall thickness values very rapidly (highest rate of increase in cell wall thickening and shortest duration to level off) (Fig. 7 and 9, Table 4). The maximum wall thickness achieved by the end of the growing season was greatest for *P. resinosa*, which differed significantly from all the other species except *A. balsamea* (Fig. 9, Table 4). Conversely, *T. occidentalis* had the smallest maximum wall thickness at the end of the growing season, differing significantly from all other species (Fig. 9, Table 4). The *P. resinosa* wall

thickness curve was the most linear and did not level off, indicating continued wall thickening late into the season.

Among the seven species, maximum wall-to-lumen ratio curves were of two types: a sigmoidal curve characterized *A. balsamea*, *B. papyrifera*, and *P. glauca*, while a near linear increase seemed to characterize the rest of the species (Fig. 7 and 9). *Abies balsamea, B. papyrifera*, and *P. glauca* curves demonstrated a more stable wall to lumen ratio than the others. The remaining species all generally increased wall thickness and/or decreased cell length to maintain an approximately constant increase in this ratio.

Discussion

Initial xylem production and development

The overall dates (late May to early June) of initial xylem cell production coincided with the findings of Tardif *et al*. (2001a) for radial growth registered by band dendrometers in a 1997 study of the same species and location. The date of completion of the first tangential row of cells was five or six days later than the timing of xylem cell production for *A. balsamea, P. glauca, P. resinosa,* and *T. occidentalis*. The delay until the production of cells was truly underway was greater for *P. mariana, P. banksiana*, and *B. papyrifera* (11,14, and 17 days respectively). Deslauriers *et al*. (2003a) observed the beginning of xylem cell division for *A. balsamea* to range from May 7 to June 7 in the boreal forest of Quebec, while Ahlgren (1957) recorded a range from April 6 to May 20 in Minnesota. The current findings of the timing of xylem cell production and the more conservative estimate provided by the date of completion of the first tangential row of cells (May 17 and 22, respectively) place *A. balsamea* within its noted range. Fraser (1952) found *P. mariana* to begin cell division by June 10, and *B. papyrifera* by June 3, both in Ontario. Ahlgren (1957) also identified a range of starting dates over five years for *B. papyrifera* (May 3-21), *P. glauca* (April 21 to May 7), *P. mariana* (May 13-21), *P. resinosa* (May 3-20), and *T. occidentalis* (May 7-23). The current results were comparable for the timing of xylem cell production (the presence of at least scattered cells) in *B. papyrifera, P. mariana, P. resinosa,* and *T. occidentalis*. We found *P. glauca* to initiate radial growth later than that recorded by Ahlgren (1957), however, those dates (from trees in north-eastern Minnesota) were based on dendrometer measurements and referred to as initial cambial activity, which lacked a clear definition. This could

represent actual cell division, or perhaps initial swelling, meaning that these comparisons must be taken with some caution. Furthermore, annual variation would be expected in one location, and more so in different locations and years. Fraser (1952) noted that the start of xylem cell production began at the top of the tree, and occurred last at the base, which could be another source of variation within a species, depending on sampling heights.

While variation has been noted, we had hypothesized that under similar conditions, the start of the growing season would be similar across species. This was reported by Tardif *et al*. (2001a) with the same species, and Linkosalo (2000) observed that the timing of phenological events of different species respond similarly to climate. Differences among species, however, were observed in the timing of xylem cell production and completion of the first tangential row of cells, particularly the late start of *B. papyrifera*. The late start of *B. papyrifera* has also been observed by Tardif *et al*. (2001a), Belyea *et al*. (1951), and Schmitt *et al*. (2004); however, this species did not differ from others in all cases (Belyea *et al*. 1951; Ahlgren 1957). Also contrary to our findings, two studies found *T. occidentalis* to start at least two weeks earlier than our observations (Bannan 1955; Forster *et al*. 2000). The timing of the start of cell production for different species can be variable (Belyea 1951; Ahlgren 1957; Mäkinen *et al*. 2003), resulting from different climatic conditions each year, geographical locations, tree conditions, and sampling procedures (e.g. heights).

