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Preliminary study on phloemogenesis in Norway spruce: influence of age and selected environmental factors

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ABSTRACT: The process of phloem formation in Norway spruce (*Picea abies* [L.] Karst.) was analysed during the growing season 2009 in Rájec-Němčice locality (Czech Republic). The research series consisted of research plots with 34 and 105 years old spruce monocultures. The formation of phloem cells was determined by the examination of small increment cores taken once a week. Cross-sections of tissues were studied under a light microscope. Cambium activation was observed on 9 April both in young and old trees. On the same date the first newly formed cells of early phloem were observed in old trees but in young trees one week later. Although the time of early phloem formation was 14 days longer in old trees, there were no large differences in the numbers of formed cells. The beginning of the longitudinal axial parenchyma formation was determined in young trees on May 14. In old trees this activity was seen a week later. The influence of air temperature and soil moisture was also analysed in relation to phloemogenesis.

Keywords: cambium; environmental factors; influence of age; light microscopy; Norway spruce (*Picea abies*); phloem formation

The growth of multicellular organisms is seen as the increase in an individual's volume conditioned by the creation of new cells. This leads to an irreversible process of the expansion of plant dimensions. Secondary growth is referred to as radial growth and is conditioned by cambial activity (Larson 1994; Procházka et al. 1998). The cambium is a secondary meristem, which divides the phloem cells centrifugally and xylem cells centripetally (Zimmermann, Brown 1971; Larson 1994). Cambial activity and secondary growth in temperate regions are periodic, alternating periods of growth with winter inactivity (Fujii et al. 1999). The number of phloem mother cells produced during the season is much smaller than that of those produced on the xylem side (Zimmermann, Brown 1971). During periods of active cambial growth each xylem mother cell divides to form two daughter cells, which in turn divide once, resulting in the formation of a set of four cells, all of which eventually mature into xylem cells. Phloem mother cells are produced on the phloem side of the cambium, and these divide only once to form a pair of cells (Murmanis 1970; Isebrands, Larson 1973). This partially explains why less bark than wood is always formed (Panshin, de Zeeuw 1980). However, from the physiological point of view, the development of new phloem cells is as important as the development of xylem cells, maybe even more important. The phloem is a tissue specialized in the translocation of assimilates which are essential for the nutrition of heterotrophic, non-photosynthesizing parts of plant and also for the storage materials (Evert 2006). The phloem consists of parenchyma cells and sieve-tube cells (Panshin, de Zeeuw 1980).

Both phloem and xylem cells are subjects of secondary growth, but there are considerable differences in the process of their development. The processes of xylogenesis are more affected by the changes in climatic conditions; therefore, the structure of a tree ring represents a kind of climatic data archives. This serves as a basis for the field of dendroclimatology (Schweingruber 1990; Kaennel,

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Schweingruber 1995). The development of phloem is probably more affected by endogenous factors than the development of xylem (Gričar, Čufar 2008). A possible cause can be the fact that only the cells which developed in the current vegetation period are functional in the phloem, the older ones are compressed and thus non-functional as a consequence of the secondary growth of the stem (Panshin, de Zeeuw 1980). That is why the creation of new phloem cells is vital for the tree as otherwise the entire individual would die. This is in contrast to xylem, where even the cells developed in previous periods perform their physiological functions, although most of them are dead. This leads to the fact that the variability of xylem cell formation in the last tree ring does not affect the function of xylem tissues as a whole considerably.

The conducting phloem of *Pinaceae* consists of living, mature sieve cells and various types of parenchyma cells. Within each growth increment, the phloem parenchyma strands are arranged in a more or less conspicuously interrupted tangential band, usually one or two cells in radial direction, as seen in transverse section. The portion of each growth increment external to the parenchyma strands has been designated early phloem, and the remainder of the growth increment late phloem (Alfieri, Evert 1973).

