1 **Cellular phenology of annual ring formation of *Abies balsamea* (L.) Mill. in the**

2 **Québec boreal forest (Canada).**

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1 **Abstract**

2

3 Cell formation in growth rings of balsam fir in the boreal forest was studied to describe

4 the timing of ring formation and the development patterns of earlywood and latewood.

5 Wood micro-cores were extracted during the growing season from 1998 to 2000. The

6 micro-cores were stained with cresyl fast violet to facilitate the counting of the number of

7 cells in the radial enlargement, wall thickening phases, and mature cell phases. The

8 periods required to complete these various phases were then estimated. Variations in

9 the beginning of the growing season (May 7-June 7), the earlywood-latewood transition

10 (July 2-July 19) and the end of the growing season (Aug. 20-Sep. 20) were observed.

11 Short cell enlargement durations, of less than a week for earlywood and 5-10 days for

12 latewood, were observed. Time required for cell wall thickening was about 20 days for

13 earlywood and longer than 10-15 days for latewood. A certain flexibility was observed in

14 the rings formation patterns and in the cells development rate, providing an advantage

15 in the boreal forest where growth optimal conditions changes from year to year. These

16 findings on the spatial and temporal pattern of ring development may be useful for

17 understanding tree relationships with climate or other environmental parameters.

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1 **Résumé**

2

3 La formation cellulaire du cerne de croissance du sapin de baumier a été étudiée en

4 forêt boréale pour décrire les temps de formation et le développement du bois initial et

5 final. Des micro-carottes ont été extraites pendant les saisons 1998 à 2000. Des

6 sections ont été colorées à l'aide du *cresyl fast violet* pour dénombrer les cellules dans

7 les phases d'élargissement radial, de formation des parois secondaires et de cellules

8 matures. Les temps requis aux cellules pour compléter ces phases ont été estimés.

9 Des variations dans le début de la croissance (7 mai–7 juin), dans la transition bois

10 initial-final (2 juillet-19 juillet) et dans la fin de la croissance (20 août–20 septembre) ont

11 été observées. De courtes durées d'élargissement, de moins d'une semaine pour le

12 bois initial et de 5-10 jours pour le bois final ont été observées. Les temps requis pour

13 l'épaississement des parois secondaires étaient d'environ 20 jours et 30-35 jours pour

14 le bois initial et final respectivement. Les résultats démontrent une flexibilité dans le

15 développement du cerne procurant un avantage en forêt boréale où les conditions

16 optimales de croissance changent d'années en années. Ces résultats pourront être

17 utiles pour mieux comprendre la relation avec le climat ou avec d'autres paramètres

18 environnementaux.

19

1 **Introduction**

2 Most dendroecological studies with balsam fir (*Abies balsamea* [L.] Mill.) in North

3 American boreal forest are related to growth patterns caused by spruce budworm

4 outbreaks (*Choristoneura fumiferana* [Clem.]) (Blais 1958, 1962; Morin and Laprise

5 1990; Morin 1994; Krause and Morin 1995). These studies provide information about

6 the annual variation of growth but not on the intra-annual variation and the period of ring

7 development. Although research has been undertaken to understand balsam fir's

8 reactions to massive foliage loss, basic mechanisms of tree physiology and cell

9 development recorded in the sequence of ring widths are not well understood and need

10 further investigation. By establishing a detailed calendar of cell division and

11 development from wood cores taken over short time intervals, it is possible to link tree-

12 ring development with other measured environmental parameters (Antonova and

13 Stasova 1993,1997) or tree disturbance (Park and Morin 2001). Such a detailed

14 approach will assist dendroclimatological and dendroecological studies by providing

15 details on the mechanism of ring development.

16

17 In North America, few studies have been published on the seasonal formation of xylem.

18 None of these studies were conducted in the ecological zone of the boreal forest and

19 only a few concern the balsam fir ring formation (Kutscha and *al.* 1975; Riding and Little

20 1986). An attempt was made with balsam fir to characterize the growing season, ring

21 development, and the influence of climate (Deslauriers 1999), but information is still

22 lacking. The beginning of the growing period, rate of cambial activity, period of

23 secondary cell wall development, and beginning of the dormant season, along with

24 temporal variations of these processes, are still unknown for balsam fir and other major

1 conifers species in the boreal forest including black spruce (*Picea mariana* [Mill.] BSP)

2 and jack pine (*Pinus divaricata* [Ait.] Dumond). These species are considered the most

3 important ones with respect to forest yield and management. Balsam fir produce a

4 higher number of cell in respect with other boreal species, that is more easy to set up an

5 adequate methodology.

6

7 Repeated cellular analyses during the growing season is one of the best methods to

8 understand and document the intra-annual development of growth rings. Secondary

9 xylem production is a complex process derived from cell cataclinal division in the

10 vascular cambium. The differentiation of annual rings in conifers involves the formation

11 of tracheids that go through several phases before reaching their final form. A

12 differentiating tracheid reaches its final radial diameter during a phase of radial

13 enlargement after it has emerged from the cambial zone (Wilson et al. 1966). The

14 secondary wall is formed during a subsequent maturation phase (Mahmood 1971;

15 Wodzicki 1971; Creber and Chaloner 1990).

16

17 This study is a contribution to the intra-annual analysis of the balsam fir growth ring

18 development by weekly micro-core sampling during three successive growing seasons,

19 with two main objectives. The first was to characterize the phase of ring development

20 by following the development of cells from their division-radial enlargement to their

21 maturation. This analysis provided information on the dynamics of cambium and xylem

22 cell differentiation through the growing season. The second objective was to analyze

23 the period of earlywood and latewood development variation with respect to the duration

24 of cell formation.

1   **Material and methods**

2

3   *Study area*

4   This study was conducted in two permanent plots (Lib-23, Lib-24) (Morin, 1994) located

5   near the 50th parallel (49°58 ' N; 72°30 ' W), about 100 km north of Lac-Saint-Jean

6   (Québec), an area located in Thibault's (1987) black spruce (*Picea mariana* (Mill.)

7   B.S.P.) moss ecological region (No. 12b) of the boreal zone.   The vegetation

8   association is similar to the balsam fir – white birch (*Betula papyrifera* March.) type of

9   the more southern balsam fir – white birch zone (Grandtner 1966).   The mean annual

10   temperature is –0,7°C and the mean annual precipitation is 422 cm, with 357 cm falling

11   as snow (Environment Canada 1992).   The balsam fir stands were located near the

12   northern limit of the fir stands, which makes them interesting for further studies on

13   climate-growth relationships because these trees might be more sensitive to climate.

