

Xylem Phenology of *Fagus sylvatica* in Rarău Mountains (Eastern Carpathians, Romania)

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Abstract

The cambium activity and the tree ring formation of European beech (*Fagus sylvatica* L.) from the Rarău Mountains was monitored during 2009, 2010 and 2011 in a beech - coniferous stand, representative for Eastern Carpathian mixed forests. Wood microcores were collected weekly from five trees and prepared in order to describe the different phases of wood formation. Four phases of tree ring development were quantified, in number of cells and phase duration: cambial phase, cell enlargement, cell wall thickening and cell maturation. The onset of the cambial activity took place in the first week of May 2009, one week later in 2010 and in the last week of April 2011. The beech tree ring development period varies between 127 days in 2009 and 137 days in 2011.

Keywords: cambial activity, cell differentiation, European beech, xylem growth rings

Introduction

The monitoring of growth activity in terms of anatomic aspects is a complex process which allows the determination of the duration of the annual tree ring formation and its correlative relationship with the environmental factors. The xylem, developed in optimal climatic conditions, presents uniformity in its structure and annual tree rings of equal size. In most cases, the process of radial growth varies from year to year, depending on the climatic fluctuations during the growing season (Rossi *et al.*, 2006).

The beech (*Fagus sylvatica* L.) is the main forest species in Romania occupying 26% of the forest vegetation area, and 39% of the volume of standing timber (IFN, 2014). Beech forests are particularly important both from an economic and an ecological point of view (Stoiculescu, 2013). Due to the high sensitivity to climatic factors, high longevity (Di Filippo *et al.*, 2012) and the ability to grow in different sites condition, the beech is a relevant species for dendroclimatological studies at European level (Dittmar *et al.*, 2003; Piovesan *et al.*, 2005; Di Filippo *et al.*, 2007) and in the Carpathian region (Roibu and Popa, 2006; Roibu, 2010). Most dendroclimatological studies are based on the analysis of the statistical relationship between the width of tree rings and the climatic factors from current and previous growing seasons (Schweingruber, 1992). Only few studies addressed the problem of intra-annual variation of the tree-ring characteristics, respectively the dynamics of beech radial growth phases (Schmitt *et al.*, 2000; Bouriaud *et al.*, 2004; Oladi *et al.*, 2011; Prislán *et al.*, 2013; Vavřík *et al.*, 2013).

Most studies regarding the monitoring of the xylem formation processes focused on coniferous species (Rossi *et al.*, 2006, 2008; Deslauriers *et al.*, 2008; Gricar *et al.*, 2009; Moser *et al.*, 2010; Rossi *et al.*, 2013).

The structure of beech wood is complex and inhomogeneous due to the diversity of the anatomical elements. Density, distribution and vessel wall thickness can be analyzed after evaluating the vulnerability of xylem to embolism (Cochard *et al.*, 2001). The analysis of the connection between the phases of xylem formation and leaf phenological characteristics allowed the assessment of climatic factors influencing the radial growth processes in South Eastern and Central Europe (Cufar *et al.*, 2008).

The objective of this study is to analyze the dynamics of the European beech cambial activity and to assess the different stages of the annual tree ring formation over the period 2009-2011 in the Eastern Carpathians beech-conifer mixtures, thus contributing to the knowledge about the growth phenology of European beech in the Carpathian area.

Materials and methods

Study area. The study was carried out on the northern slope (47°29' N, 25°33' E) of the Rarău Mountains in a mixed forest of beech and conifers (spruce, fir), located at an altitude of 850 m. The type of soil is eutric cambisol formed on limestone bedrock. The mean annual temperature is 2.3 °C (Rarău weather station - altitude 1536 m, 2 km from the study site) with a maximum monthly temperature in July

(11.4 °C) and a minimum in January (- 6.9 °C). The mean annual precipitation is 907 mm, with a maximum in June (144 mm) (Rusu, 2002).

For xylem growth monitoring, five dominant trees with very good growing conditions, presenting no visible defects, and with diameters at breast height ranging from 30 cm to 40 cm were selected.

Sample collection, preparation and microscopic observations.