Cambial activity and subsequent initiation of xylem cell production and development at

the beginning of the growing season is sensitive to various environmental factors, primarily temperature, water availability, and/or photoperiod (Mäkinen *et al*. 2003). Generally, cell division initiation occurred while both soil and air temperatures were increasing during the second half of May, and into June. In the dendrometer study by Tardif *et al*. (2001a), the initiation of growth, identified by a consistent upward trend in the cumulative growth increment, corresponded to a rapid increase in maximum soil and air temperatures. This appears consistent with the current findings. Mäkinen *et al*. (2003), in a study utilizing both stem samples and dendrometers, found that actual xylem cell formation coincided with the timing of the greatest radial growth indicated by the dendrometers. The same authors found a significant correlation between temperature and cell production, once underway. Antonova and Stasova (1993, 1997) also found a positive correlation between initial xylem cell production and air temperature. Spring reactivation of cambial activity and subsequent initiation of xylem cell production is typically preceded by the new growth of the buds and their release of auxin throughout the stem (Esau 1960). Partanen *et al*. (1998) found bud burst to be accelerated by a combination of lengthening photoperiod and fluctuating air temperatures, conditions typical of spring, but to be delayed by a shortened photoperiod. Within most boreal environments, after the winter-spring period, soil moisture is typically not limited (Antonova and Stasova 1993, 1997). The variability in the timing of cambial activity and initiation of xylem cell production can be as much as a month or more annually (Belyea 1951; Ahlgren 1957; Mäkinen *et al*. 2003).

In this study, the beginning and early portion of cell production was observed while the

trend in temperature continued to increase, with frequent fluctuations. There was also a dip in soil hydration early in the season, which was quickly improved as rainfall resumed near the start of sampling. The dates of greatest cell production for all of the species appeared to occur after mean soil temperatures reached or exceeded 14 °C, and mean air temperatures were around 20 °C. Optimal temperatures and water availability differ for the developmental phases of xylem cells, meaning, for example, that high June temperatures will have a different effect on cell production/development than high August temperatures (Antonova and Stasova 1993, 1997). Therefore, the response of each developmental phase (cell division, radial enlargement, and cell wall thickening) to temperature and water availability will determine xylem cell and ring characteristics, such as cell division rates, numbers of earlywood and latewood cells, radial dimensions of cells, and cell wall thicknesses. Antonova and Stasova (1993, 1997) found that in the warmest period of early/mid-summer when temperatures were not limiting, moisture availability played a greater role than air temperatures in xylem cell production. Tardif *et al*. (2001a) also found soil moisture to be important, especially for *B. papyrifera*. Tardif *et al*. (2001b) observed that conifers produced larger rings when April was warm and July was cooler and wetter. Ring width for *B. papyrifera,* however, was shown to have a positive relationship to June precipitation (Tardif *et al*. 2001b).

Ring width and cell development

Typical sigmoidal curves for incremental ring width growth, total cell production, and earlywood cell production were observed in this study. Tardif *et al*. (2001b) demonstrated the uniformity of radial growth response to the local climate for the six conifers of the

same study area. In a dendrometer study of the same species in 1997 (Tardif *et al*. 2001a), similar growth rates were suggested for these species based on the slopes of the increasing mean cumulative daily radial increment over the 'grand period' of growth. Roughly speaking, the same can be seen in the present study with quantified overall rates of increment growth ranging from 0.73-1.08% of total ring width produced per day among the seven species. There were a number of differences among species; *P. glauca* and *T. occidentalis* stood out as the quickest to reach peak growth, and had the highest rates and shortest durations of increment growth/cell production. This has also been observed for *T. occidentalis* by Bannan (1955), while Belyea *et aI.* (1951) had similar observations for *P. glauca*. These two species appear to be inherently fast growing with a short season of growth. The rates and duration of growth within the season may have responded to species-specific optimal climatic conditions (Antonova and Stasova 1993, 1997; Dang and Cheng 2004). Similar rates and duration of cell production and ring width increment for *A. balsamea* were found in other studies (Deslauriers *et al*. 2003a, 2003b; Rossi *et al.* 2004). Schmitt *et al.* (2004) found the duration of *B. papyrifera* cell production to be shorter than *Pinus* species, which is contrary to the current findings, however this was in northern Finland. Furthermore, cell production around the stem of the tree is not always uniform, and variability can exist on different sides of the tree (Fraser 1952; Mäkinen *et al*. 2003).

There were differences among the species in the rates of cell level changes and duration required for the majority of these changes to occur. Decrease in minimum cell length continued at a constant rate until the end of the growing season, while thickening of the

cell wall plateaued long before the end. This suggested that the radial decrease in the cell played the main role in determining the proportion of latewood and how abrupt or gradual the transition would be. Larson (1960) indicated for red pine, that latewood was formed once terminal shoot growth ceased and therefore auxin (a growth hormone) was no longer provided, which greatly reduced the potential for radial cell expansion. The width of latewood for *Pinus* species can be wide, and the transition is typically abrupt (Panshin and de Zeeuw 1970). Earlywood production ended early for *P. banksiana* and *P. resinosa*; however, these two species continued total cell production for a long time thereafter, contributing to greater proportions of latewood. The observed dates of latewood production coincided with the ending of earlywood production for all species except *A. balsamea*, and *B. papyrifera*. It was also observed for *P. resinosa*, and *P. banksiana* only slightly less, that the increase in wall thickness was nearly linear. This indicated that the walls kept getting thicker with time, and since we saw that the cells kept getting smaller and production continued late into the season, a large proportion of latewood would be produced by these species.