14   Lib-23 has a unimodal age structure with the trees establishing between 1815 and 1850.

15   Lib-24 was severely affected by the last spruce budworm outbreak (1974 to 1988) and

16   most of the adult trees, which established between 1875 and 1890, died in the last

17   outbreak.   The Lib-24 stand is now composed mainly of young, 5-6 meter tall trees,

18   released after the spruce budworm outbreak due to stand opening (Morin 1994).

19

20   *Tree selection*

21   One micro-core at DBH (1,3 m) was taken from each of 55 trees before the beginning of

22   1998 growing season for a preliminary tree selection.   Cells of the last three rings

23   formed were counted and trees with a similar average number of cells per ring were

1 chosen in order to ensure comparable growth rates. Ten adult trees (mean height, 17,5

2 m) were selected from Lib-23 for an analysis of ring formation in 1998, 1999 and 2000.

3 At Lib-24, 10 adult (mean height, 18.7 m) and 10 young (mean height, 4,5 m) trees were

4 chosen; the young trees were analyzed for the 1998-1999 growing season while the

5 adult trees were analyzed for the 1999-2000 season.

6

7 *Ring sampling*

8 Surgical bone sampling needles DBMNI-1501 (french name, Aiguille d'aspiration, Inter V

9 médical, Montréal) were used for the extraction of small cores of wood and bark. The

10 cores are 1 mm in diameter and 15-20 mm long, containing 4 to 6 rings. These needles

11 were used instead of an increment puncher (wood samples of 10 mm in length and 2.5

12 mm in diameter, Forster et al, 2000) as its smaller diameter allows repetitive sampling

13 also on small tree (dbh < 5cm) without causing significant growth disturbance. Wood

14 cores were extracted every week for three growing seasons (1998, 1999 and 2000) from

15 early May to October. Sample were taken following a spiral trajectory up the stem, from

16 30 cm below DBH to 30 cm above. Wood cores were always taken at least 10 cm apart

17 for the adult trees and 5 cm apart for the young trees to avoid the presence of resin

18 ducts on the next core, which is a common disturbance reaction with balsam fir. A total

19 of 1410 wood cores were fixed in paraffin and transverse sections of 10-12 µm

20 thickness were prepared with a microtome. Several sections of the same cores were

21 put on 3-4 glass slides. The sections were immerged in two baths of Histo-Clear™ for

22 10 minutes and two baths of alcohol (100%) for 2 minutes to remove the paraffin and

23 dehydrate the sections, The sections were then put in a water bath before applying

1   stain.   Two different types of analysis were conducted on each sample: ring

2   development analysis and cell measurement.

3

4   ***Ring development analysis***

5   Sections were stained with a water solution of 0,05% cresyl fast violet and kept in water

6   to assess the ring development.   This staining procedure helps differentiate the

7   developing phloem and xylem cells (Antonova and Shebeko 1981, in Russian).   For

8   each sample, the number of cells in the radial enlargement phase, cell wall thickening

9   phase, and mature cell phase were counted along three radial files.   The cells had to be

10   kept in water and counted immediately after staining until the microscopic analysis was

11   done as the coloration disappears after only a short period.   The dormant cambium

12   before the beginning and at the end of the growing season was easily identified by 2 to

13   4 radially flattened fusiform cells (Riding and Little 1984).   Both cells from cambium

14   zone and cells in radial enlargement phase (xylem) have primary pink walls.   The

15   cambial zone and cells in radial enlargement, are easily identifiable as they posses only

16   primary walls, while both seive cells and tracheid cells have secodnary walls that show

17   strong birefringence in polarized light (Zimmermann 1964; Riding and Little 1984).   Cells

18   in the secondary wall thickening phase change from light violet in the beginning of the

19   process to deep violet near the mature cell state.   Lignification is characterized by the

20   appearance of blue, initiating first in middle lamella and spreading into the secondary

21   walls of differentiating tracheids.   Xylem cells are considered mature or entirely lignified

22   when they are completely blue.

23

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1 *Cell measurements*

2 Sections were stained with safranin (1% in water) and permanently fixed with

3 Permount® to asses cell measurement with the computer program Wincell™. A

4 Polaroid camera fixed on an optical microscope and connected to a computer was used

5 for numerical image analysis. The measured parameters were lumen area (μm^2), single

6 cell wall thickness (µm), lumen diameter (µm) and total cell width (µm).   Three radial

7 files were measured and the average cell size (or any wood parameter measured) was

8 computed on each section. Radial files with larger tracheids were subjectively chosen

9 to ensure that the cell section passed through or near the limit of the middle part of their

10 length. Because the cores were taken along a spiral, the ring width varied within the

11 tree circumference and therefore between the different samples. Hence, the number of

12 cells was counted on 3 radial files on the 3 rings preceeding the ring in formation and

13 used subsequently for a cell number standardization for each sample.

14

15 *Statistical analysis: ring formation*

16 The mean cell number of the three previous years per sample and the mean of all

17 samples per tree were computed and used for a cell number circumference

18 standardization. The extreme values (maximum and minimum) were removed by using

19 a stem leaf graph created by the Univariate procedure of SAS package to ensure a

20 normal distribution. A ratio was obtained for each sample by dividing the mean cell

21 number of the sample by the mean cell number of all samples per tree. The number of

22 cells in each phase (radial enlargement, wall thickening and mature cells) was then

23 multiplied by the ratio to standardize the data according to the sample's relative position

1 on the stem. Before computing the mean cell number in each phase per date of

2 sampling, another Univariate procedure (SAS package) was used to remove the

3 extreme values.

4

5 The approximate date of entrance and the number of days spent in the differentiation

6 phases (enlargement and wall thickening) of each consecutive tracheid formed during

7 the growing season were found following Wodzicki (1971). The methodology is based

8 on differences between (1) total cells number (radial elongation+wall thickening+mature

9 cells), (2) wall thickening+mature cells and (3) mature cells, at a 7 day sampling interval.

10 The calculations were done with fitted data using NLIN (NonLINear regression,

11 Marquardt iterative option) of the SAS statistical package as the number of cells

12 fluctuates near the end of the season. This method regresses the residuals onto the

13 model partial derivatives in respect to the Gompertz parameters until the estimates

14 converge (SAS Institute Inc. 1990). The examination of the R-square, the asymptotic *t*-

15 statistic for the parameters, and the plots of the residual showed that the Gompertz

16 function was appropriate to describe growth and time relationships (Zeide 1993; Huang,

17 Titus and Wiens 1992). Gompertz equation has been used to model tree-ring growth

18 (Camarero and al. 1998). The equation was fitted to establish the cell increase profile

19 of each total described.

