MONITORING OF XYLEM FORMATION IN NORWAY SPRUCE IN THE CZECH REPUBLIC 2009

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ABSTRACT

The progress of xylem formation in Norway spruce (Picea abies (L.) Karst.) was measured during one growing season in Rájec-Němčice in the Czech Republic. Tissue samples were taken at weekly intervals by means of Trephor tool, from April to October 2009. The formation of new tracheid was studied with light microscope. The cambium began its division activity between 16th and 30th April and the development of new cells finished between 27th August and 17th September. The average length of time of the cambium activity was 4.8 months. The total time of wood formation was 153 days, i.e. 5.1 months. The cambium achieved maximum width (9.9 cell layers) in June, dormant cambium contained 4.6. Fitting xylem increments to the Gompertz function showed that the period of most intensive cell formation was at the turn of June and July. The total time necessary for the development of most cells differs when the WCDcalc script (153 days) is used and when the Gompertz function (174 days) is used.

KEYWORDS: Norway spruce (Picea abies (L.) Karst.), cambium, wood formation, cell differentiation, xylem growth rings, Gompertz function, WCDcalc.

INTRODUCTION

Radial increment of wood species is a result of the activity of two lateral meristematic tissues – cambium and phellogen. Phellogen divides the cells of the periderm; cambium divides new xylem cells centripetally and phloem cells centrifugally.

Some authors use the term cambium for cambial initials only, whereas the term cambial zone includes both cambial initials and mother cells of wood and phloem – this is the uniseriate concept (Wilson et al. 1966, Barlow et al. 2002). On the other hand, the multiseriate concept contains both the initials and the mother cells of wood and phloem in the term cambium (Panshin and de Zeeuw 1980, Larson 1994). Catesson (1984) considered the terms cambium and cambial zone to be synonymous. This paper uses the multiseriate concept terminology.
The cycle of seasons is marked by the periodicity of radiation, length of day, temperature, and soil moisture (precipitation); in each season there are favourable or unfavourable conditions for the cambium activity, i.e. for the growth of plants (Larcher 2003). Under normal climatic conditions the cambium of temperate-zone trees is inactive in the winter. While in the dormant state, the walls of cambial initials thicken considerably and the protoplast becomes viscous, a condition termed the gel state. In the spring, the cambium becomes activated – cell walls in the cambial zone become thinner and more plastic, cytoplasm gets into the sol state (Panshin and de Zeeuw 1980).

The activity of cambium depends on many internal (e.g. genetic properties of plants, their health, age and the position in the plant) and external factors (Panshin and de Zeeuw 1980). Wodzicki (1971) divided the external factors of the environment into (1) basic conditions for xylogenesis (temperature, moisture, nutrients in the soil, gravity, photoperiod) and (2) occasional factors (wind, fire, frost, floods, defoliation, forest management, air pollution).

It is generally accepted that the renewal of cell activity in the cambial zone in the spring in temperate climates begins when the mean temperature rises above 4.4°C for about a week (Wilcox 1962). Matovič (1990) and Horáček et al. (1999) found out that when average daily temperatures reach 8°C for several days (5 up to 6), the cambium activity starts. According to Gričar (2007), the limit for cambium activation is the minimum temperature above 5°C for several days. The threshold temperature at which xylogenesis had a 0.5 probability of occurrence was 5.5°C for minimum air temperature as it was calculated by Deslauriers et al. (2008). Rossi et al. (2007) reported that xylogenesis was active in conifers at high altitudes when the mean daily air temperature was 5.6–8.5°C.

The structure of tree rings in wood represents a record of the influence of the main environment factors which affected the cambium activity in the specific growing season. To be able to analyse the record, it is necessary to know the dynamics of the cambial activity and the dynamics of wood cell development (Horáček et al. 1999, Fonti et al. 2007).

A cell which gets divided from the cambial zone cannot divide any more and it differentiates. The differentiation consists of the phase of postcambial growth and the phase of the synthesis of secondary cell wall formation and lignification (Wilson et al. 1966, Panshin and de Zeeuw 1980, Larson 1994). Consequent phases of formation of the cells by cambium and their development in each of the differentiation phases are independent processes and their reactions to environmental factors can be different (Antonova and Stasova 1997).