The decrease in cell length was contrasted by a near constant increase in the cell wall-to-lumen ratio for all but *A. balsamea, B. papyrifera*, and *P. glauca*. These species had ratio curves that rapidly increased and then plateaued. This indicates a more stable wall-lumen relationship for these species. The earlywood cell production curve also plateaued quickly for *P. glauca* and *T. occidentalis* since they had a short growing season. *Thuja occidentalis* also showed one of the greatest rates of increase in maximum wall thickening, and the shortest duration to achieve this. The *T. occidentalis* wall thickening

curve levelled off early, leaving latewood determination up to a decrease in radial cell diameter. This agrees with literature, which indicates latewood is narrow for both *P. glauca* and *T. occidentalis* (Panshin and de Zeeuw 1970).

The start of latewood production did not significantly differ among the species and it coincided with a warm and dry period. This suggested that this process was controlled by air temperature and/or soil moisture. Air temperatures are considered as one of the triggers as well as soil moisture and temperature (Abe and Nakai 1999; Dang and Cheng 2004). Air temperatures may also influence the cessation of shoot elongation/growth, which ends auxin production and results in subsequent cell production with limited-to-no radial enlargement, and therefore latewood production begins (Larson 1960). Antonova and Stasova (1997) found latewood cell enlargement to be negatively influenced by warm midsummer temperatures, resulting in the decreased cell sizes. Temperatures also influence both the rate and duration of cell wall thickening (Antonova and Stasova 1997; Deslauriers *et al*. 2003a). Increasing temperatures positively affects the rate, but decreases the duration of secondary cell wall thickening; furthermore, a longer duration was found to be more important for producing thick cell walls. This corresponds well to the long duration of cell wall thickening observed in both *Pinus* species and their larger proportions of latewood. Deslauriers *et al*. (2003a) found the formation of the last latewood cells for *A. balsamea* to occur in August or September, which agrees with our findings for this species, which produced cells into September.

The *A. balsamea* earlywood curve was anomalous. *Abies balsamea* is known to produce between 65 and 80% earlywood in a given growth ring (Panshin and de Zeeuw 1970; Deslaurier *et al*. 2003a). The earlywood curve nearly matched the total cell production curve in our findings, indicating that essentially the whole ring was earlywood cells. This was contradicted by the observed starting date of latewood production, which occurred with approximately 20% of cell production remaining, which is more in line with literature. It was also noted that the maximum cell wall-to-lumen ratio exceeded 0.25 early in the growing season, indicating that at least some latewood cells had been produced at that time. The duration of earlywood cell production for *A. balsamea* was greater than total cell production; furthermore, the date of greatest earlywood cell production was reached after the date of greatest total cell production. The date of greatest earlywood cell production should be the same date or sooner than for the number of cells curve, and duration should be less. It seems probable that the total number of cells value would be more reliable as it is based on three radial rows of cells, while earlywood determination was acquired from only one radial row of cells per sample. It could be an artifact of a reduced sample size for the earlywood curve compared to the total cell production curve. Another issue present in our results was the fact that the fibres of *B. papyrifera* were commonly identified by the software to be latewood cells, even in the beginning of the growing season. Therefore the earlywood curve for this species should not be considered accurate. This is a hazard of the latewood definition being used on fibres instead of tracheids.

Conclusions

Xylem cell production for the seven tree species studied was observed to start in mid-May to early June in 1999 in Quebec. The start of latewood production in late August-early September appeared to be subject to a warm and dry period as would be expected. Modelled xylem development among species over the growing season was typically sigmoidal in shape for ring width, number of cells, number of earlywood cells, maximum wall thickness, and maximum cell wall-to-lumen ratio. Trees of *P. glauca* and *T. occidentalis* stood out as the quickest to develop their growth rings. The constant decrease in minimum cell length seemed to play an important role in determining the latewood proportion and transition. Of greatest interest were the differences seen in timing, rates, and duration of developmental processes.

When comparing these results to those of other studies, variability seems to be recurrent; however, given the varying optimal growing conditions in the boreal forest from year to year, flexibility in the growing process is a great advantage (Deslauriers *et al.* 2003). Further study could focus on calibrating band dendrometers to actual cell production and development as dendrometers are much easier to use and can potentially provide similar information. Also, timing of phenological events (e.g. bud burst, shoot elongation and cessation) could be assessed in relation to cell production and developmental stages, and climatic conditions, for these species.

