Cellular phenology of annual ring formation of *Abies balsamea* (L.) Mill. in the Québec boreal forest (Canada).

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Abstract

Cell formation in growth rings of balsam fir in the boreal forest was studied to describe the timing of ring formation and the development patterns of earlywood and latewood. Wood micro-cores were extracted during the growing season from 1998 to 2000. The micro-cores were stained with cresyl fast violet to facilitate the counting of the number of cells in the radial enlargement, wall thickening phases, and mature cell phases. The periods required to complete these various phases were then estimated. Variations in the beginning of the growing season (May 7-June 7), the earlywood-latewood transition (July 2-July 19) and the end of the growing season (Aug. 20-Sep. 20) were observed. Short cell enlargement durations, of less than a week for earlywood and 5-10 days for latewood, were observed. Time required for cell wall thickening was about 20 days for earlywood and longer than 10-15 days for latewood. A certain flexibility was observed in the rings formation patterns and in the cells development rate, providing an advantage in the boreal forest where growth optimal conditions changes from year to year. These findings on the spatial and temporal pattern of ring development may be useful for understanding tree relationships with climate or other environmental parameters.

Résumé

La formation cellulaire du cerne de croissance du sapin de baumier a été étudiée en forêt boréale pour décrire les temps de formation et le développement du bois initial et final. Des micro-carottes ont été extraites pendant les saisons 1998 à 2000. Des sections ont été colorées à l'aide du *cresyl fast violet* pour dénombrer les cellules dans les phases d'élargissement radial, de formation des parois secondaires et de cellules matures. Les temps requis aux cellules pour compléter ces phases ont été estimés. Des variations dans le début de la croissance (7 mai–7 juin), dans la transition bois initial-final (2 juillet-19 juillet) et dans la fin de la croissance (20 août–20 septembre) ont été observées. De courtes durées d'élargissement, de moins d'une semaine pour le bois initial et de 5-10 jours pour le bois final ont été observées. Les temps requis pour l'épaississement des parois secondaires étaient d'environ 20 jours et 30-35 jours pour le bois initial et final respectivement. Les résultats démontrent une flexibilité dans le développement du cerne procurant un avantage en forêt boréale où les conditions optimales de croissance changent d'années en années. Ces résultats pourront être utiles pour mieux comprendre la relation avec le climat ou avec d'autres paramètres environnementaux.

Introduction

Most dendroecological studies with balsam fir (*Abies balsamea* [L.] Mill.) in North American boreal forest are related to growth patterns caused by spruce budworm outbreaks (*Choristoneura fumiferana* [Clem.]) (Blais 1958, 1962; Morin and Laprise 1990; Morin 1994; Krause and Morin 1995). These studies provide information about the annual variation of growth but not on the intra-annual variation and the period of ring development. Although research has been undertaken to understand balsam fir's reactions to massive foliage loss, basic mechanisms of tree physiology and cell development recorded in the sequence of ring widths are not well understood and need further investigation. By establishing a detailed calendar of cell division and development from wood cores taken over short time intervals, it is possible to link tree-ring development with other measured environmental parameters (Antonova and Stasova 1993,1997) or tree disturbance (Park and Morin 2001). Such a detailed approach will assist dendroclimatological and dendroecological studies by providing details on the mechanism of ring development.

In North America, few studies have been published on the seasonal formation of xylem. None of these studies were conducted in the ecological zone of the boreal forest and only a few concern the balsam fir ring formation (Kutscha and *al.* 1975; Riding and Little 1986). An attempt was made with balsam fir to characterize the growing season, ring development, and the influence of climate (Deslauriers 1999), but information is still lacking. The beginning of the growing period, rate of cambial activity, period of secondary cell wall development, and beginning of the dormant season, along with temporal variations of these processes, are still unknown for balsam fir and other major

conifers species in the boreal forest including black spruce (*Picea mariana* [Mill.] BSP) and jack pine (*Pinus divaricata* [Ait.] Dumond). These species are considered the most important ones with respect to forest yield and management. Balsam fir produce a higher number of cell in respect with other boreal species, that is more easy to set up an adequate methodology.

Repeated cellular analyses during the growing season is one of the best methods to understand and document the intra-annual development of growth rings. Secondary xylem production is a complex process derived from cell cataclinal division in the vascular cambium. The differentiation of annual rings in conifers involves the formation of tracheids that go through several phases before reaching their final form. A differentiating tracheid reaches its final radial diameter during a phase of radial enlargement after it has emerged from the cambial zone (Wilson et al. 1966). The secondary wall is formed during a subsequent maturation phase (Mahmood 1971; Wodzicki 1971; Creber and Chaloner 1990).

This study is a contribution to the intra-annual analysis of the balsam fir growth ring development by weekly micro-core sampling during three successive growing seasons, with two main objectives. The first was to characterize the phase of ring development by following the development of cells from their division-radial enlargement to their maturation. This analysis provided information on the dynamics of cambium and xylem cell differentiation through the growing season. The second objective was to analyze the period of earlywood and latewood development variation with respect to the duration of cell formation.

Material and methods

Study area

This study was conducted in two permanent plots (Lib-23, Lib-24) (Morin, 1994) located near the 50th parallel (49°58 ' N; 72°30 ' W), about 100 km north of Lac-Saint-Jean (Québec), an area located in Thibault's (1987) black spruce (*Picea mariana* (Mill.) B.S.P.) moss ecological region (No. 12b) of the boreal zone. The vegetation association is similar to the balsam fir – white birch (*Betula papyrifera* March.) type of the more southern balsam fir – white birch zone (Grandtner 1966). The mean annual temperature is –0,7°C and the mean annual precipitation is 422 cm, with 357 cm falling as snow (Environment Canada 1992). The balsam fir stands were located near the northern limit of the fir stands, which makes them interesting for further studies on climate-growth relationships because these trees might be more sensitive to climate. Lib-23 has a unimodal age structure with the trees establishing between 1815 and 1850. Lib-24 was severely affected by the last spruce budworm outbreak (1974 to 1988) and most of the adult trees, which established between 1875 and 1890, died in the last outbreak. The Lib-24 stand is now composed mainly of young, 5-6 meter tall trees, released after the spruce budworm outbreak due to stand opening (Morin 1994).