20   The Gompertz function is defined as:

21   [1] $y = a \exp(-e^{(\beta - \kappa t)})$

22

1 Where **y** is the weekly cumulative sum of growth (expressed in number of cells), **t** is

2 time computed in days since the first sampling date where t=0, **a** is the upper asymptote

3 of the maximum number of cells where at t_i $y \cong a$, β is the *x*-axis placement parameter,

4 and κ is the rate of change parameter (Cheng and Gordon 2000). Two biologically

5 useful variables were calculated from the fitted statistics, both defined by Richards

6 (1959). A weighted mean absolute cell formation rate **r** can be obtained by equation 2,

7 and the time required for the major portion of cell formation **d** to occur can be obtained

8 by equation 3. Parameter ν was fixed to 0.0001 since Gompertz function is a special

9 case of the Richards function when $\nu \cong 0$.

10 [2] $r = a.\kappa / 2(\nu+2)$

11 [3] $d = 2 (\nu+2) / \kappa$

12

13 ***Statistical analysis: cell measurement***

14 Curves of cell size variation in radial files of xylem, called tracheidograms, were

15 constructed following Vaganov (1990). Standardization is required to compare tree ring

16 structures as different rings have different numbers of cells of varying dimensions. The

17 standardization method decreases or increases the initial tracheidogram along the

18 abscissa leaving the ordinate unchanged (Vaganov 1990). The average number of

19 mature cells was used to calculate the number of cells to standardize for each sampling

20 date.   Classification of cells into latewood were conducted using Mork's formula

21 described in Denne (1988):

22 [4]   single cell wall thickness x 4 $\geq$ tracheid lumen

23

1 **Results**

2

3 *Tree ring characterization*

4 The weekly averages of differentiating cells and mature cells, during the growing season

5 from 1998 to 2000, are presented in figure 1. An important variation was observed

6 between the starting and ending periods of the growing seasons (table 1). The starting

7 periods vary from May 7, observed in Lib-23 1999, and June 7, observed at both sites in

8 2000, a month's difference.

9

10 The mean number of cells counted each week varied from 1 to 3 in the radial

11 enlargement phase (figure 1). The highest values (4-5) counted on some samples in

12 the second and the third week after the start, might reflect an active cell division at the

13 beginning. However, the cambium zone at the first weeks of the growing season was

14 easily ruptured and a few radially enlarging xylem could have been missed. The first

15 cell in the wall thickening phase was observed between one and three weeks after the

16 start of the growing season (table 1). After increasing regularly, they reached a plateau

17 of around 10 cells for a month and a half (figure 1). The low variation during this long

18 period indicates a constant flow of cells going in and out of the wall-thickening phase.

19 Close to the end of the growing season these number decreased rapidly indicating that

20 less cells were entering the wall thickening phase and that cell division was coming to

21 an end.

22

23 In all cases, the total number of cells regularly increases until a mean maximum number

24 defined as the upper asymptote. Past that particular point, cell numbers start to

1   fluctuate because samples were taken at different positions on the trees. The upper

2   asymptote, representing the fitted mean total maximum number of cells or a plateau, is

3   reached at different dates in July or August (table 1, figure 1) and seemed to arrive

4   earlier when the earlywood-latewood transition was earlier. However, even if this

5   number is reached, it does not mean that it is the end of cell division. Cell division

6   could be masked by differences in the ring width around the trunk of the trees even if

7   standardization was done to make it more regular. The end of cell division in ring

8   development was the hardest variable to determine because the cessation of tracheid

9   production varies within and between trees (Sundberg and *al.* 1987).

10

11   ***Growth model***

12   Cell increase fitted well with the Gompertz equation as shown by the R^2 variation

13   between 73.9% and 96.5%. The cell formation rate (r) varied between sites and

14   between the young and adult trees. For the total cell number, the values varied from

15   0,49 to 0,70 cell/day and in general, the cell formation rate decreased with a longer

16   period of cell division. The shortest length and the highest rate were observed in 1998

17   when the upper asymptote was reached near July 23 at both sites. The mature cell

18   formation rate, representing the rate at which the cells enter into a mature state, is

19   normally lower than that of the total number of cells because it excludes the radially

20   enlarging and wall thickening cells. The mature cell formation rate also lasts longer

21   because its upper asymptote (*a*) is reached later in the growing season.

22

1 ***Earlywood and latewood formation period***

2 The separation of ring development into two parts, earlywood and latewood formation

3 period, was determined from weekly cell measurements. The cell classification was

4 conducted using only mature cells to avoid the comparison of non-mature cells with

5 mature ones. In figure 2, cells that are still in differentiation phases (radial enlargement

6 or cell wall thickening) are shown to illustrate more precisely the transition between

7 earlywood and latewood. At the end of the growing season, when the number of cells

8 starts to fluctuate, the number of cells was standardized at mature cell fitted values

9 corresponding to the last sampling date.

10

11 Earlywood growth was observed to be a fast process with cell division taking between 1-

12 2 months from the start of the growing season to the transition time (table 1). The

13 earlywood formation length is almost the same for 1998 and 2000 (6 to 7 weeks) but it

14 is longer for 1999 (9-10 weeks) because of a very early growing season. Thus, most of

15 the ring width, 65-75% earlywood for mature trees and 80% for young trees, is formed in

16 a very short period of time. The time at which we observed the first mature earlywood

17 cells was constant at 3 to 4 weeks after the beginning of the growing season. This

18 gives a good indication of the time required for the complete formation of an earlywood

19 cell which is independent of the beginning of the growing season. Complete earlywood

20 formation, including the time necessary for cell wall thickening, took 10-12 weeks in

21 1998-2000, and 13-14 weeks in 1999. Variation in the transition time does not seem to

22 be related with the start of the growing season. In fact, even if the 1999 growing season

23 started sooner, its earlywood-latewood shift was two weeks later than the one observed

24 in 1998, and was close to the one observed in 2000 (table 1, figure 2). Total latewood

1 formation (including the cell wall thickening) lasted 9 weeks in 1998 and 2000, and 6 to

2 7 weeks in 1999, which is less than for earlywood.   Latewood have less cells then

3 earlywood, but more time is required to complete single cell formation because of the

4 longer duration of the cell wall thickening phase.

5

6 *Single cell formation period*

7 Fitted sums of data (table 2) were used to interpolate between sampling dates and to

8 set an approximate period of formation for each cell during the growing season (figures

9 3, 4 and 5). These estimates can be considered quite reliable for most of the earlywood

10 cells since the earlywood-latewood transition date is approximately the same as the one

11 observed (table 1).   For latewood, the estimation of the end of the wall thickening

12 phase, including the last 1-3 cells, is estimated later than the one graphically observed

13 (figure 2) in 1998 and 1999 in both site. This problem is due to the difference between

14 the convergence of the upper asymptote of the total cells and the one of mature cells

15 that cause a bias for the last cell (table 2).