References

Abe H, Nakai T (1999) Effect of the water status within a tree on tracheid morphogenesis in *Cryptomeria japonica* D. Don. Trees 14:124-129

Ahlgren CE (1957) Phenological observations of nineteen native tree species in northeastern Minnesota. Ecology 38:622-628

Antonova GF, Stasova VV (1993) Effects of environmental factors on wood formation in Scots pine stems. Trees 7:214-219

Antonova GF, Stasova VV (1997) Effects of environmental factors on wood formation in larch (*Larix sibirica* Ldb.) stems. Trees 11:462-468

Antonova GF, Cherkashin VP, Stasova VV, Varaksina TN (1995) Daily dynamics in xylem cell radial growth of Scots pine (*Pinus sylvestris* L.). Trees 10: 24-30

Bannan MW (1955) The vascular cambium and radial growth in *Thuja occidentalis* L. Can J Bot 33:113-138

Bäucker E, Bues C-T, Vogel M (1998) Radial growth dynamics of spruce (*Picea abies*) measured by micro-cores. IAWA J 19:301-309

Belyea RM, Fraser DA, Rose AH (1951) Seasonal growth of some trees in Ontario. For Chron 27:300-305

Bergeron Y, Brisson J (1990) Fire regime in red pine stands at the northern limit of the species' range. Ecology 71:1352-1364

Buckhout WA (1907) The formation of the annual ring of wood in the european larch and the white pine. For Q 5:259-267

Camarero JJ, Guerrero-Campo J, Gutierrez E (1998) Tree-ring growth and structure of *Pinus uncinata* and *Pinus sylvestris* in the Central Spanish Pyrenees. Arctic Alpine Res 30:1-10

Cheng C, Gordon IL (2000) The Richards function and quantitative analysis of germination and dormancy in meadowfoam (*Limnanthes alba*). Seed Sci Res 10:265-277

Dang QL, Cheng S (2004) Effects of soil temperature on ecophysiological traits in seedlings of four boreal tree species. For Ecol Manag 194:379-387

Daubenmire RF (1949) Relation of temperature and daylength to the inception of tree growth in spring. Bot Gaz 110:464-475

Daubenmire RF (1950) A comparison of season of cambial growth in different geographic races of *Pinus ponderosa*. Bot Gaz 112:182-188

Daubenmire RF, Deters ME (1947) Comparative studies of growth in deciduous and evergreen trees. Bot Gaz 109:1-12

Deslauriers A, Morin H, Bégin Y (2003a) Cellular phenology of annual ring formation of *Abies balsamea* in the Quebec boreal forest (Canada). Can J For Res 33:190-200

Deslauriers A, Morin H, Urbinati C, Carrer M (2003b) Daily weather response of balsam fir (*Abies blasamea* (L.) Mill.) stem radius increment from dendrometer analysis in the boreal forests of Quebec (Canada). Trees 17:477-484

D**h**nisch O, Schulte M, Kruse K (2003) Cambial growth of *Swietenia macrophylla* King studied under controlled conditions by high resolution laser measurements. Holzforschung 57:196-206

Environment Canada. (2004) Climate normals information 1971-2000. (Online) Available at: http://www.climate.weatheroffice.ec.gc.ca/climate_normals/results_e.html (updated 25 February 2004; accessed 11 October 2004)

Esau K (1965) Plant Anatomy, 2nd edn. John Wiley & Sons, Inc., New York

Ford ED, Robards AW (1976) Short term variation in tracheid development in the early wood of *Picea sitchensis* Leiden Bot Series 3:212-221

Forster T, Schweingruber FH, Denneler B (2000) Increment puncher. A tool for extracting small cores of wood and bark from living trees. IAWA J 21:169-180

Fraser DA (1952) Initiation of cambial activity in some forest trees in Ontario. Ecology 33:259-273

Fritts HC (1976) Tree rings and climate. Academic Press, New York

Gindl W, Grabner M, Wimmer R (2000) The influence of temperature on latewood lignin content in treeline Norway spruce compared with maximum density and ring width. Trees 14:409-414

Gompertz B (1825) On the nature of the function expressive of the law of human mortality and on a new mode of determining life contingencies. Phil Trans R Soc 2:513–585

Hanson HC, Brenke B (1926) Seasonal development of growth layers in *Fraxinus campestris* and *Acer saccharinum*. Bot Gaz 82:286-305

Husch B, Beers TW, Kershaw JA (2003) Forest Mensuration, 4th edn. John Wiley & Sons, Inc., Hoboken, N.J. p 187

Jackson LWR (1952) Radial growth of forest trees in the Georgia piedmont. Ecology 33:336-341