Tree selection

One micro-core at DBH (1,3 m) was taken from each of 55 trees before the beginning of 1998 growing season for a preliminary tree selection. Cells of the last three rings formed were counted and trees with a similar average number of cells per ring were

chosen in order to ensure comparable growth rates. Ten adult trees (mean height, 17,5 m) were selected from Lib-23 for an analysis of ring formation in 1998, 1999 and 2000. At Lib-24, 10 adult (mean height, 18.7 m) and 10 young (mean height, 4,5 m) trees were chosen; the young trees were analyzed for the 1998-1999 growing season while the adult trees were analyzed for the 1999-2000 season.

Ring sampling

Surgical bone sampling needles DBMNI-1501 (french name, Aiguille d'aspiration, Inter V médical, Montréal) were used for the extraction of small cores of wood and bark. The cores are 1 mm in diameter and 15-20 mm long, containing 4 to 6 rings. These needles were used instead of an increment puncher (wood samples of 10 mm in length and 2.5 mm in diameter, Forster et al, 2000) as its smaller diameter allows repetitive sampling also on small tree (dbh < 5cm) without causing significant growth disturbance. Wood cores were extracted every week for three growing seasons (1998, 1999 and 2000) from early May to October. Sample were taken following a spiral trajectory up the stem, from 30 cm below DBH to 30 cm above. Wood cores were always taken at least 10 cm apart for the adult trees and 5 cm apart for the young trees to avoid the presence of resin ducts on the next core, which is a common disturbance reaction with balsam fir. A total of 1410 wood cores were fixed in paraffin and transverse sections of 10-12 µm thickness were prepared with a microtome. Several sections of the same cores were put on 3-4 glass slides. The sections were immerged in two baths of Histo-Clear™ for 10 minutes and two baths of alcohol (100%) for 2 minutes to remove the paraffin and dehydrate the sections, The sections were then put in a water bath before applying

stain. Two different types of analysis were conducted on each sample: ring development analysis and cell measurement.

Ring development analysis

Sections were stained with a water solution of 0,05% cresyl fast violet and kept in water to assess the ring development. This staining procedure helps differentiate the developing phloem and xylem cells (Antonova and Shebeko 1981, in Russian). For each sample, the number of cells in the radial enlargement phase, cell wall thickening phase, and mature cell phase were counted along three radial files. The cells had to be kept in water and counted immediately after staining until the microscopic analysis was done as the coloration disappears after only a short period. The dormant cambium before the beginning and at the end of the growing season was easily identified by 2 to 4 radially flattened fusiform cells (Riding and Little 1984). Both cells from cambium zone and cells in radial enlargement phase (xylem) have primary pink walls. The cambial zone and cells in radial enlargement, are easily identifiable as they posses only primary walls, while both seive cells and tracheid cells have secodnary walls that show strong birefringence in polarized light (Zimmermann 1964; Riding and Little 1984). Cells in the secondary wall thickening phase change from light violet in the beginning of the process to deep violet near the mature cell state. Lignification is characterized by the appearance of blue, initiating first in middle lamella and spreading into the secondary walls of differentiating tracheids. Xylem cells are considered mature or entirely lignified when they are completely blue.

Cell measurements

Sections were stained with safranin (1% in water) and permanently fixed with Permount® to asses cell measurement with the computer program Wincell™. A Polaroid camera fixed on an optical microscope and connected to a computer was used for numerical image analysis. The measured parameters were lumen area (μm^2), single cell wall thickness (µm), lumen diameter (µm) and total cell width (µm). Three radial files were measured and the average cell size (or any wood parameter measured) was computed on each section. Radial files with larger tracheids were subjectively chosen to ensure that the cell section passed through or near the limit of the middle part of their length. Because the cores were taken along a spiral, the ring width varied within the tree circumference and therefore between the different samples. Hence, the number of cells was counted on 3 radial files on the 3 rings preceeding the ring in formation and used subsequently for a cell number standardization for each sample.

Statistical analysis: ring formation

The mean cell number of the three previous years per sample and the mean of all samples per tree were computed and used for a cell number circumference standardization. The extreme values (maximum and minimum) were removed by using a stem leaf graph created by the Univariate procedure of SAS package to ensure a normal distribution. A ratio was obtained for each sample by dividing the mean cell number of the sample by the mean cell number of all samples per tree. The number of cells in each phase (radial enlargement, wall thickening and mature cells) was then multiplied by the ratio to standardize the data according to the sample's relative position

on the stem. Before computing the mean cell number in each phase per date of sampling, another Univariate procedure (SAS package) was used to remove the extreme values.

The approximate date of entrance and the number of days spent in the differentiation phases (enlargement and wall thickening) of each consecutive tracheid formed during the growing season were found following Wodzicki (1971). The methodology is based on differences between (1) total cells number (radial elongation+wall thickening+mature cells), (2) wall thickening+mature cells and (3) mature cells, at a 7 day sampling interval. The calculations were done with fitted data using NLIN (NonLINear regression, Marquardt iterative option) of the SAS statistical package as the number of cells fluctuates near the end of the season. This method regresses the residuals onto the model partial derivatives in respect to the Gompertz parameters until the estimates converge (SAS Institute Inc. 1990). The examination of the R-square, the asymptotic *t*-statistic for the parameters, and the plots of the residual showed that the Gompertz function was appropriate to describe growth and time relationships (Zeide 1993; Huang, Titus and Wiens 1992). Gompertz equation has been used to model tree-ring growth (Camarero and al. 1998). The equation was fitted to establish the cell increase profile of each total described.