The monitoring of xylem formation phases was performed at weekly intervals from mid-April to late September, over the period 2009-2011. Microcores with a thickness of 2.5 mm and a length of 3-5 cm containing phloem, cambium, developing xylem, and 1-3 fully formed rings were taken weekly from each selected tree at the breast height, 5 cm from each other, with a Trepfor tool (Rossi *et al.* 2006). After sampling, the microcores were placed in a solution of 1:1 ethanol -water in Eppendorf microtubes.

Microcores were dehydrated in ethanol of various concentrations immersed in RotiClear and paraffin (Rossi *et al.*, 2006) by using STP 120 Microm tissue processor. Subsequently, the samples mounted in paraffin blocks were processed with Microm HM335 microtome in order to obtain sections of 7-8 µm thickness. To highlight the tissues and different phases of formation of the annual tree ring, the microsections were immersed in ROTICLEAR (for paraffin removal), dehydrated (in ethanol) and stained with 0.32% solution of Cresyl Violet acetate. The analysis of microsections was performed with an AxioImager.A1m Zeiss microscope in a polarized visible light at an x50-400 magnification, identifying the cells in different stages of development.

The analysis of the growing xylem was made on three radial rows, and the cambial and postcambial areas (cell differentiation), lignification (thickening of cell walls) and mature cells were assessed (Rossi *et al.*, 2006a; Cufar *et al.*, 2008; Prislán *et al.*, 2011). The cross-sectional analysis included some quantitative (number of cambial cells) and qualitative observations (day of the year - DOY) on the onset and cessation phases of cambial cell growth, cell differentiation, lignification and mature cells. The cells from the cambial area (CC) were identified by the flat shape with thin cell walls. The xylem elements (vessels, fibers, radial and axial parenchyma) found in the stage of differentiation or postcambial stage (PC) present a double size and thinner cell walls (Cufar *et al.*, 2008) in comparison to CC. The observation in polarized light allows differentiation of cells found in secondary wall deposition phase (SW) characterized by the thickening and cell wall glow. The lignification process is considered complete when the wall of mature cells (MT) is fully colored in blue as opposed to the CC, PC and SW phases, when it is colored in purple.

Results

Cambium. Mature xylem formation is a process which takes place in three successive phases during the growing season: procambial and cambial cell activity, development of parenchymal cells and vessels, thickening of cell walls, respectively completely lignified cells and vessels (Cufar *et al.*, 2008). All stages are separated in space or time, although they partially overlap. A detailed analysis of the cells in the

developing ring enables the highlighting of some cell types derived from the meristematic procambium cell division. The new cells derived from the procambium represent more rows of cambial cells of different size and layout (Larson, 1994). During the dormant season, the cambial cells are made up of 3-5 rows of cells. During the radial growth (when the cell division activity is intense) the cambial cell layer reaches about 7-8 rows of cells with thinner cell walls (Fig. 1).

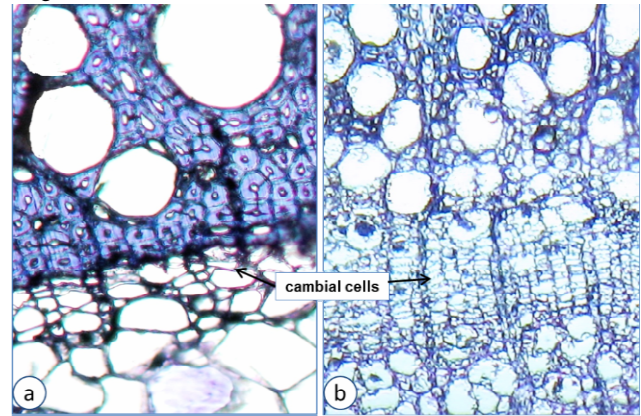


Fig. 1. Cambial zone in dormant season (a - April) and growing season (b - June)

In 2009, an early cambial activity was recorded, as the initially fusiform cells began to divide in early May (DOY 126), thus generating new cells (up to 6-7 cambial cells). In 2010, the cambial cells began to grow in length and diameter about a week later (DOY 131). Compared to the previous years, in 2011 an early cambial division was noticed in the last week of April (DOY 101). The maximum cambial activity was recorded in the second half of June, the differences between years being of one week maximum. Later, the cambial activity decreased and the cell division process ended in the last half of July (DOY 209 in 2010 and 191 in 2011). The duration of the cambial cell production processes ranged from 71 days in 2009 to 90 days in 2011.