Jagels R, Telewski FW (1990) Computer-aided image analysis of tree rings. In: Cook ER, Kairiukstis LA (eds) Methods of Dendrochronology. Kluwer Academic Publishers, Dordrecht, pp 76-93

Jones B, Tardif J, Westwood R (2004) Weekly xylem production in trembling aspen (*Populus tremuloides*) in response to artificial defoliation. Can J Bot 82:590-597

Knudson L (1913) Observations on the inception, season, and duration of cambium development in American larch [*Larix laricina* (Du Roi) Koch.]. Bull Torrey Bot Club 40:271-293

Korstian CF (1921) Diameter growth in box elder and blue spruce. Bot Gaz 71:454-461

Kozlowski TT, Winget CH (1964) Diurnal and seasonal variation in radii of tree stems. Ecology 45:149-155

Larson PR (1960) A physiological consideration of the springwood summerwood transition in red pine. For Sci 6:110-122

Linkosalo T (2000) Mutual regularity of spring phenology of some boreal tree species: predicting with other species and phenological models. Can J For Res 30:667-673

Mäkinen H, Nöjd P, Saranpää P (2003) Seasonal changes in stem radius and production of new tracheids in Norway spruce. Tree Physiol 23:959-968

Mork E (1928) Die Qualit@t des Fichtenholzes unter besonderer R**h**chsichtnahme auf Schleif und Papierholz. Der Papier Fabrikant 26:741-747

Motulsky H, Christopoulos A (2004) Fitting Models to Biological Data Using Linear and Nonlinear Regression, A practical guide to curve fitting. Oxford University Press, Inc., New York

Nobuchi T, Ogata Y, Siripatanakilok S (1995) Seasonal characteristics of wood formation in *Hopea odorata* and *Shorea henryana*. IAWA J 16:361-369

Oribe Y, Funada R, Shibagaki M, Kubo T (2001) Cambial reactivation in locally heated stems of the evergreen conifer *Abies sachalinensis* (Schmidt) Masters. Planta 212:684-691

Oribe Y, Funada R, Kubo T (2003) Relationship between cambial activity, cell differentiation and the localization of starch in storage tissues around the cambium in locally heated stems of *Abies sachalinensis* (Schmidt) Masters. Trees 17:185-192

Panshin AJ, de Zeeuw C (1970) Textbook of wood technology, 3rd edn. McGraw-Hill, New York

Partanen J, Koski V, H@nninen H (1998) Effects of photoperiod and temperature on the timing of bud burst in Norway spruce (*Picea abies*). Tree Physiol 18:811-816

Pearson GA (1924) The growing season of western yellow pine. J Agri Res 29:203-204

Régent Instruments, Inc. (2001) WinCELL pro 2001: image analysis software [computer program]. Quebéc, Que

Richards, FJ (1959) A flexible growth function for empirical use. J Exp Bot 10:290-300

Rossi S, Deslauriers A, Morin H (2003) Application of the Gompertz equation for the study of xylem cell development. Dendrochronologia 21:33-39

Rowe JS (1972) Forest regions of Canada. Canadian Forestry Service, Ottawa. Publication No. 1300

Schmitt U, Jalkanen R, Eckstein D (2004) Cambium dynamics of *Pinus sylvestris* and *Betula* spp in the northern boreal forest in Finland. Silva Fennica 38:167-178

Schmitt U, M`ller R, Eckstein D (2000) Seasonal wood formation dynamics of beech (*Fagus sylvatica* L.) and black locust (*Robinia pseudoacacia* L.) as determined by the “pinning” technique. J App Bot 74:10-16

Schweingruber FH (1996) Tree rings and environment. Dendroecology. Birmensdorf, Swiss Federal Institure for Forest, Snow and Landscape Research, Berne

Sheskin DJ (1997) Handbook of parametric and nonparametric statistical procedures. CRC Press LLC, Boca Raton

Skene DS (1969) The period of time taken by cambial derivatives to grow and differentiate into tracheids in *Pinus radiata* D. Don. Ann Bot 33:253-262

Sutton A, Tardif J (2005) Distribution and anatomical characteristics of white rings in *Populus tremuloides* Michx. IAWA J 26:00-00

Tardif J, Flannigan M, Bergeron Y (2001a) An analysis of the daily radial activity of 7 boreal tree species, northwestern Québec. Environ Monit Assess 67:141-160

Tardif J, Conciatori F, Bergeron Y (2001b) Comparative analysis of the climatic response of seven boreal tree species from northwestern Québec, Canada. Tree-Ring Res 57:169-181