In the 1980s and the 1990s an extensive research of wood formation was conducted at the Rájec-Němčice research site in the Czech Republic (Matovič 1990, Horáček 1994, Horáček et al. 1999, Vavrčík 2002). The model wood species was Norway spruce (Picea abies (L.) Karst.) grown in a monoculture. In 2009 the monitoring of wood formation at the same research site was resumed.

MATERIAL AND METHODS

Research was carried out at the Rájec-Němčice research site of the Department of Forest Ecology, Mendel University in Brno, 3 km north of Němčice village (49°29’31”N and 16°43’30”E). The soil type of the area is modal oligotrophic Cambisol (Fabiánek et al. 2009, Menšík et al. 2009). The research plot is situated at an altitude of 600–660 m a.s.l., with mean annual air temperature 6.5°C and mean annual precipitation 717 mm (Hadaš 2002).

For the experiment, six specimens of Norway spruce (Picea abies (L.) Karst.) with a stem of
30–46 cm in diameter, 34.5–37 m in height, and 106 years of age were chosen (first generation after mixed forest).

The selected specimens were of good health and the absence of compression wood was assumed. Tissue samples were taken at weekly intervals, from April to October 2009.

Sampling was carried out by means of the Trephor tool (Rossi et al. 2006). The microcores (1.8 mm in diameter) were taken at the breast height around the stem perimeter, so that they contained phloem, cambium and xylem of the developing growth ring. The distance between two neighbouring microcores was 2 cm so that the samples did not contain traumatic tissue. Immediately after sampling, the microcores were immersed in FAA (formalin-ethanol-acetic acid), where they were left for a week; afterwards, they were stored in 30 % ethanol.

Microcores were dehydrated in an alcohol series (70 %, 70 %, 90 %, 95 %, 100 %, and 100 %), then clearing in xylene followed. The actual paraffin infiltration of microcores was carried out in a laboratory drying oven at a temperature of 60°C for 4 hours. Paraffin was poured by means of the Leica EG 1120 dispenser and the microcores were connected with histological cases to be mounted in the microtome. Then the Leica RM 2235 rotation microtome was used to make cross sections 10–12 µm thin. An adhesive (egg white and glycerine, 1:1) was used for better adhesion of the sections on glass slides. The sections were dried in an oven at a temperature of 60°C for 30 minutes. Further steps were the removal of the paraffin (xylene), dehydration (ethanol) and staining of the sections by safranin and astra blue. The sections were mounted in Canada balsam.

To monitor and scan the microsections we used the Leica DMLS microscope with the Leica DFC 280 digital camera. To measure wood radial increment we used the ImageJ open source program.

The cross sections were used to identify cambial cells and differentiating cells (tracheids) in postcambial growth, secondary cell wall deposition and lignification, and mature cells. The numbers of these cells were recorded for three randomly chosen files and then the wood radial increment was measured. Further, the average was calculated.

The xylem formation of the growth ring has been analysed with Gompertz function (Dufour and Morin 2007, Rossi et al. 2003) using equation:

\[ y = A \cdot e^{-e^{B-t/k}} \]

where: \( y \) – weekly cumulative cells, \( t \) – day of year, \( A \) – upper asymptote, representing the maximum number of cells, \( B \) – place on x axis, estimating the beginning of cambial activity, \( k \) – inflection point on the curve.

To calculate the timing of differentiation phases of individual cells (tracheids), the script in R WCDcalc (Vavrčík and Gryc 2010), based on the calculations of Wodzicki (1971), was used.