16

17 The duration of the radial cell enlargement phase might not have a strong influence on

18 the final, mature cell width as there is a non-relevant difference between the wide

19 earlywood cells and the narrow latewood cells (figure 3, 4 and 5). Radial enlargement

20 was relatively uniform between study sites and years as shown by the values of less

21 than a week.   Normally these values vary from 2-6 days for earlywood with a slight

22 increase at 5 to 10 days for latewood. Only a small difference was observed for the

23 year 2000 where the lengths were slightly longer for both study plots. Times spent in

24 radial enlargement are also quite similar between young and adult trees. However, wall

1   thickening varied between earlywood and latewood. The duration of earlywood wall

2   thickening exhibited a constant increase during the growing season from 10 to 20-25

3   days before the earlywood –latewood transition. Latewood wall thickening then varied

4   from 20-25 to 30-35 days depending on tree age (young or adult) and the year.

5

6   *Pattern of ring formation*

7   Interesting patterns of ring development were observed for the different study years

8   and specific study plots. First, the overall growing season may almost be of the same

9   length (comparing the beginning and the end) but may possess different progressions

10 into the cell division rates and cell wall thickening periods. Young trees at Lib-24 started

11 their 1998 and 1999 growth season on May 20-21 (table 1), but the transition between

12 earlywood and latewood occurred later in 1999 because of a slower cell division rate

13 (table 2 and figure 4). This delay cannot be attributed to significant difference in radial

14 enlargement, consequently the cell division rate and the cell development phase seem

15 independent.

16

17 A second pattern observed showed that when a delay was present at the beginning of

18 the growing season, it could be maintained through to the end. In fact, the whole ring

19 pattern of development throughout the 2000 growing season is delayed. The year 2000

20 cell division rate was also slower (0,515 cell per day, table 2), and the time spent in

21 radial enlargement was longer, making it difficult to catch up. Similar situations were

22 also present when comparing the years 1999 and 2000 at Lib-23 and Lib-24. Delays of

23 about 3 weeks to a month were observed at the beginning and at the end of wall

24 thickening phase but not at the earlywood-latewood transition.

1 **Discussion**

2

3 *Earlywood growth*

4 A month of difference was observed for the beginning of tracheid formation, from May 7

5 to June 7, which is a long delay. However, these delays are commonly observed

6 because the beginning of the growing season varies considerably, depending mostly on

7 climate variation (Creber and Chaloner 1984, 1990; Zabuga and Zabuga 1990; Vaganov

8 and al. 1994). For Scots pine in Russia, the start of the growing season (1978-1982)

9 varied between the first 10 days of May to the second 10 days of June, with temperature

10 being the main factor that influenced the initiation of tracheid formation (Zabuga and

11 Zabuga, 1990). The temperature around 25 °C or more at the beginning of May 1999

12 could explain the very early initiation of tracheid formation at Lib-23. In spite of this,

13 detailed growth-climate analysis must be undertaken to fully understand the influence of

14 climatic factors.

15

16 As reported for red pine (Whitmore and Zahner 1966) and Norway spruce (Horäcek

17 1994), the number of cells in the radial enlargement and wall thickening phases were

18 relatively constant during most of the active growing period of earlywood following a

19 sharp increase at the beginning. However, in our study a low number of cells in radial

20 enlargement (maximum of 5) were counted every week. Other studies, including

21 balsam fir, have found many more cells in this phase, especially in very active cambia

22 (Whitmore and Zahner 1966; Sundberg and *al.* 1987; Horacek 1994). Even if some

23 radially enlarging xylem could have been missed because of a rupture of the cambium

1   zone, cell numbers remain low compared with that of other studies, resulting in a quite

2   short estimated enlargement time of less than a week for earlywood and from 5-10 days

3   for latewood. By comparison, in Siberia, the growth of pine tracheid primary walls lasts

4   from 3 to 17 days (Antonova and *al.* 1995).

5

6   Between 65-80% of ring width was produced in less than 2 months, from the mid-end of

7   May to the mid-end of July, with June being the most important month for ring

8   development. The short period needed for earlywood growth might be an adaptation to

9   the cold climatic conditions of the boreal forest where cells require a very short time for

10   their radial growth and start their wall thickening rapidly. This situation could leave a

11   certain safety margin at the end of the growing season used to complete the lignification

12   before frosts occur. Antonova and *al.* (1995) found that tracheid radial growth depends

13   more on the rate of development, especially at the beginning of tracheid growth when

14   the rate is higher, than on the duration of the process. Therefore, local and daily

15   weather conditions at the beginning of cell enlargement should be crucial for

16   determining both cell width and consequent ring width, because of its influence on the

17   enlargement rate. As the rate and period of earlywood development changes from year

18   to year, the understanding of the growth mechanism could improve significantly our

19   comprehension of the growth and climate relationship. For example, ring width is a

20   parameter poorly correlated with monthly climatic average in Eastern North-America

21   (Conkey 1986; D'Arrigo and *al.* 1992; Schweingruber and *al.* 1993). Monthly climatic

22   averages may not be precise enough to characterize the short crucial moment at the

23   beginning of cell enlargement.

24

1 ***Latewood growth***

2 Latewood initiation time is very important because it marks the end of the wide

3 earlywood cell enlargement phase that largely defines ring width and marks the close

4 end of cell division. The earlywood-latewood transition varies in time depending on the

5 start of the growing season (early or late) and on the cell formation rate. The same

6 transition date between study plots for a given year might reflect a climatic "signal" that

7 affects a wide area. In our studies, it had varied from July 2 to July 19, a range of two

8 weeks. Zabuga and Zabuga (1990) have observed a variation as long as a month with

9 Scot pine in Siberia.

10

11 Latewood formation, that involves a reduction in radial expansion and an increase in

12 wall thickening was strongly influenced by the increase in the duration of the cell wall

13 thickening phase, which was about 10 days longer than earlywood. Other investigations

14 have also observed that it is the duration and not the rate of wall material deposition that

15 causes the thicker cell walls (Wodzicki 1971; Denne 1974, 1976). In addition, Uggla et

16 al. (2001) recently concluded that latewood formation is under developmental, rather

17 than metabolic, control. Moreover, year-to-year climatic variations, such as a decrease

18 in soil moisture (Conkey 1986; Zabuga and Zabuga 1990), was found to explain the

19 variations in earlywood-latewood transition time.

