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Phenology of wood formation in different coniferous species from Obcina Feredeului, Romania

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Abstract. The dynamics of the cambial activity and the formation of the xylem of Norway Spruce *(Picea abies)*, Silver fir (*Abies alba)*, European larch *(Larix decidua)* and Scots pine *(Pinus sylvestris)* from Obcina Feredeului region (experimental plot Deia), were studied during the 2013 growing season. The tree ring formation phases have been analyzed using wood microcores, collected weekly. The number of cells found in the cambial phase, cells enlargement, cell wall thickening and mature cells have been analyzed and discussed in terms of onset, cessation and duration of development. The onset of the cambial activity in spruce occurred around April 5th, while the most growth through a number of cambial cells occurred around June 28th. The onset of the cambial cell division in fir and larch occurred during the third week of March. In the case of Scots pine, the cambial activity began around March 15th, while at the end of April the cambial cells have reached their maximum number of cells. The research findings have confirmed the varied dynamics of the tree ring formation stages among species.
Key Words: Cambial activity, cell wall thickening, lignification, xylem growth ring.

Introduction. The xylological monitoring of the radial growth processes is a complex endeavour that enables the identification of both the tree ring formation period and the correlation with the environmental factors. Knowing the variation of the radial growth in various species is of particular scientific interest.

Various coniferous species in Europe have been acknowledged as an important source for identifying the factors determining radial growth. Numerous species have been analyzed in order to establish their sensitivity to the climate variations (Lebourgeois et al 2010; Gutiérrez 1991; Bonaimé et al 2008; Bigler et al 2006).

The research studies based on a comparative inter-species analysis of the tree ring formation stages have confirmed important findings concerning the xylem formation. (Deslauriers et al 2008; Rossi et al 2006a).

The onset and the duration of various development phases were analyzed for fir (*Abies balsamea*), in the Quebec-Canada boreal forest, and differences have been identified upon each stage of the tree ring formation, both in terms of experiment sites and years as well (Deslauriers et al 2003). The research conducted on Scots pine in Russia has confirmed that the temperature has had a clear impact on the development of the tracheids (Zabuga & Zabuga 1990).

The high temperatures in spring induce an early debut of the cambial activity and the increasing number of cells with impact on the formation of the xylem (Deslauries et al 2008). According to Körner & Paulsen (2004) high radial growth has occurred as a result of the high temperatures in the summer. The differences identified at the beginning and at the end of the tracheid development are mostly due to the climate variability (Vaganov et al 2006). The period of the year and the duration of the tree ring formation highly depend on the geographical area, on the climate and also on the physiology of the species (Michelot et al 2012). The objective of our case study is to analyze the intra-annual variation of the radial growth processes in spruce, fir tree, larch and Scots pine from Obcina Feredeului region.

Material and Method. In order to analyze the growth processes, we have sampled 1-2 mm thick and 1-2 cm long micro-cores. These growth samples encompass both the tree ring area under formation but also 3-4 previously formed tree rings. Five trees of Spruce, Silver fir, European larch and similar five of Scots pine were chosen to take weekly samples from April to October 2013. After the sampling itself, the recently extracted micro cores were put into Eppendorf tubes, in a 1:1 mixture of ethyl alcohol and water (50% water and 50% alcohol), thus avoiding the dehydration of the xylem. We have cut fine 7-10 micron thick micro sections by means of the semi-automatic microtome. The samples were stained with a 0.32% solution of cresyl violet acetate. After consecutively going through the lab procedures, we have conducted the measurements under a microscope. The microscopic observations refer to counting of cell number in different phonological phases: the cambial area, the cells under development, the cells undergoing lignification and the cells that had reached maturity (Rossi et al 2006a,b).

Results

Dynamics of cambial activity. With each of the species under analysis, the cambium exhibited a different dynamics, both in terms of the number of cells and as concerns the intensity of the cambial division. In spruce, the first samples have shown the onset of the cambial activity, around the 5th of April, while the most intense growth through cambial cells occurred around June 28th, 2013. The onset of the cambial period in silver fir occurred around April 19th. The cessation of the cambial period occurred sooner in fir (around September 3rd) as compared to spruce (30.09.2013). In the case of Scots pine, the cambium activity started around March 21st, while at the end of April, the cambial cells have already reached their maximum number of cells. The cessation of the cambial period in larch occurred around March 19th, while during the third week of June, the number of cells in the cambial area had increased up to 10 cells. The cessation of the cambial activity occurred at the end of September, both for Scots pine and larch.