Vantornhout I, Minnaert HL, Tirry L, De Clercq P (2004) Influence of diet on life table parameters of *Iphiseius degenerans* (Acari: Phytoseiidae). Exp App Acarol 00:1-13 Uncorrected proof

Wang L, Payette S, Bégin Y (2002) Relationships between anatomical and densitometric characteristics of black spruce and summer temperature at tree line in northern Quebec. Can J For Res 32:477-486

Wight W (1933) Radial growth of the xylem and the starch reserves of *Pinus sylvestris*: a preliminary survey. New Phytologist 32:77-96

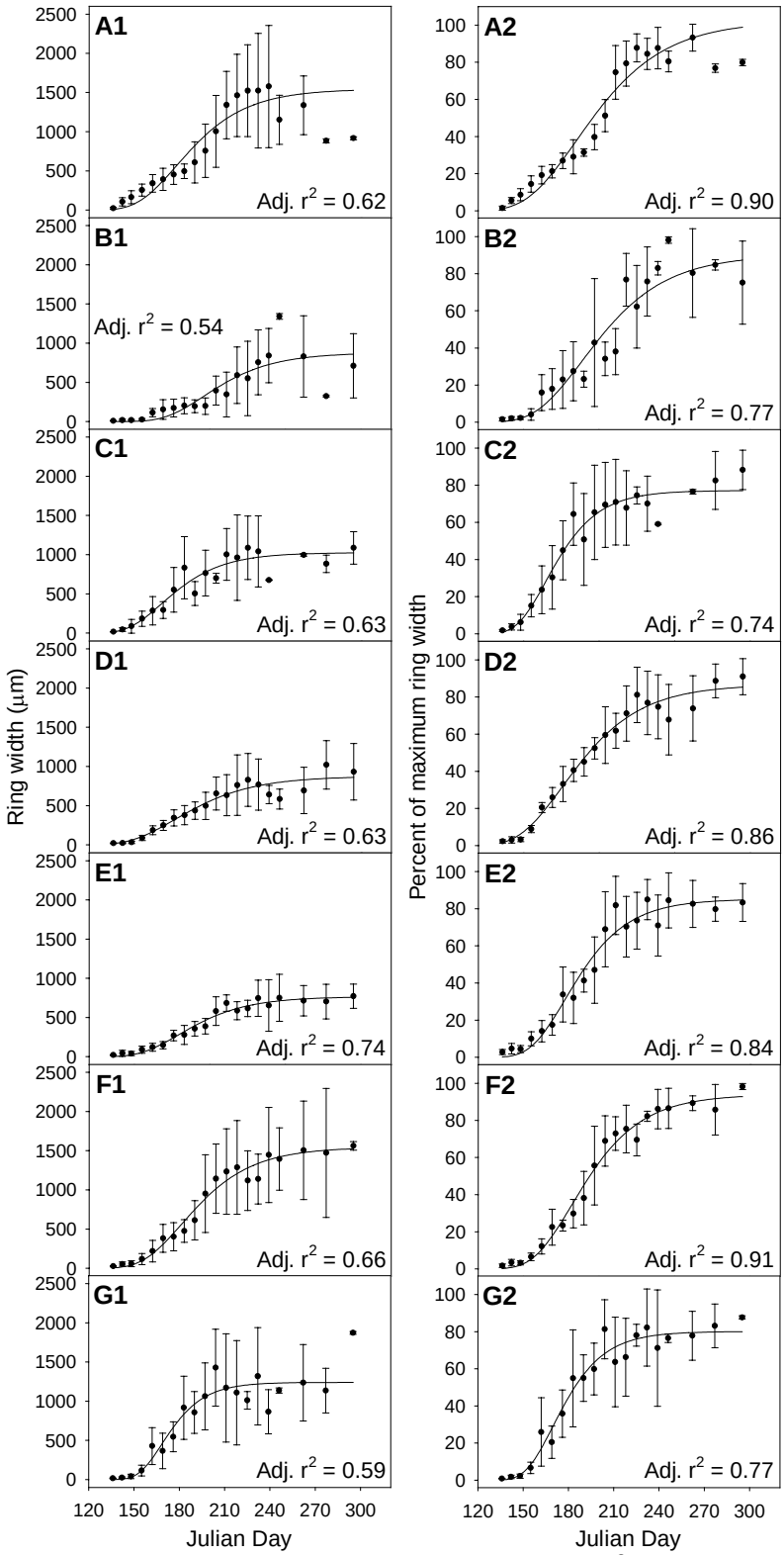
Winsor CP (1932) The Gompertz curve as a growth curve. Proc Nat Acad Sci 18:1-8