20

21 Several studies have found good correlations with maximum tree-ring wood density and

22 August temperatures (mean-max) (D'Arrigo and *al.* 1992; Schweingruber and *al.* 1993;

23 Splechtna and *al.* 2000). However, the period to obtain the best correlation can vary

24 from mid-July to mid-August, as found in 1998 and 1999 or from mid-August to mid-

1   September in 2000 (figure 3, 4 and 5). Our results suggest that July and September,

2   can also have a significant effect on latewood density. The growing season starting

3   date (early-late) and also the earlywood-latewood transition date could have had an

4   influence on the end of the cell wall thickening phase. However, climatic conditions at

5   the end of the process are not excluded from explaining the variation. Lignin deposition

6   in latewood cells still persists after the end of the cell wall thickening phases (Gindl and

7   *al.* 2000), therefore complete ring density was probably attained before the period

8   observed, from August 20 to September 20, which is the end of lignin deposition in cell

9   wall.

10

11   ***Control of the growing season length: duration of rate ?***

12   Some questions arise from the observation of the growing season process. First, does

13   a delay in the beginning of growing season make it shorter and result in fewer cells

14   produced?   For example, Vaganov and *al.* (1994) found that in severely limited

15   temperature conditions a narrower ring is formed when a delay is observed in the

16   beginning of the growing season.   They attributed the narrow ring to a growth

17   suppression in the middle of June due to decreasing temperature and light.   By

18   comparing 1998-1999 with 2000, it seems that the "conditions" needed for radial growth

19   can be delayed without strong negative effects as long as they eventually occurs.

20   Conversely, Lib-23 1999 shows that if the "conditions" or some kind of "climatic signal"

21   are present very early in spring, trees will slowly start growing even if some snow

22   patches are still present and frost can still occur. The growing season was longer for

23   that site/year because of a very early start but did not necessarily end sooner as shown

24   by the latewood initiation date and the end of the cell wall thickening period and did not

1 result in a greater number of cells produced nor in a wider ring. The results obtained

2 show that the cell division rate is also very important in the progress of the growing

3 season. Our results suggest that climate can have an effect on both the rate and the

4 duration of the cell differentiation process, independent of the beginning of the growing

5 season. Thus, a certain flexibility exists in the growing process, which is an advantage

6 in boreal forests where optimal growth conditions change from year to year.

7

8 **Conclusion**

9 Repeated cellular analyses during the growing season is one of the best methods to

10 understand the mechanism of growth ring development but requires meticulous

11 methodological work. On the other hand, wood growth estimation on a daily scale will

12 make it possible to carry out precise relationships with climate, or to understand the

13 effect of a perturbation such as insect defoliation. The data obtained by this approach

14 will also help to understand which environmental parameters are responsible for the

15 formation of the ring and the ring width, thus improving our prediction of forest

16 productivity, and consequently forest management planning.

17

24

Deslauriers

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9

1 **Table 1.** Date of the principal events of the growing season. The values show the date

2 to which 50% of the sample trees had the specified characteristics.

Observation	1998		1999			2000	
	Lib-23	**Lib-24y**	**Lib-23**	**Lib-24y**	**Lib-24a**	**Lib-23**	**Lib-24a**
Start - radial enlargement	May 28	May21	May 7	May 20	May 20	June 7	June 7
Start – cell wall thickening	June 11	June 11	May 27	Mai 27	June 3	June 14	June 21
Earlywood-latewood transition	July 2	July 2	July 15	July 15	July 15	July 19	July 19
Cell number reaching the upper asymptote	July 23	July 23	Aug. 5	Aug. 12	Jul. 29	Aug. 23	Aug. 9
Dormant cambium	Aug. 20	Aug. 27	Aug.26	Sep. 2	Sep. 2	Sep.13	Sep.13
End - cell wall thickening	Aug. 20	Aug. 27	Sep. 2	Sep. 2	Sep. 2	Sep. 20	Sep. 20

3
4 **Note:** Lib-24y, young trees; Lib24a, adult trees.
5

6

1   **Table 2.** Parameters for the Gompertz function fitted for every site and year for 3

2   distinct cell sums.

|	1998		1999			2000	
	Lib-23	**Lib24 y**	**Lib-23**	**Lib24 y**	**Lib24 a**	**Lib-23**	**Lib24 a**
Total cells (radial elongation+wall thickening+mature cells)							
R^2 **(%)**	75,7	90,7	75,9	91,8	89,5	74,8	79,9
a	36,58	49,19	37,61	48,20	41,35	41,12	44,89
β	1,66	1,18	2,09	1,80	2,61	1,51	1,74
κ	0,065	0,063	0,052	0,046	0,061	0,050	0,056
r (cell/day)	0,593	0,70	0,49	0,555	0,627	0,515	0,624
d (day)	61,7	63,9	76,6	86,8	65,9	79,8	71,9
Mature cell							
R^2 **(%)**	80,3	96,5	85,9	95,2	92,2	82,0	88,3
a	38,08	47,82	36,83	48,99	41,20	43,30	38,91
β	2,17	1,88	2,65	2,17	2,70	1,84	2,65
κ	0,045	0,051	0,040	0,037	0,042	0,033	0,055
r (cell/day)	0,430	0,613	0,369	0,449	0,433	0,353	0,534
d (day)	88,5	78,1	99,7	109,1	95,1	122,8	72,9
Wall thickening + mature cells							
R^2 **(%)**	73,9	93,4	79,0	93,2	90,2	78,2	84,7
a	36,1	48,37	36,79	47,67	40,64	40,20	42,3
β	1,82	1,39	2,28	1,91	2,72	1,77	2,00
κ	0,067	0,063	0,053	0,046	0,060	0,050	0,059

3

4   **Note:** *a* is the upper asymptote of the maximum number of cells where at t_i $y \cong a$; β is

5   the *x*-axis placement parameter; κ is the rate of change parameter; **r** is the rate of cell

6   formation by day; **d** is the total time required to complete the cell increase process, in

7   days.