RESULTS

Cambial activity

At the beginning of the growing season certain changes in the cell morphology in comparison with the dormant cambium were observed. These changes are shown in Fig. 1. Fig. 1–A shows the swollen cambium at the beginning of the growing season. The cells have a larger radial dimension than in the cambium at the end of the growing season (Fig. 1–C). Fig. 1–B shows the active cambium formed from up to 10 layers of cells (the state of 18th June 2009).
The cambium at the beginning of the growing season (2\textsuperscript{nd} April 2009) consisted of 3 up to 6 layers of cells in a file, the average number of cells was 4.6. In the last sampling (8\textsuperscript{th} October 2009), the number of cells was very similar, ranging between 4 and 6 cells, the average value being 4.4 cells. The differences in the number of cells at the beginning and the end of growing season fall within the variability of this characteristic. During the growing season, the number of the cells in the cambial zone first grew (Fig. 2), e.g. at the end of April (30\textsuperscript{th} April) to 5.6 and at mid-May (14\textsuperscript{th} May) to 8.11 cells. The maximum number of cells in the cambium was observed in June – 9.9 cells – and July – 9.2 cells. In August, the number of cells in the cambium decreased gradually and at the end of September it was stabilized at values similar to those observed at the beginning of the growing season.

The starting cambial activity in microsamples was manifested by radial expansion of cells, slight narrowing of their tangential cell walls and sometimes by a well visible cell content. After a short time, their number rose and then the first phloem and xylem cells were differentiated. The interval between cambium activation and the first xylem cell differentiation varied in different trees. We assign the beginning of the cambial activity to the time when the first cell in postcambial growth appeared, i.e. 16\textsuperscript{th}–30\textsuperscript{th} April.

In the week before 9\textsuperscript{th} April the temperature did not fall below 4.9°C and the average daily temperatures were within 11.1 and 14.1°C. In the week before 16\textsuperscript{th} April the temperature did not fall below 6.2°C and the average daily temperatures were within 11.8 and 14.4°C. It is necessary
to note that in 2009 there was a sharp increase in temperature at the turn of March and April.

From mid-August, we observed a decrease in the formation of new cells and a gradual reduction of the cambium activity. The activity finally stopped during the first half of September. It means that the cambium was active in individual trees for 18–23 weeks, i.e. 4.2 up to 5.4 months (site average 4.8 months).

**Tracheid differentiation**

Process of differentiation and the number of tracheid cells in individual phases are shown in Fig. 3 and Fig. 4.
The first early wood cells, which appeared in the phase of postcambial growth, were observed in sample tree 4 as soon as on 16th April. In the following weeks new cells in postcambial growth were observed in the other sample trees as well. At the end of April (30th April) there were cells in postcambial growth in all sample trees. The number of cells at this phase grew till the end of June when it reached the maximum (6.11 cells on average), then the number of cells at this stage went down gradually (Fig. 4).

The synthesis of secondary cell walls of tracheids started 3 weeks later (14th–21st May 2010). The first signs of cell lignification were observed at the turn of May and June. At this time the lignification started in the corners of the cells. The first fully lignified cells were observed in sample trees 2, 3, and 4 on 18th June. The latest (9th July) fully lignified cells were found in sample tree 1.

The times necessary for individual phases of differentiation – the enlarging phase and the maturing phase – were calculated by means of the WCDcalc script. Fig. 5 shows the graphical representation of the script. The time of each phase and their general trends were different and they changed during the growing season.

The length of the postcambial growth phase increased slightly from the first cell until about 25% of cells formed in the growing season were produced (11–22 days), then it dropped to 9 days in which 80% of all cells were produced. At the end of the growing season, the time for which cells stayed at the postcambial growth phase slightly increased. As regards the maturing phase, the following trend was recorded: at the beginning of the growing season the time for which cells remained at this phase was constant, ranging between 21 and 29 days; then there was a gradual but considerable increase in the time of the maturing phase (25 days). As soon as 80% of all cells were formed, the time in the maturing phase dropped to 10 days.

The number of cells during the growing season

The changes in the number of cells during the growing season are shown in Fig. 6. It is obvious that the actual numbers of formed cells among individual sample trees differ; however, the dynamics of the increase in the number of cells in all sample trees is very similar.

Fig. 4: Average dynamics of xylem growth ring formation and individual phases of xylogenesis; PC – postcambial growth, SW – formation of secondary wall and lignification, MT – mature tracheids, TOTAL – total number of formed xylem cells.