Wolter KE (1968) A new method for marking xylem growth. For Sci 14:102-104

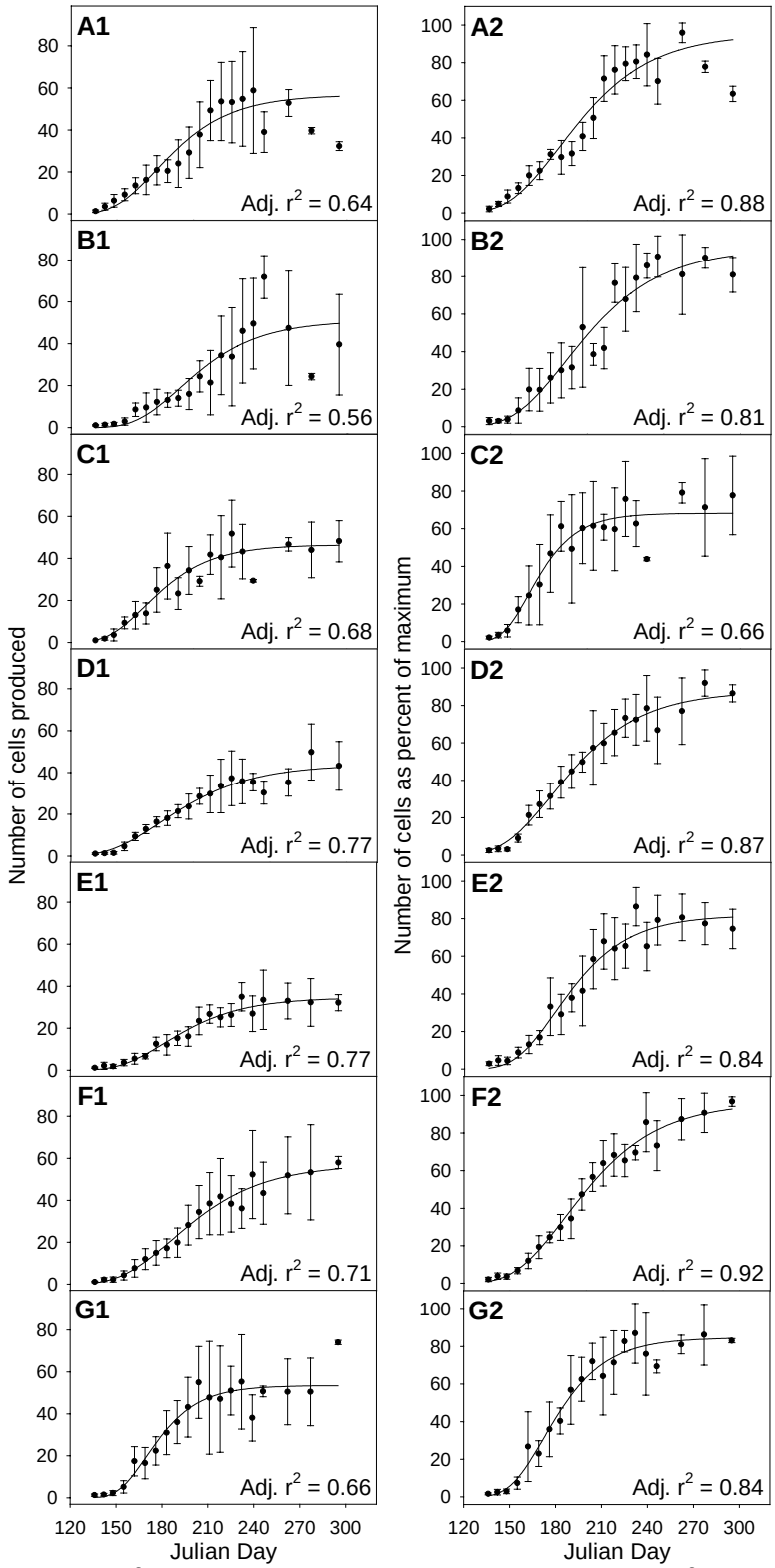
Yasue K, Funada R, Kobayashi O (2000) The effects of tracheid dimensions on variations in maximum density of *Picea glehnii* and relationships to climatic factors. Trees 14:223-229

Zar JH (1974) Biostatistical analysis, 3rd edn. Prentice Hall, Englewood Cliffs, N.J.