The Gompertz function is defined as:

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

Where ***y*** is the weekly cumulative sum of growth (expressed in number of cells), **t** is time computed in days since the first sampling date where t=0, ***a*** is the upper asymptote of the maximum number of cells where at t_i $y \cong a$, β is the *x*-axis placement parameter, and κ is the rate of change parameter (Cheng and Gordon 2000). Two biologically useful variables were calculated from the fitted statistics, both defined by Richards (1959). A weighted mean absolute cell formation rate **r** can be obtained by equation 2, and the time required for the major portion of cell formation **d** to occur can be obtained by equation 3. Parameter ν was fixed to 0.0001 since Gompertz function is a special case of the Richards function when $\nu \cong 0$.

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

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

Statistical analysis: cell measurement

Curves of cell size variation in radial files of xylem, called tracheidograms, were constructed following Vaganov (1990). Standardization is required to compare tree ring structures as different rings have different numbers of cells of varying dimensions. The standardization method decreases or increases the initial tracheidogram along the abscissa leaving the ordinate unchanged (Vaganov 1990). The average number of mature cells was used to calculate the number of cells to standardize for each sampling date. Classification of cells into latewood were conducted using Mork's formula described in Denne (1988):

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

Results

Tree ring characterization

The weekly averages of differentiating cells and mature cells, during the growing season from 1998 to 2000, are presented in figure 1. An important variation was observed between the starting and ending periods of the growing seasons (table 1). The starting periods vary from May 7, observed in Lib-23 1999, and June 7, observed at both sites in 2000, a month's difference.

The mean number of cells counted each week varied from 1 to 3 in the radial enlargement phase (figure 1). The highest values (4-5) counted on some samples in the second and the third week after the start, might reflect an active cell division at the beginning. However, the cambium zone at the first weeks of the growing season was easily ruptured and a few radially enlarging xylem could have been missed. The first cell in the wall thickening phase was observed between one and three weeks after the start of the growing season (table 1). After increasing regularly, they reached a plateau of around 10 cells for a month and a half (figure 1). The low variation during this long period indicates a constant flow of cells going in and out of the wall-thickening phase. Close to the end of the growing season these number decreased rapidly indicating that less cells were entering the wall thickening phase and that cell division was coming to an end.

In all cases, the total number of cells regularly increases until a mean maximum number defined as the upper asymptote. Past that particular point, cell numbers start to

fluctuate because samples were taken at different positions on the trees. The upper asymptote, representing the fitted mean total maximum number of cells or a plateau, is reached at different dates in July or August (table 1, figure 1) and seemed to arrive earlier when the earlywood-latewood transition was earlier. However, even if this number is reached, it does not mean that it is the end of cell division. Cell division could be masked by differences in the ring width around the trunk of the trees even if standardization was done to make it more regular. The end of cell division in ring development was the hardest variable to determine because the cessation of tracheid production varies within and between trees (Sundberg and *al.* 1987).

Growth model

Cell increase fitted well with the Gompertz equation as shown by the R^2 variation between 73.9% and 96.5%. The cell formation rate (r) varied between sites and between the young and adult trees. For the total cell number, the values varied from 0,49 to 0,70 cell/day and in general, the cell formation rate decreased with a longer period of cell division. The shortest length and the highest rate were observed in 1998 when the upper asymptote was reached near July 23 at both sites. The mature cell formation rate, representing the rate at which the cells enter into a mature state, is normally lower than that of the total number of cells because it excludes the radially enlarging and wall thickening cells. The mature cell formation rate also lasts longer because its upper asymptote (*a*) is reached later in the growing season.

Earlywood and latewood formation period

The separation of ring development into two parts, earlywood and latewood formation period, was determined from weekly cell measurements. The cell classification was conducted using only mature cells to avoid the comparison of non-mature cells with mature ones. In figure 2, cells that are still in differentiation phases (radial enlargement or cell wall thickening) are shown to illustrate more precisely the transition between earlywood and latewood. At the end of the growing season, when the number of cells starts to fluctuate, the number of cells was standardized at mature cell fitted values corresponding to the last sampling date.

Earlywood growth was observed to be a fast process with cell division taking between 1-2 months from the start of the growing season to the transition time (table 1). The earlywood formation length is almost the same for 1998 and 2000 (6 to 7 weeks) but it is longer for 1999 (9-10 weeks) because of a very early growing season. Thus, most of the ring width, 65-75% earlywood for mature trees and 80% for young trees, is formed in a very short period of time. The time at which we observed the first mature earlywood cells was constant at 3 to 4 weeks after the beginning of the growing season. This gives a good indication of the time required for the complete formation of an earlywood cell which is independent of the beginning of the growing season. Complete earlywood formation, including the time necessary for cell wall thickening, took 10-12 weeks in 1998-2000, and 13-14 weeks in 1999. Variation in the transition time does not seem to be related with the start of the growing season. In fact, even if the 1999 growing season started sooner, its earlywood-latewood shift was two weeks later than the one observed in 1998, and was close to the one observed in 2000 (table 1, figure 2). Total latewood

formation (including the cell wall thickening) lasted 9 weeks in 1998 and 2000, and 6 to 7 weeks in 1999, which is less than for earlywood. Latewood have less cells then earlywood, but more time is required to complete single cell formation because of the longer duration of the cell wall thickening phase.

Single cell formation period

Fitted sums of data (table 2) were used to interpolate between sampling dates and to set an approximate period of formation for each cell during the growing season (figures 3, 4 and 5). These estimates can be considered quite reliable for most of the earlywood cells since the earlywood-latewood transition date is approximately the same as the one observed (table 1). For latewood, the estimation of the end of the wall thickening phase, including the last 1-3 cells, is estimated later than the one graphically observed (figure 2) in 1998 and 1999 in both site. This problem is due to the difference between the convergence of the upper asymptote of the total cells and the one of mature cells that cause a bias for the last cell (table 2).