Dynamics of cell differentiation and maturation. The onset of the radial increment in spruce occurred around May 4th, the time when the first cells have been observed in the enlargement phase. The developing cells in spruce have reached their maximum around June 21st, while the number of enlarged cells has increased to 6-7 cells. The emergence of the last enlarging cells occurred at the end of July. The onset of the enlarging cells in fir on May 5th and larch occurred on May 8th. The start of the cell expansion occurring on May 4th in the case of Scots pine. The maximum number of expanding cells in fir, Scots pine and larch was reached around May 10th. The last enlarging cells in fir, Scots pine and larch were identified around July 13th, as in the case of spruce.

The first cell in lignification stage in spruce can be identified around May 28th, while the maximum number of cells I this phase was reached during the first week of July. The onset of cell lignification with fir and Scots pine occurred around May 24th, while the maximum number of cells was reached during the second week of June with both species. Note the cessation of cell lignification occurring about one week earlier in Scots pine, on September 9th, as compared September 16th in the case of fir. The onset of cell lignification occurred around May 24th in larch, in which case the maximum number of cells was reached during the third week of June. The cessation of the lignification process in spruce (September 2nd) and larch (August 26th) occurred much earlier as compared to the other species (Figure 1).

The first fully lignified cells in spruce can be identified around June 19th. The highest increase through a number of mature cells in spruce reached its maximum point around September 30th.

In the case of Scots pine, the first cells with lignified walls were identified around June 19th. Unlike Scots pine, the first samples in larch have already shown the presence of the mature cells on June 16th. The last cells undergoing lignification were identified in the last week of August, with larch. Moreover, there is a one week difference between fir, spruce and Scots pine, in terms of the last cell lignification. Entirely lignified cells with the species under analysis have been identified at the end of September.