The time necessary for maturation of cell in the last tracheids was again a bit longer.
Sample tree 1 is an exception. In the second half of the growing season, sample trees 2 and 3 continue the same trend of cell number increment, sample trees 4, 5, and 6 show a slower increment. The cambium of sample tree 1 only formed 26.6 cells during the entire growing season. Sample trees 2 and 3 formed more than a double. The observed drops in the number of cells during the growing season are caused by the fact that the samples were taken gradually during the growing season from various parts of the stem perimeter. At the end of July the cambium formed 70% of cells from the total number of cells on average. However, 70% of the total width of tree ring was observed as soon as at the beginning of July (Fig. 7). This border also corresponds to the transition between early and late wood, it means that also first late tracheids were formed in this time.
We created the model for the Rájec-Němčice site which describes the radial increment of spruce wood during the growing season of 2009 by means of the Gompertz function (Fig. 8). The following values were calculated on its basis: (1) the final width of xylem growth ring − 59.5 cells, (2) the maximum weekly increment − 3.51 (2nd July 2009), (3) the average number of newly formed cells in one day of the growing season − 0.34 cells, and (4) the number of days necessary for the formation of most xylem cells − 174 days.

DISCUSSION

Cambial activity

Cambial activity (cambium swelling) at research site Rájec-Němčice, Czech Republic, in 2009 probably started in the first half of April. To establish the exact time of the beginning of cambium activation in mature trees is highly difficult in natural conditions. Wilson (1964) states, regarding to Douglas fir, that the division including mitosis takes 26.4 hours (mitosis 5.6 hours). Due to the used interval of sampling − one week − it is very difficult to capture such a short moment.

In 2009 the first cells in postcambial growth appeared in one of the sample trees as soon as on 16th April, in the other trees on 30th April. This outcome is in agreement with the previous studies conducted at the same site (Matovič 1985, 1990; Vavrčík 2002), which considered the appearance of the first cell in postcambial growth to be the beginning of cambium activity. Matovič (1985, 1990) found out that cambium activity in the examined growing seasons in years 1981–1989 was observed at the turn of April and May. Also Vavrčík (2002) puts the beginning of cambium activity at the beginning of May in 1998.

The determining factor of the beginning of cambium activity is the temperature of air (Oribe et al. 2001, 2003, Gričar et al. 2006). Cambium at a height of 1.3 m starts its activity when the average daily temperatures remain at values around 8˚C for several days (5–6) (Matovič 1985, 1990). In 2009, starting on 2nd April, there was a longer series of days with the average daily temperature over 8˚C − the average daily temperatures reached 12.5˚C. These higher average daily temperatures caused the earlier activation of cambium and the division of the first xylem cells from the cambial zone in the second half of April.

The number of cells in the cambial zone was highly variable and changed during the growing season. The ascertained average number of cells in dormant cambium was 4.6 and this corresponds to the results published before concerning the same site Rájec-Němčice (Matovič 1985, 1990). Gričar (2007), Rossi et al. (2007), Gričar and Čufar (2008) stated that the dormant cambium consisted of 6 up to 8 layers of cells. The difference may be caused by the adaptation of the species (ecotype) to the differing conditions of the climate and the site. The number of cells in the cambial zone at the explored site in 2009 gradually increased; in June, the number was doubled (9.9); and from the half of August there was a decrease in the number of cells in the cambial zone, which is connected with the gradual reduction of cambial activity and related to the overall procedure of tree ring development. Gričar (2007) says that after cambium activation the number of cells doubles and the maximum number is reached within a month. Deslauriers (2003) and Larson (1994) obtained similar results − they state that after the activation of cambium the number of cells in the cambial zone doubles.

The total time of cambium activity depends on the climate and soil conditions of the growing season and the social position of the tree in the stand (Larson 1994). Temperature (fall of average daily temperatures below 8˚C) seems to be the determining factor in the ending of cambium
activity, in critical situations it is also the decrease in soil moisture (the decrease in water storage to the limit or below the limit of reduced availability), or the combination of both factors (Matovič 1985). In the examined growing season we observed a gradual decrease in cambium activity during August and the end of the activity in the first half of September, which corresponds to the model created by means of Gompertz function. The average daily temperatures in September were relatively high (average of 16.2°C), therefore the temperature was probably not the determining factor in the ending of the cambium activity. The more probable factor was the decrease in the soil water storage to the point of reduced availability at the end of the growing season in August and September.