The duration of the radial cell enlargement phase might not have a strong influence on the final, mature cell width as there is a non-relevant difference between the wide earlywood cells and the narrow latewood cells (figure 3, 4 and 5). Radial enlargement was relatively uniform between study sites and years as shown by the values of less than a week. Normally these values vary from 2-6 days for earlywood with a slight increase at 5 to 10 days for latewood. Only a small difference was observed for the year 2000 where the lengths were slightly longer for both study plots. Times spent in radial enlargement are also quite similar between young and adult trees. However, wall

thickening varied between earlywood and latewood. The duration of earlywood wall thickening exhibited a constant increase during the growing season from 10 to 20-25 days before the earlywood –latewood transition. Latewood wall thickening then varied from 20-25 to 30-35 days depending on tree age (young or adult) and the year.

Pattern of ring formation

Interesting patterns of ring development were observed for the different study years and specific study plots. First, the overall growing season may almost be of the same length (comparing the beginning and the end) but may possess different progressions into the cell division rates and cell wall thickening periods. Young trees at Lib-24 started their 1998 and 1999 growth season on May 20-21 (table 1), but the transition between earlywood and latewood occurred later in 1999 because of a slower cell division rate (table 2 and figure 4). This delay cannot be attributed to significant difference in radial enlargement, consequently the cell division rate and the cell development phase seem independent.

A second pattern observed showed that when a delay was present at the beginning of the growing season, it could be maintained through to the end. In fact, the whole ring pattern of development throughout the 2000 growing season is delayed. The year 2000 cell division rate was also slower (0,515 cell per day, table 2), and the time spent in radial enlargement was longer, making it difficult to catch up. Similar situations were also present when comparing the years 1999 and 2000 at Lib-23 and Lib-24. Delays of about 3 weeks to a month were observed at the beginning and at the end of wall thickening phase but not at the earlywood-latewood transition.

Discussion

Earlywood growth

A month of difference was observed for the beginning of tracheid formation, from May 7 to June 7, which is a long delay. However, these delays are commonly observed because the beginning of the growing season varies considerably, depending mostly on climate variation (Creber and Chaloner 1984, 1990; Zabuga and Zabuga 1990; Vaganov and al. 1994). For Scots pine in Russia, the start of the growing season (1978-1982) varied between the first 10 days of May to the second 10 days of June, with temperature being the main factor that influenced the initiation of tracheid formation (Zabuga and Zabuga, 1990). The temperature around 25 °C or more at the beginning of May 1999 could explain the very early initiation of tracheid formation at Lib-23. In spite of this, detailed growth-climate analysis must be undertaken to fully understand the influence of climatic factors.

As reported for red pine (Whitmore and Zahner 1966) and Norway spruce (Horäcek 1994), the number of cells in the radial enlargement and wall thickening phases were relatively constant during most of the active growing period of earlywood following a sharp increase at the beginning. However, in our study a low number of cells in radial enlargement (maximum of 5) were counted every week. Other studies, including balsam fir, have found many more cells in this phase, especially in very active cambia (Whitmore and Zahner 1966; Sundberg and *al.* 1987; Horacek 1994). Even if some radially enlarging xylem could have been missed because of a rupture of the cambium

zone, cell numbers remain low compared with that of other studies, resulting in a quite short estimated enlargement time of less than a week for earlywood and from 5-10 days for latewood. By comparison, in Siberia, the growth of pine tracheid primary walls lasts from 3 to 17 days (Antonova and *al.* 1995).

Between 65-80% of ring width was produced in less than 2 months, from the mid-end of May to the mid-end of July, with June being the most important month for ring development. The short period needed for earlywood growth might be an adaptation to the cold climatic conditions of the boreal forest where cells require a very short time for their radial growth and start their wall thickening rapidly. This situation could leave a certain safety margin at the end of the growing season used to complete the lignification before frosts occur. Antonova and *al.* (1995) found that tracheid radial growth depends more on the rate of development, especially at the beginning of tracheid growth when the rate is higher, than on the duration of the process. Therefore, local and daily weather conditions at the beginning of cell enlargement should be crucial for determining both cell width and consequent ring width, because of its influence on the enlargement rate. As the rate and period of earlywood development changes from year to year, the understanding of the growth mechanism could improve significantly our comprehension of the growth and climate relationship. For example, ring width is a parameter poorly correlated with monthly climatic average in Eastern North-America (Conkey 1986; D'Arrigo and *al*. 1992; Schweingruber and *al.* 1993). Monthly climatic averages may not be precise enough to characterize the short crucial moment at the beginning of cell enlargement.

Latewood growth

Latewood initiation time is very important because it marks the end of the wide earlywood cell enlargement phase that largely defines ring width and marks the close end of cell division. The earlywood-latewood transition varies in time depending on the start of the growing season (early or late) and on the cell formation rate. The same transition date between study plots for a given year might reflect a climatic "signal" that affects a wide area. In our studies, it had varied from July 2 to July 19, a range of two weeks. Zabuga and Zabuga (1990) have observed a variation as long as a month with Scot pine in Siberia.

Latewood formation, that involves a reduction in radial expansion and an increase in wall thickening was strongly influenced by the increase in the duration of the cell wall thickening phase, which was about 10 days longer than earlywood. Other investigations have also observed that it is the duration and not the rate of wall material deposition that causes the thicker cell walls (Wodzicki 1971; Denne 1974, 1976). In addition, Uggla et al. (2001) recently concluded that latewood formation is under developmental, rather than metabolic, control. Moreover, year-to-year climatic variations, such as a decrease in soil moisture (Conkey 1986; Zabuga and Zabuga 1990), was found to explain the variations in earlywood-latewood transition time.