Tree-ring formation. The cells derived from cambium expand themselves generating new cells, with high lumen and thin cell walls, thus marking the onset of xylem differentiation. Newly differentiated cells of different shape and size represent the postcambial area. Next to them, parenchymal cells develop, representing wood parenchyma cell clusters and forming a large part of the xylem. Medullary rays are derived from the parenchymal cells, consisting of several rows of cells showing different sizes and shapes.

On May 15, 2009 (DOY 135) the first wooden developing vessels were distinguished, with larger sizes, with thin cell walls, found in the postcambial area, the cell differentiation occurring in the initial phase. In 2010, the onset of postcambial activity took place over the same period (DOY 137) and, in 2011, it took place at the beginning of May (DOY 122) (Fig. 2). Generally, the first xylem elements appeared 1-2 weeks after the onset of the cambial activity. The duration of the cell differentiation process ranged from 40 to 80 days.

The onset of lignification occurred in late May-early June, when the first vessels undergoing lignification were observed in the polarized light; the yearly differences were of 7-14 days (DOY 143 in 2011 and 158 in 2010). Following the deposition of the lignin in the vessel walls, the side wall

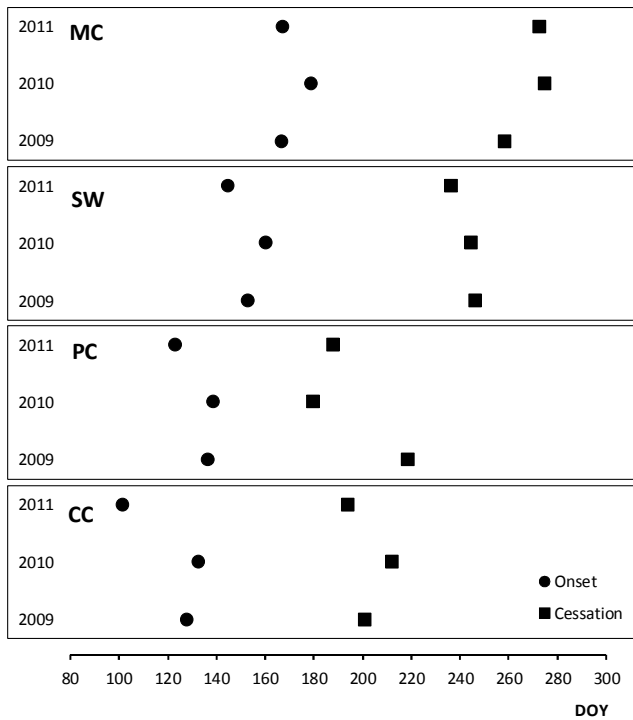


Fig. 2. Onset and cessation of tree ring formation phase in European beech (CC – cambial cells; PC – postcambial phase; SW – secondary wall deposition phase; MC – mature cells)

was formed and it displayed a blue color (Fig. 3). A crowded arrangement of the parenchymal cells in the storage phase of the lignin in the secondary cell wall was observed near the vessels and the wood fibers. The lignification process continued after the cessation of the cambial activity until mid-September (DOY 232-242). The duration of the lignification process was of 82-90 days.

In 2009, the appearance of the first fully lignified mature cells, was observed around June 12 (DOY 163) with a maximum at the end of June and the first week of July, when the number of cells with the walls fully formed (lignified) reached 25 (wood vessels). In 2010, the first mature vessels and the completely lignified parenchyma cells respectively were observed in mid-June (DOY 176), 9 days later than in 2011 (DOY 165). The time required for a vessel element to be fully formed (between early cell differentiation process and complete development) was 30-40 days.

The complete formation of the annual tree ring was noticed on 11/09/2009 (DOY 254), when the number of cambial cells was reduced to 3-4 cells and all anatomical elements were mature. The annual ring formation process was considered complete in September (DOY 270 in 2010 and 268 in 2011). However in 2010, the microscopic analyses revealed the presence of a few axial parenchyma cells still in the process of lignification at the end of September, a similar situation being noticed in 2011. The duration of the mature xylem development varied between 93 and 103 days. The average length of the annual ring formation process considered between the onset of the cambial division and the cessation of the lignification process ranged between 127 days in 2009 and 137 days in 2011.