In 2009 the cambium in sample trees was active for 4.2 up to 5.4 months (4.8 months on average). The period of cambium activity at the explored site is longer than it was at sites of spruce in Slovenia – 3.5 months (Gričar 2007). The differences can be explained by different conditions of the climate and the site.

**Differentiation of tracheids and the numbers of newly formed cells**

During the tracheid differentiation, after they are divided from the cambial zone, we can distinguish two phases: (1) postcambial growth, and (2) formation secondary cell wall and lignification. The times for which individual newly formed tracheids remained in the phases during the growing season were different. Tracheidogram (Fig. 5) describing the procedure of the tracheid differentiation during the growing season differs from the models published before (Wodzicki 1971, Matovič 1985, 1990, Horáček et al. 2003) by the time for which the late tracheids remained in the maturing phase. We can assume that higher average daily temperatures in the second half of the growing season affected the speed of lignin synthesis in the cell wall. The tracheids then remained in the maturing phase for a shorter time.

The cumulative increment of cells was fitted by the Gompertz function, which had been used in the previous studies (Gričar et al. 2008, Gričar 2007, Deslauriers 2003, Deslauriers et al. 2003, Rossi et al. 2003). The calculated average number of cells a day formed during the time of active growth in Rájec in 2009 was 0.34. This is the average of the entire growing season and the dynamics of the development of new cells will be different at the beginning, during, and at the end of the growing season. The calculated final width of xylem growth ring in Rájec-Němčice in 2009 was around 60 cells. The resulting number of cells is a function of the climatic conditions of the site and the ecotype of the species.

The final average wood increment and the average percentage increment of cells during the growing season was expressed by a growth S curve, which is in correspondence with Heinrichs et al. (2007), Mäkinen et al. (2003) and Schmitt et al. (2004). Rossi et al. (2006) found out that the maximum increment of conifers (growing both in Europe and North America) occurs at the time of the summer solstice, when the photoperiod is the longest. The maximum number of newly formed cells in Rájec-Němčice, as calculated by means of the Gompertz function, agrees with this – the maximum speed of new cell production was in the second half of June and in July (0.5 cell/day).

Deslauriers and Morin (2005) estimated from a weekly sampling the daily cell production rate was higher in June, or in July, when the transition from earlywood to latewood was observed later in the growing season. In Rájec-Němčice the transition between early and latewood was observed immediately when the cell production rate reached the maximum value.

The decreases in the number of cells in cumulative sums are caused by the procedure of sampling around the stem perimeter during the growing season. The smallest number of fully developed cells in sample tree 1 can be put in relation to the number of cells in the cambial zone.
at the half of the growing season (Fig. 2 and 6).

The total time necessary for the development of most cells differs when the WCDcalc script (153 days) is used and when the Gompertz function (174 days) is used. This is caused by the different approach of these methodologies. The WCDcalc script is based on the calculation using the method by Wodzicki (1971), where real tracheids are taken into account, whereas the Gompertz function procedure consists in the statistical fitting of the resulting data with the function and then the estimated parameters are processed.

CONCLUSIONS

The results of monitoring of wood formation during the growing season of 2009 in mature Norway spruce (*Picea abies* (L.) Karst.) specimens at the research site in Rájec-Němčice, the Czech Republic, are presented in the paper. The cambium began its division activity between 16th and 30th April and the development of new cells finished between 27th August and 17th September. The average length of time of the cambium activity was 4.8 months. The total time of wood formation was 153 days, i.e. 5.1 months. The average number of cells in the dormant cambium was 4.6. The maximum average number of cells of 9.9 was observed in June, and then the number of cells in the cambial zone dropped to values similar to those observed at the beginning of the growing season.

The analysis by the Gompertz function showed that the highest speed of division of new cells was calculated at the turn of June and July (3.51 cells a week). The maximum number of formed cells was estimated to be 59.5.

The results confirm that the behaviour of the sample trees is very similar in some criteria (e.g. the beginning of cambium activity, the increment trend) to the previous studies which were conducted at the same research site in 1981–1989 in the same stand with specimens which were then younger.

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WOOD RESEARCH


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