Several studies have found good correlations with maximum tree-ring wood density and August temperatures (mean-max) (D'Arrigo and *al.* 1992; Schweingruber and *al.* 1993; Splechtna and *al.* 2000). However, the period to obtain the best correlation can vary from mid-July to mid-August, as found in 1998 and 1999 or from mid-August to mid-

September in 2000 (figure 3, 4 and 5). Our results suggest that July and September, can also have a significant effect on latewood density. The growing season starting date (early-late) and also the earlywood-latewood transition date could have had an influence on the end of the cell wall thickening phase. However, climatic conditions at the end of the process are not excluded from explaining the variation. Lignin deposition in latewood cells still persists after the end of the cell wall thickening phases (Gindl and *al.* 2000), therefore complete ring density was probably attained before the period observed, from August 20 to September 20, which is the end of lignin deposition in cell wall.

Control of the growing season length: duration of rate ?

Some questions arise from the observation of the growing season process. First, does a delay in the beginning of growing season make it shorter and result in fewer cells produced? For example, Vaganov and *al.* (1994) found that in severely limited temperature conditions a narrower ring is formed when a delay is observed in the beginning of the growing season. They attributed the narrow ring to a growth suppression in the middle of June due to decreasing temperature and light. By comparing 1998-1999 with 2000, it seems that the “conditions” needed for radial growth can be delayed without strong negative effects as long as they eventually occurs. Conversely, Lib-23 1999 shows that if the “conditions” or some kind of “climatic signal” are present very early in spring, trees will slowly start growing even if some snow patches are still present and frost can still occur. The growing season was longer for that site/year because of a very early start but did not necessarily end sooner as shown by the latewood initiation date and the end of the cell wall thickening period and did not

result in a greater number of cells produced nor in a wider ring. The results obtained show that the cell division rate is also very important in the progress of the growing season. Our results suggest that climate can have an effect on both the rate and the duration of the cell differentiation process, independent of the beginning of the growing season. Thus, a certain flexibility exists in the growing process, which is an advantage in boreal forests where optimal growth conditions change from year to year.

Conclusion

Repeated cellular analyses during the growing season is one of the best methods to understand the mechanism of growth ring development but requires meticulous methodological work. On the other hand, wood growth estimation on a daily scale will make it possible to carry out precise relationships with climate, or to understand the effect of a perturbation such as insect defoliation. The data obtained by this approach will also help to understand which environmental parameters are responsible for the formation of the ring and the ring width, thus improving our prediction of forest productivity, and consequently forest management planning.

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

Note: Lib-24y, young trees; Lib24a, adult trees.

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

Note: ***a*** is the upper asymptote of the maximum number of cells where at t_i $y \cong a$; **β** is the *x*-axis placement parameter; **κ** is the rate of change parameter; **r** is the rate of cell formation by day; **d** is the total time required to complete the cell increase process, in days.