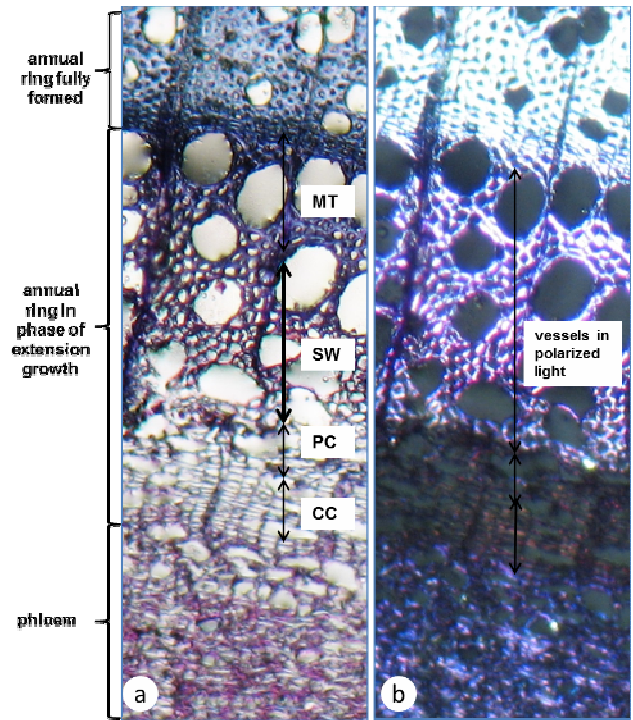


Fig. 3. Process of ring formation in European beech: a) different developing phases; b) differentiation between cell enlargement phase and thickening of the cell wall phase (12.06.2011)

Discussion and conclusions

The formation of the annual rings is under genetic control and is influenced by environmental factors (Kozłowski and Pallardy, 1997). The differentiation process of the vessels, i.e. of the main anatomical parts of the annual rings in hardwood species, may be separated in three successive phases: (1) differentiation and postcambial widening which determine the size and shape of the cell, (2) the formation of the secondary wall by the deposition of lignin on a matrix of polysaccharides, and (3) the cell death and protoplasm autolysis (Rossi *et al.* 2006; Gricar *et al.*, 2005).

In the investigated area, the onset of the cambial division occurred between the last week of April and early May. Compared to other studies carried out under similar conditions, there is a delay of about 1-2 weeks (Cufar *et al.*, 2006; Vavrcik *et al.*, 2013; Prislán *et al.*, 2013). The maximum production rate of xylem cells (the maximum number of cambial cells) took place between 31st of May and 21st of June, similarly to the results obtained in studies carried out in beech forests in Slovenia (Prislán *et al.*, 2011) and the Czech Republic (Vavrcik *et al.*, 2013). The summer solstice (June 21) represents the day with a maximum photoperiod and coincides with the maximum cell production in softwoods (Rossi *et al.*, 2006a). The present research indicates a gap of about 1-2 weeks between the maximum cell production and the summer solstice, similar to other studies (Marion *et al.*, 2007; Cufar *et al.*, 2008). In their studies, Cufar *et al.* (2008a) found that about 76% of the annual ring was formed by the end of June, whilst, in

similar studies in Czech Republic, the percentage reached 65% (Vavrcik *et al.*, 2013). The results showed that June had the highest importance in the radial growth, an observation that has also been confirmed by other studies (Bouriaud *et al.*, 2004; Michelot *et al.*, 2012; Oladi *et al.*, 2011). However, the maturation of cells (complete lignification process) continues even after the cessation of the cambial division (Gricar *et al.*, 2005; De Luis *et al.*, 2007).

A significant statistical relationship was observed between the leaf phenophases and the phases of annual ring formation, especially among the unfolding process and the activation of cambium (Cufar *et al.*, 2008; Michelot *et al.*, 2012; Prislán *et al.*, 2013).

The phenology of the annual ring formation varies from year to year, in direct correlation with the variation of the environmental factors. The development of some statistical models regarding the relationship between climate and bioaccumulation processes (measured by the annual tree ring) requires long-term series and the corroboration of the information derived from dendroclimatological studies with those obtained through xylological analyses (Rossi *et al.*, 2008). The investigations of the annual ring formation at the cellular level could provide essential information that might explain the statistical links between the annual ring parameters and the dynamics of environmental factors.

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