8
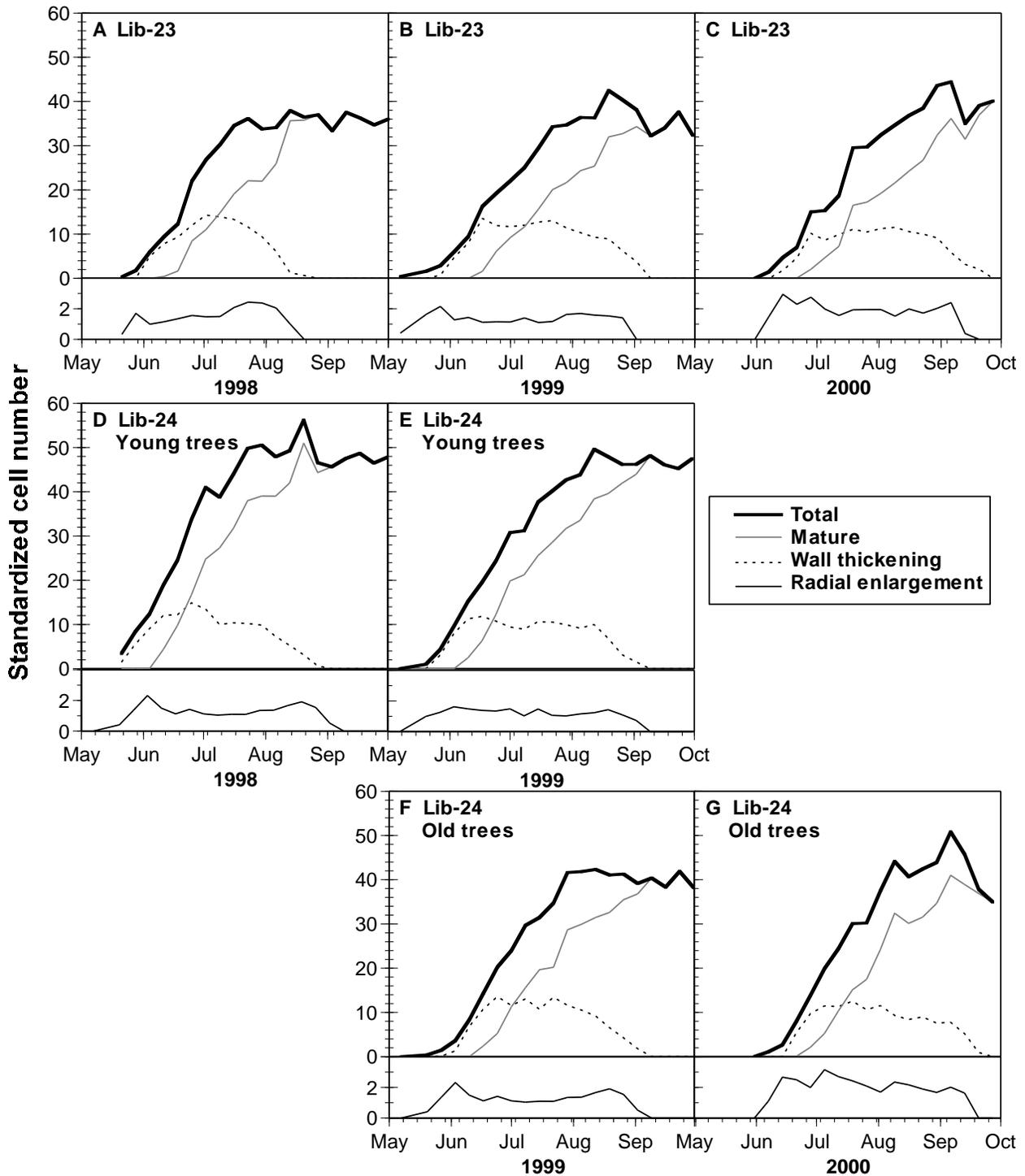
Figure 1. Number of cells in radial enlargement phase, wall thickening phase, mature cells and total number at Lib-23 and Lib-24 in 1998, 1999 and 2000. The horizontal axis major ticks marks show one month and minor tick marks show one week intervals.
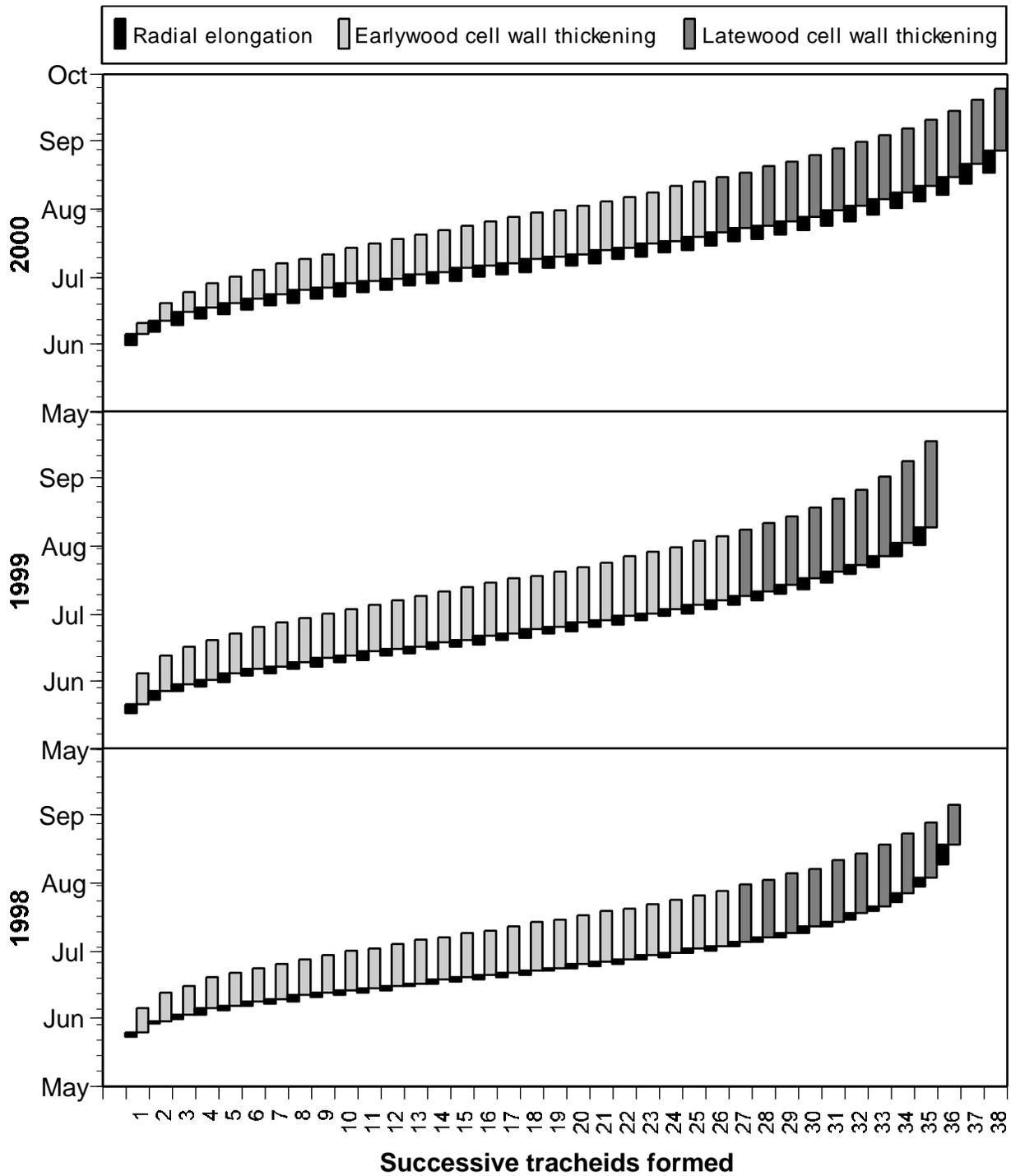
Deslauriers