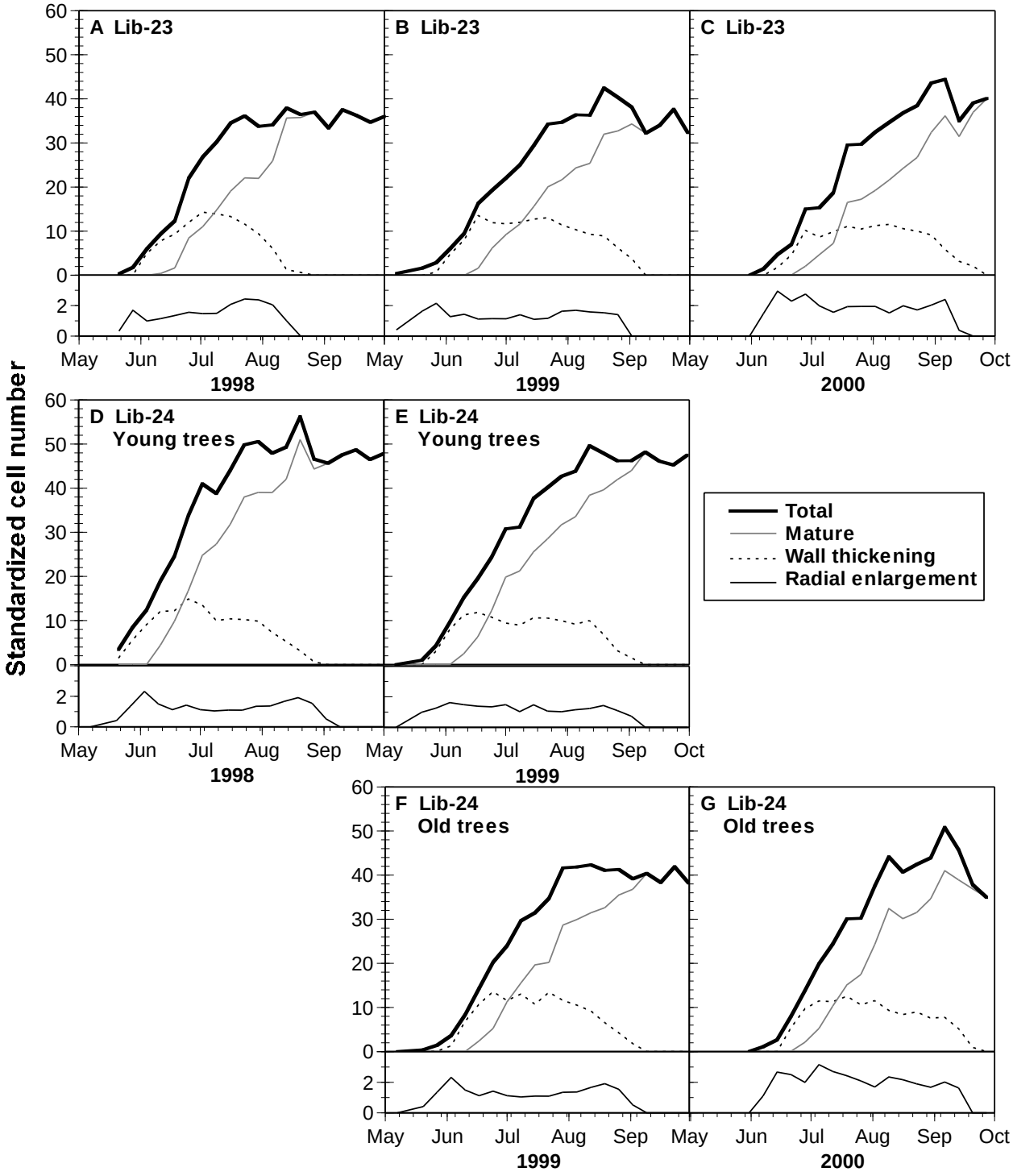

Figure 1. Number of cells in radial enlargement phase, wall thickening phase, mature cells and total number at Lib-23 and Lib-24 in 1998, 1999 and 2000. The horizontal axis major ticks marks show one month and minor tick marks show one week intervals.

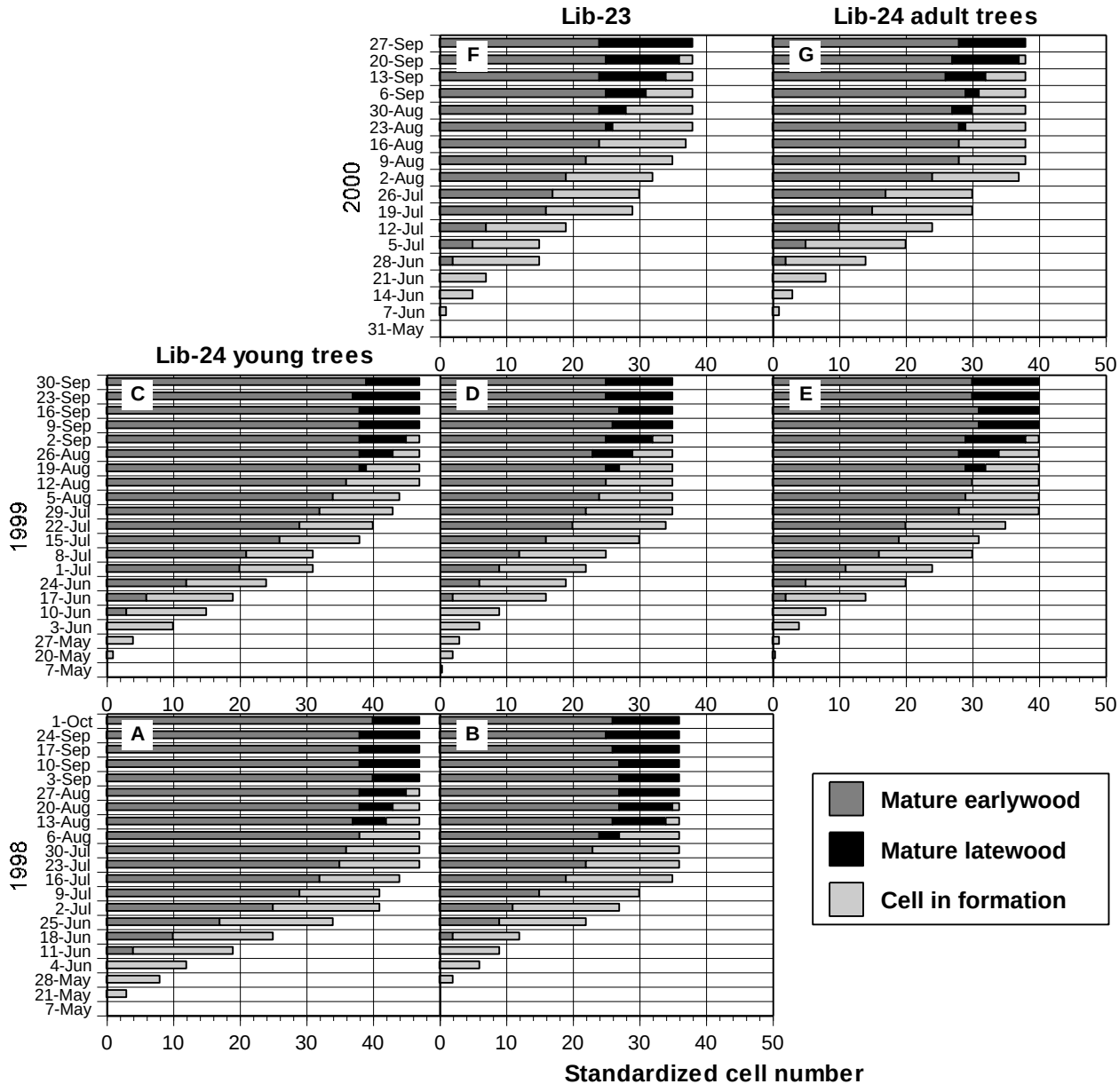

Figure 2. Number of mature earlywood and latewood cells and number of cells in formation (radial enlargement and wall thickening) for each sampling date from 1998 to 2000 for adult and young trees at Lib-23 and Lib-24 sites.