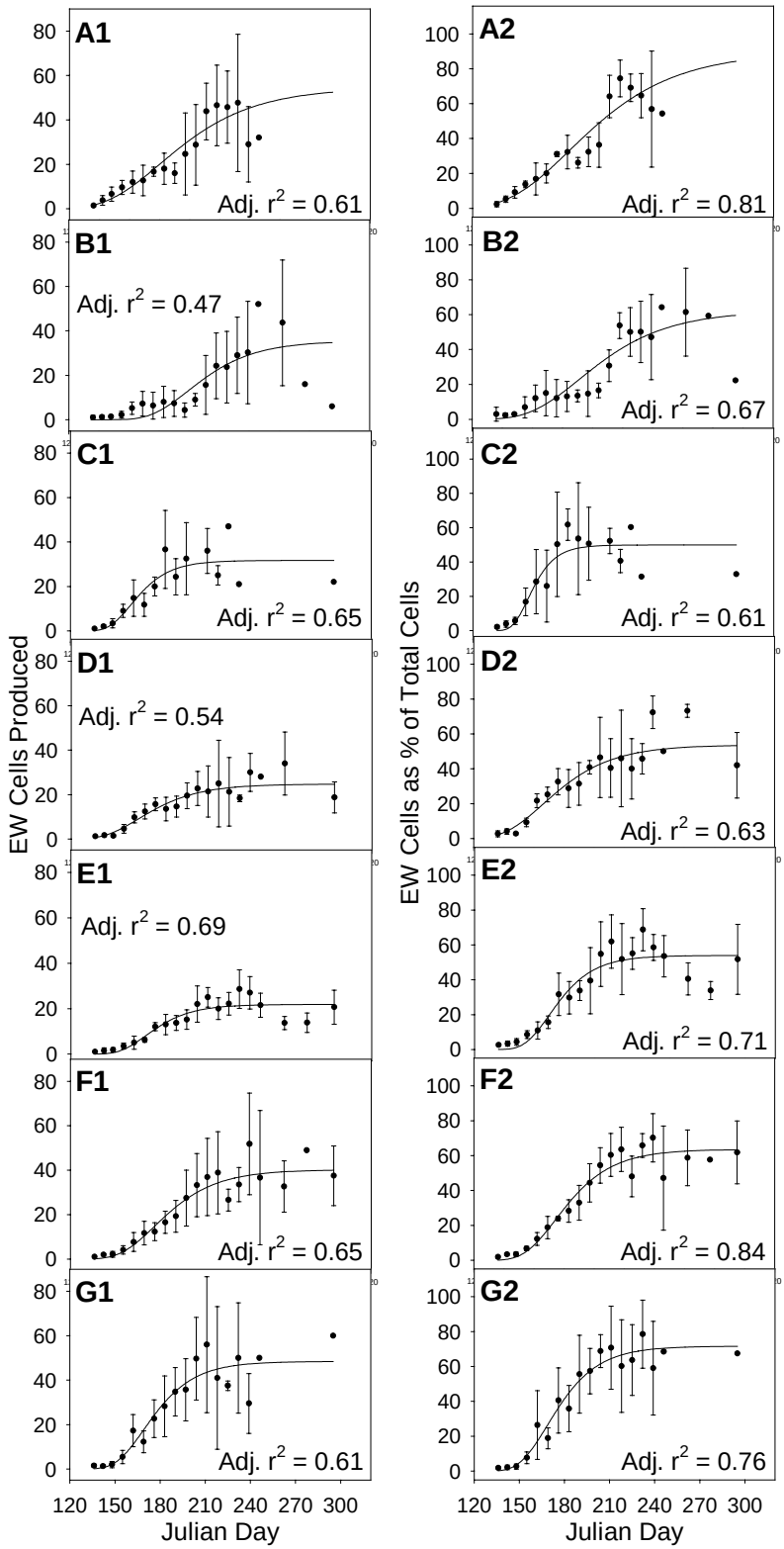
Appendices



Appendix A. Ring width: Raw (1) and standardized (2) data of seven tree species fit with Gompertz curves. A: *Abies balsamea* (n=5) B: *Betula papyrifera* (n=5) C: *Picea glauca* (n=5) D: *Picea mariana* (n=5) E: *Pinus banksiana* (n=6) F: *Pinus resinosa* (n=5) G: *Thuja occidentalis* (n=5).



Appendix B. Number of cells: Raw (1) and standardized (2) data of seven tree species fit with Gompertz curves. A: *Abies balsamea* (n=5) B: *Betula papyrifera* (n=5) C: *Picea glauca* (n=5) D: *Picea mariana* (n=5) E: *Pinus banksiana* (n=6) F: *Pinus resinosa* (n=5) G: *Thuja occidentalis* (n=5).



Appendix C. Number of earlywood cells: Raw (1) and standardized (2) data of seven tree species fit with Gompertz curves. A: *Abies balsamea* (n=5) B: *Betula papyrifera* (n=5) C: *Picea glauca* (n=5) D: *Picea mariana* (n=5) E: *Pinus banksiana* (n=6) F: *Pinus resinosa* (n=5) G: *Thuja occidentalis* (n=5).