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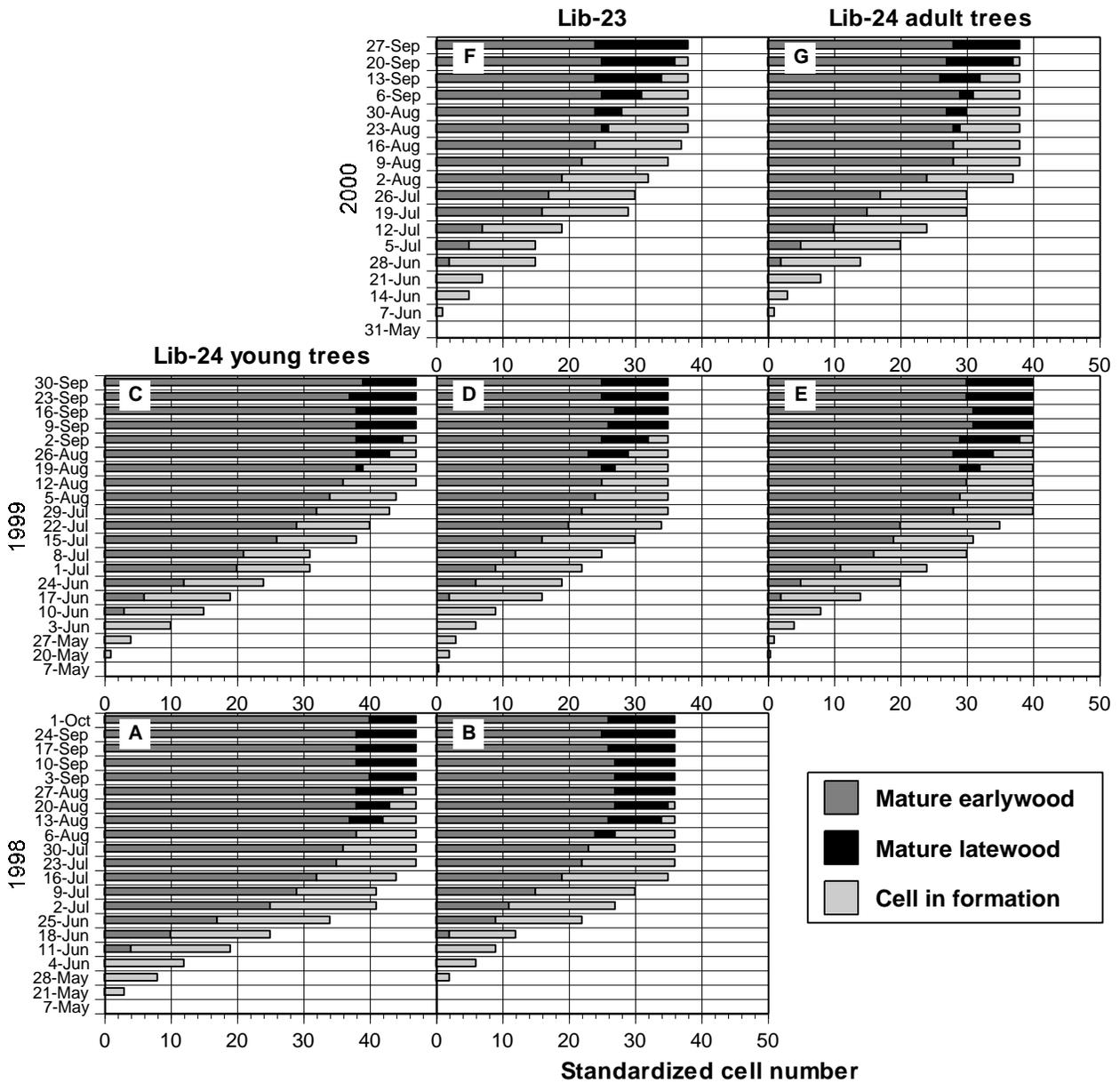
Figure 2. Number of mature earlywood and latewood cells and number of cells in

formation (radial enlargement and wall thickening) for each sampling date from 1998 to

2000 for adult and young trees at Lib-23 and Lib-24 sites.