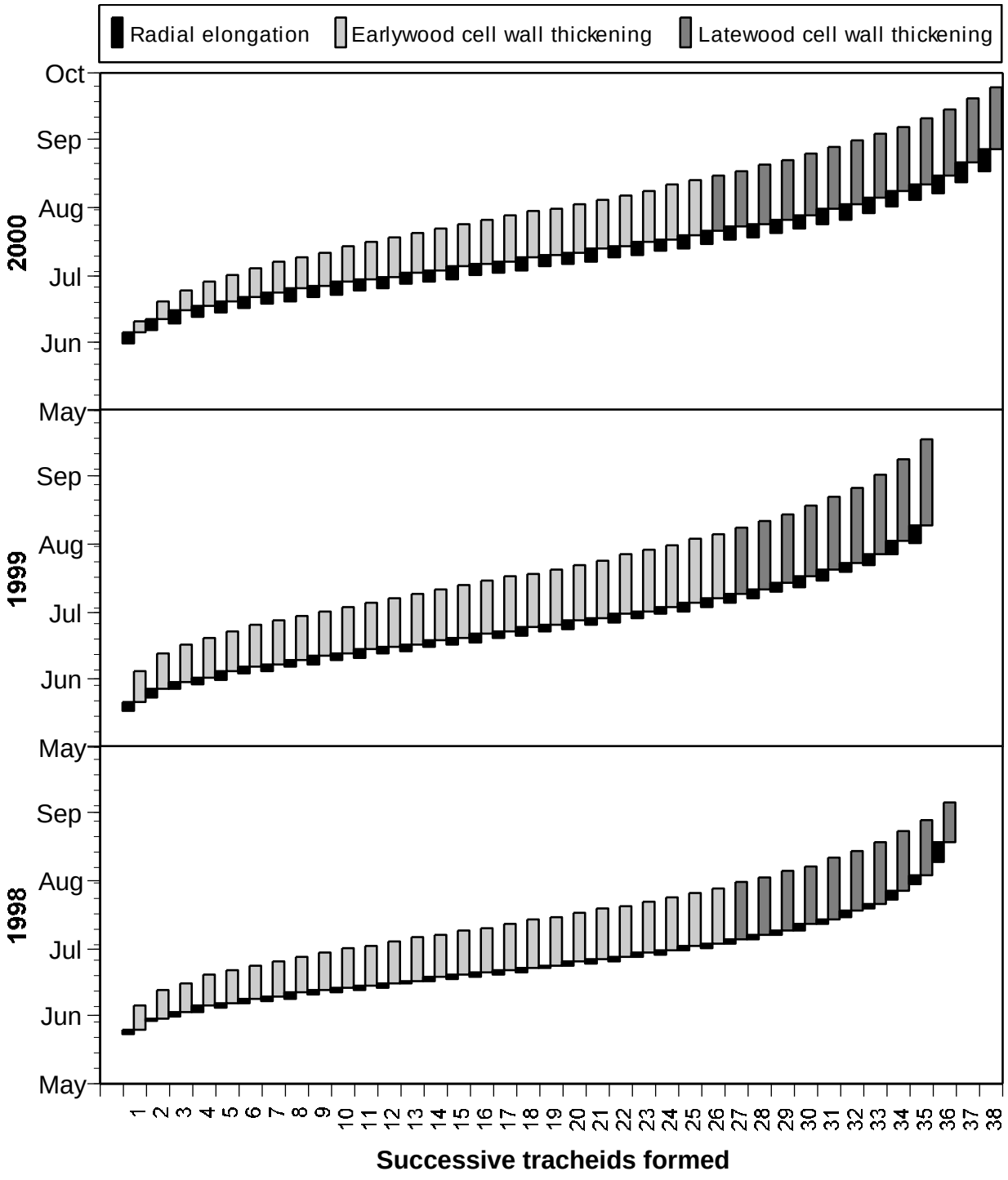

Figure 3. Duration of radial cell enlargement and cell wall thickening phases for adult trees at Lib-23 plot. The vertical axis major tick marks show one month intervals and minor tick marks show one week intervals.