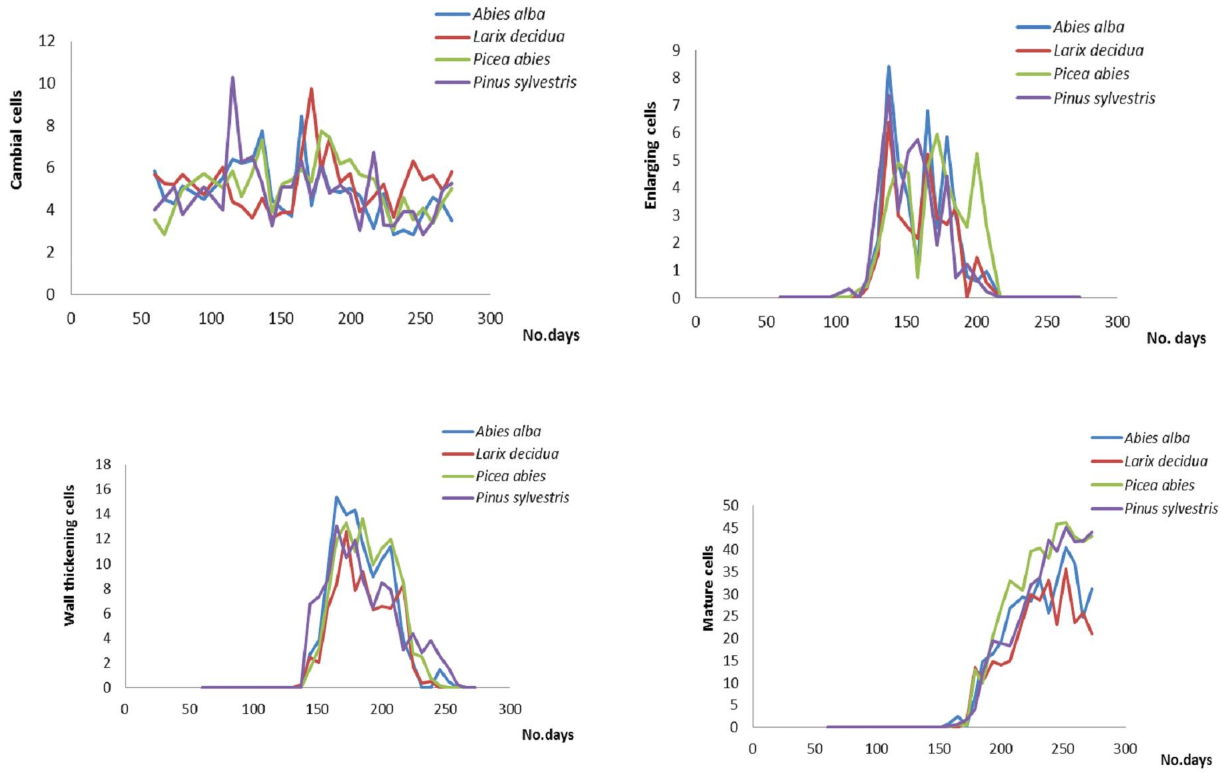

Figure 1. Intra-annual dynamics of radial growth processes.

Discussion. Similar studies identified an important variability on the onset and the cessation of the radial growth period between vegetation seasons in fir (Deslauries et al 2003; Deslauriers & Morin 2005; Gricar et al 2009). Moreover, an approximately 14 day delay had been identified with every stage of the tree ring formation, both among experiment sites and depending on each year. The onset of the cells undergoing changes, measured across three experiment sites, occurred during May 7th - June 7th (Deslauriers et al 2003). The monitoring of the beginning of the vegetation period for four years (Zabuga & Zabuga 1990) has revealed a variation between the first 10 days of May and the second part of June in the case of pine, when the temperature has clearly influenced the beginning of the tracheid development.

Temperatures of approximately 25°C, recorded at the beginning of May in the Canadian boreal forest (Quebec), have triggered the early start of the cell development. The xylem cells were formed in less than two months (end of May, until the middle of July), while the month of June proved to be the most important for the development of the annual ring (Deslauriers et al 2003). One hypothesis concerning the short period during which the annual ring was formed can be confirmed by the ability to adapt to the cold climate, when cells have a quick radial development, followed by a quick start in terms of cell wall thickening. An analysis of this situation has revealed that the lignification ended before the frost, thus signalling the end of the vegetation season (Deslauriers et al 2003). As far as our research is concerned, the tree ring was formed in about 3 months in spruce, fir and larch, while the radial growth lasted for about 4 months in the case of Scots pine. The high temperatures in spring reveal an answer concerning the early beginning of the cambial activity and the increased number of cells that play an important part in the xylem formation (Deslauriers et al 2008). In a different paper, the findings have revealed high increments, as a result of the high temperatures in summer, while at lower altitudes, a slower growth in coniferous species was identified (Körner & Paulsen 2004). The wood formation period for Scots pine amounted to 7 weeks in the southern site, and six weeks in the northern part of Finland, where the wood formation intensity is identified during the middle of the vegetation period, decreasing towards the end and until the first part of August, when the cell wall thickens (Schmitt et al 2004). The inter species radial growth differences are found not only in the synchronised onset and cessation of the cell division, but also in the seasonal growth dynamics (Ladefoged 1952).

Conclusions. A comparative analysis of the radial growth in the Deia experiment site has revealed variations in the onset and cessation of the tree ring formation period among different species. Thus, in the case of fir, the dynamics of the cambial activity has confirmed a late start as compared to the other species. In the case of Scots pine, the cambial division was more accelerated, as revealed by the analysis of the maximum number of cells reached in April. Moreover, we have also noted an early start in cell expansion, as compared to fir and larch.

A research study conducted on fir, spruce and Scots pine in a temperate climate region has shown differences in the radial growth of the three species, under climate change conditions. The decisive months for the tree ring formations are June and July, under optimum rainfall conditions. In the case of spruce, the tree ring width depends on the temperatures in September of the previous year and on the rainfall quantities in spring and summer. In Scots pine, the annual increments depend solely on the rainfall quantities in the summer (Lebourgeois et al 2010). The findings have confirmed a different dynamics in the tree ring formation both in terms of species and across different years as well. Moreover, after a qualitative and quantitative analysis of the xylem anatomical elements, there was evidence of a different reaction of each species to the climate changes throughout the vegetation season.

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