6

7

1

Legend: Radial elongation · Earlywood cell wall thickening · Latewood cell wall thickening
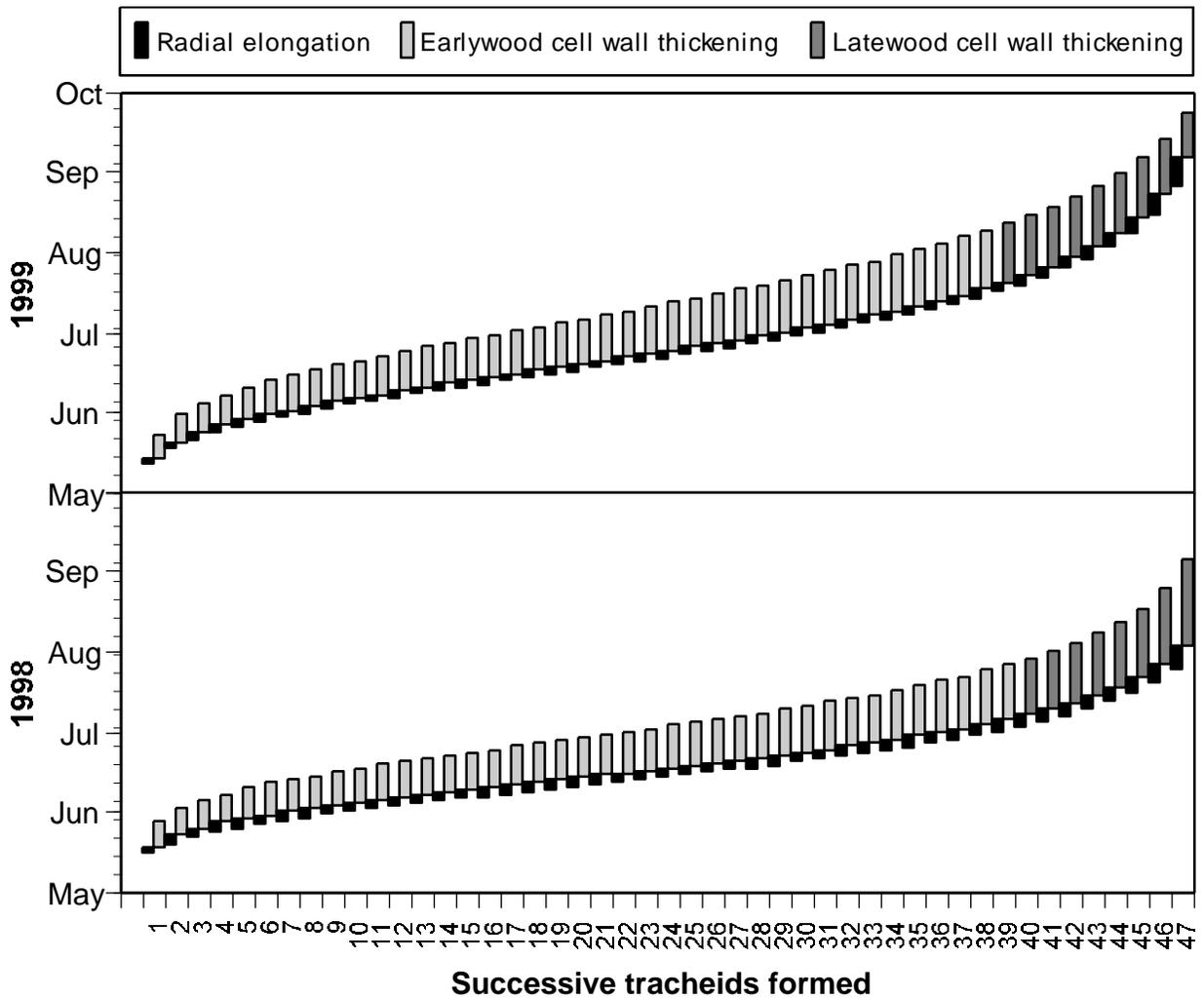
Successive tracheids formed

2

3 **Figure 3.** Duration of radial cell enlargement and cell wall thickening phases for adult

4 trees at Lib-23 plot. The vertical axis major tick marks show one month intervals and

5 minor tick marks show one week intervals.

1

2 **Successive tracheids formed**

3 **Figure 4.** Duration of radial cell enlargement and cell wall thickening phases for young

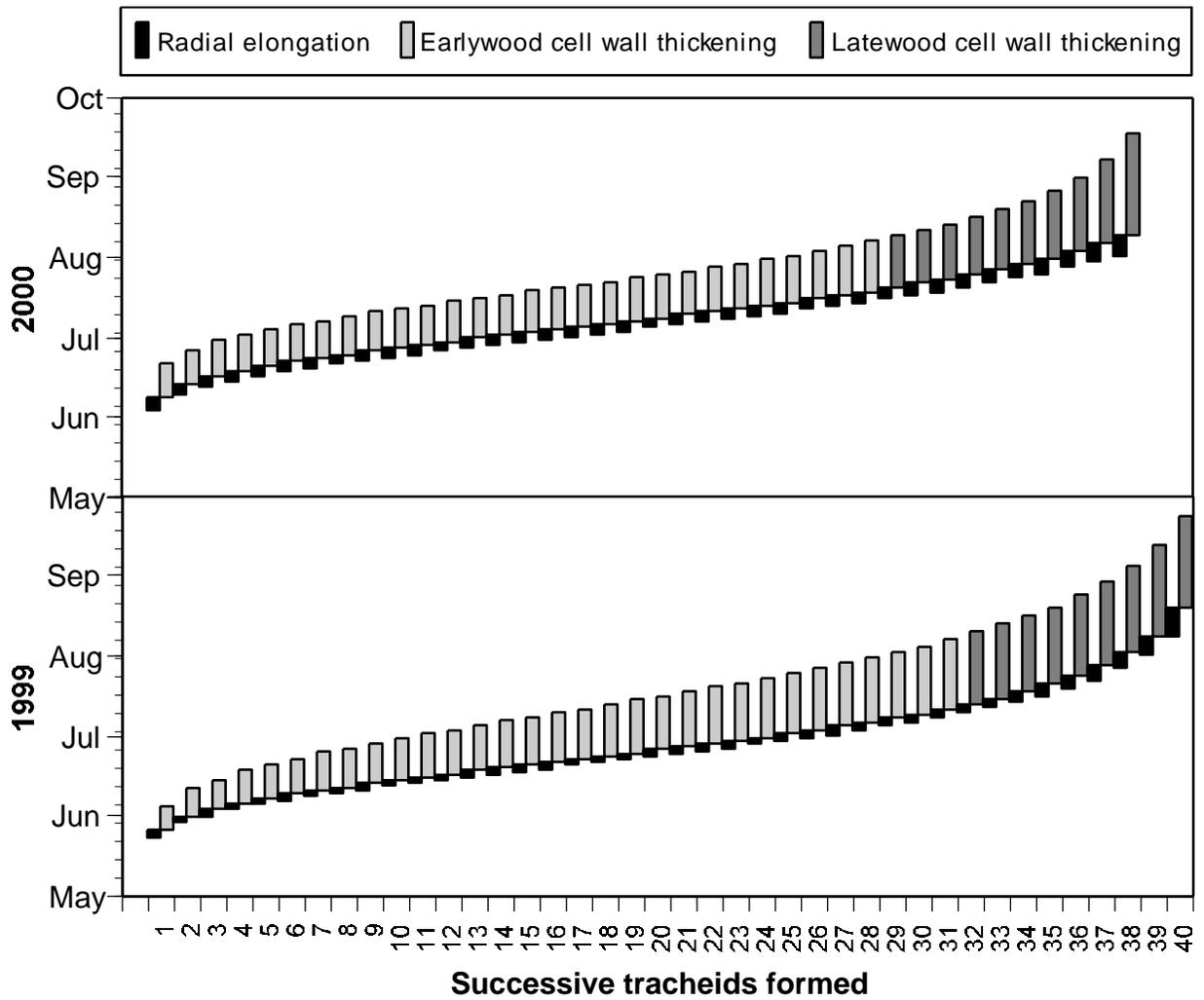4 trees at Lib-24 plot. The vertical axis major tick marks show one month intervals and

5 minor tick marks show one week intervals.

6

7

1

2 **Successive tracheids formed**

3 **Figure 5.** Duration of radial cell enlargement and cell wall thickening phases for adult

4 trees at Lib-24 plot. The vertical axis major tick marks show one month intervals and

5 minor tick marks show one week intervals.

6

7