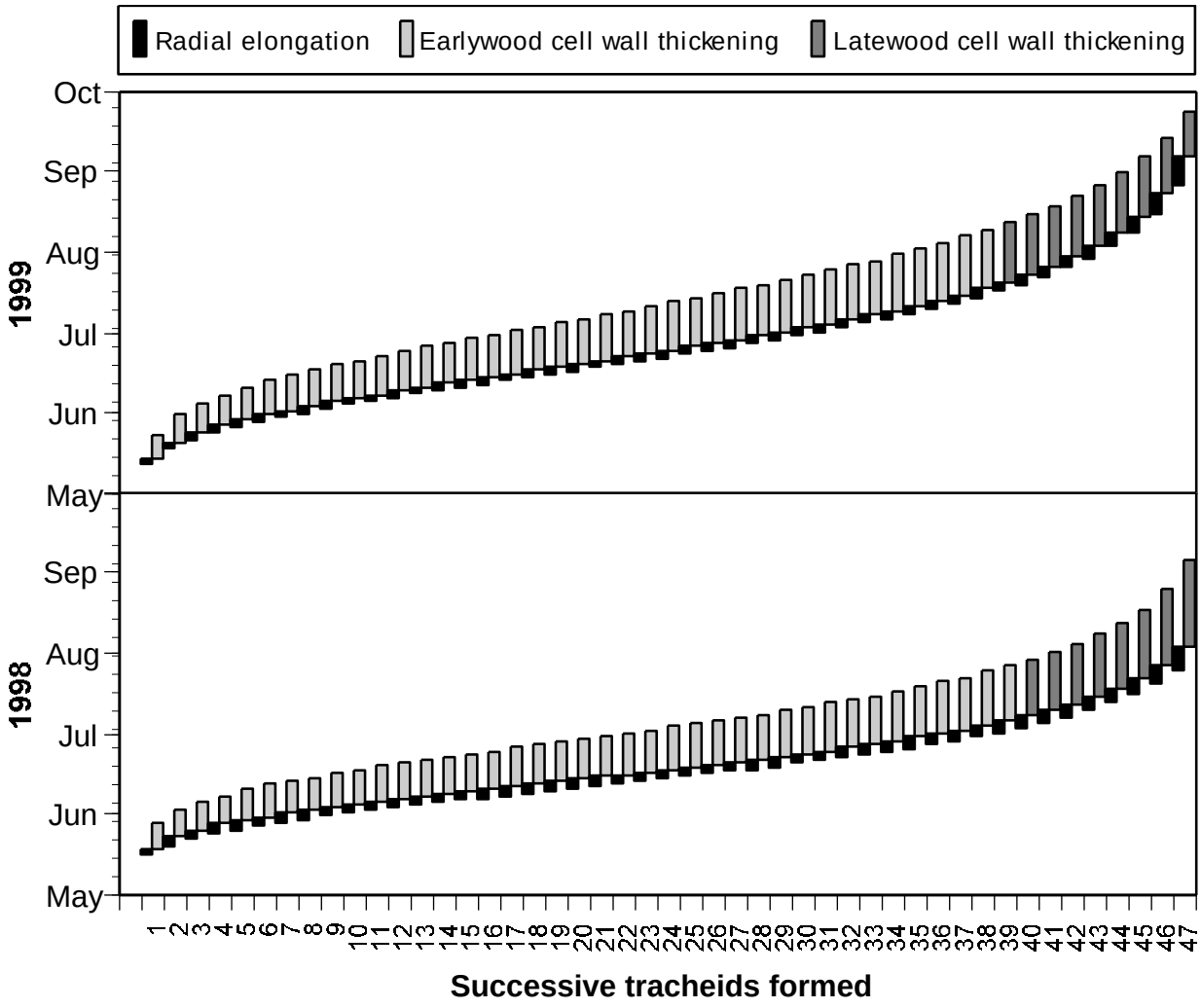

Figure 4. Duration of radial cell enlargement and cell wall thickening phases for young trees at Lib-24 plot. The vertical axis major tick marks show one month intervals and minor tick marks show one week intervals.

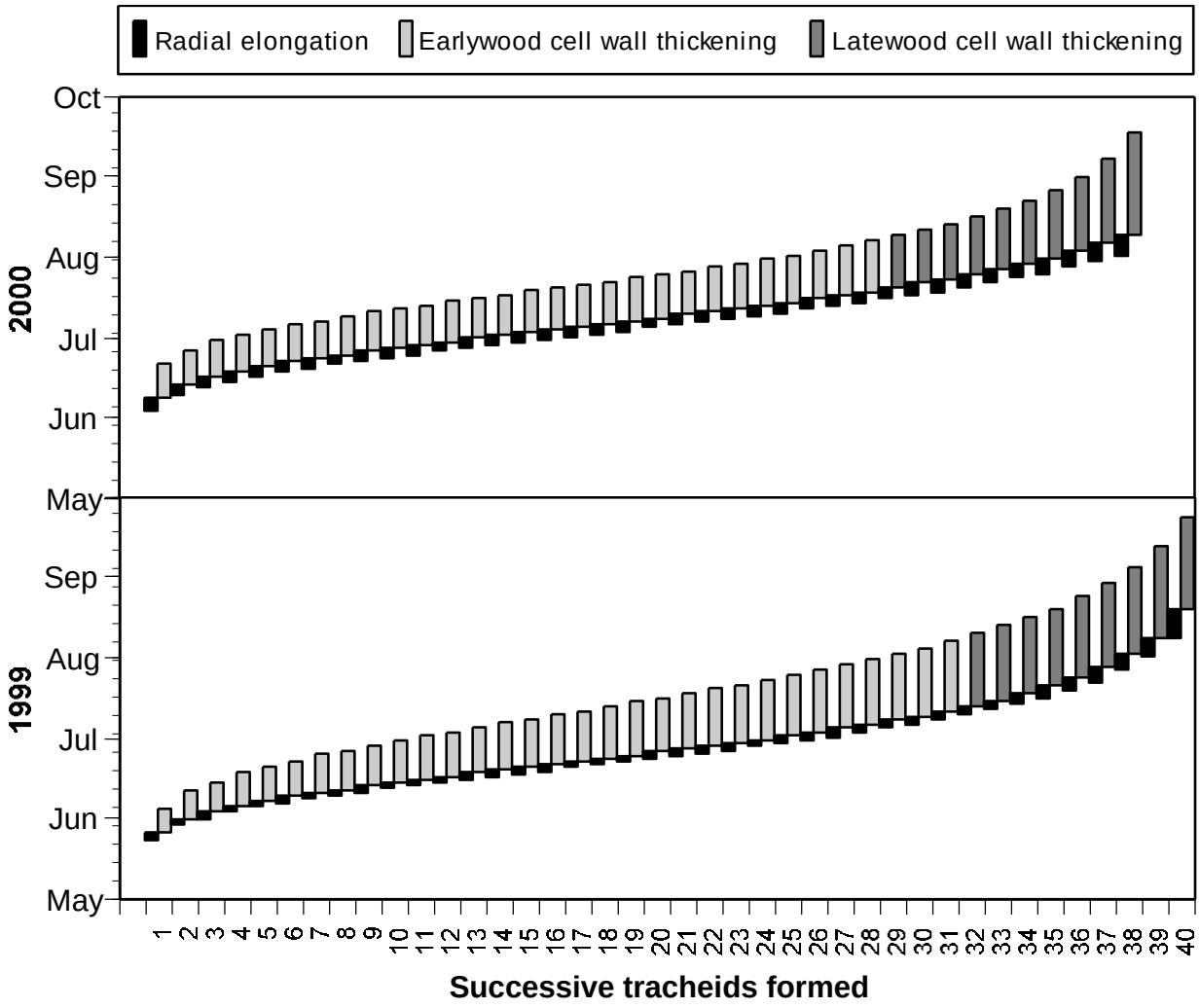

Figure 5. Duration of radial cell enlargement and cell wall thickening phases for adult trees at Lib-24 plot. The vertical axis major tick marks show one month intervals and minor tick marks show one week intervals.