The variability of the anatomical structure and phloem and xylem increment widths in relation to growth conditions was described in experiments when changes in these characteristics of Norway spruce (*Picea abies* [L.] Karst.) were observed in controlled conditions. Experiments with the controlled warming and cooling of a part of the Norway spruce stem proved that the phloem increment contains more cells of late phloem if the cambial activity finishes later (Gričar 2007). This research demonstrated that the rate of phloem cell formation was stable regardless whether the cambium was cooled, warmed up or unaffected. On the xylem side, the temperature had a significant influence on the cell production in the initial stage of the vegetation period, whereas the other factors, which were not considered, exerted probably the main effect during the formation of late xylem (Gričar 2007). This means that the formation of phloem cells in the stem of Norway spruce is very homogeneous and does not manifest any significant deviations of growth. Therefore, it is useful to describe the dynamics of phloem formation in quite a detail, at the cell level. To verify this hypothesis, the objective of our research was to analyse the impact of external factors on the formation and development of phloem cells. Further, the influence of an internal factor – age – on the phloem cell formation was explored.

MATERIAL AND METHODS

The samples of phloem were taken from stems of Norway spruce (*Picea abies* [L.] Karst.) at a field research station of the Department of Forest Ecology, Mendel University in Brno. The plots were located about 30 km to the north of Brno (coordinates 49°29'31"N, 16°43'30"E). The research plot Rájec-Němčice is located in Natural Forest Area 30 Drahanská vrchovina. It is a considerably forested region (668 km^2 = over 40% of its total area) covering mainly the highlands Drahanská and Konická vrchovina as well as the Moravian Karst of Devonian origin and a part of the Adamovská vrchovina highland. As regards the type of relief, this is a part of broken highlands of folded and faulted structures and intrusive igneous rocks of Czech highlands. The bedrock is intrusive igneous acid granodiorite of the Brno Massif (Klimo, Maršálek 1992); the soil is oligotrophic modal Cambisol with moder form of forest floor (Menšík et al. 2009; Fabiánek et al. 2009). The plots are at the altitude of 600–660 m a.s.l. and the climate is temperate (Quitt 1971). As regards climatic parameters, the average annual air temperature is 6.5°C and the average annual precipitation is 717 mm (Hadaš 2002). The forest type is *Abieto-Fagetum mesotrophicum* with *Oxalis acetosella* (5S1) (Plíva 1987).

Our study was conducted on two adjacent research plots (spruce monocultures) differing in age. The first stand (of the second generation after mixed forest) was 34 years old, the second one (of the first generation after mixed forest) was 105 years old. These two stands were used for sampling for the purposes of our research. The basic dendrometric characteristics of both stands, characterized by average values, are presented in Table 1.

From each stand six trees with values close to the mean tree of the stand were chosen. Samples in the form of microcores were taken in regular weekly intervals. The microcores (cylinders of 1.8 mm in diameter and 1.5 cm in length) were taken by means of a specialized increment borer – Trephor (Rossi

Table 1. The basic dendrometric characteristics

	Breast-height diameter (cm)	Tree height (m)	Crown base height (m)
Young stand	22.5	17.6	8.0
Old stand	36.9	35.3	20.1

et al. 2006). Sampling was conducted at the height of 1.3 m ± 20 cm. It means the first sample was taken at 1.10 m (the space division was calculated with the consideration of the number of planned samplings and their layout). Individual samplings continued upwards, always in the angle of 20–30 degrees from the previous one, 2 cm far from each other. The resulting shape of sampling spots was a spiral around the stem.

Samples, put separately in histological cassettes, were immersed into FAA (formaldehyde-acetic acid-ethanol) fixative solution for a week. For longer storage, the samples were immersed into the solution of 96% ethanol and distilled water at the ratio of 30:70. Before further processing, redundant wood and bark were cut off, and then the samples went through an alcohol series consisting of ethanol of various concentrations and xylene. The reason for this step is the preparation for the stage when the samples are impregnated in paraffin so that they could be cut using the rotation microtome. Paraffin is not soluble in water, therefore the samples are dehydrated by ethanol. Then the ethanol has to be displaced by xylene which is mixable with paraffin. The samples are left in paraffin for four hours at least. Times of the soaking of microcores before paraffin impregnation were 1.5 h for (ethanol 70%, ethanol 90%, ethanol 95%, ethanol 100% and xylene).

The samples were put in metal moulds, with the cross-section (the darker part) towards the bottom, and the moulds were filled by means of paraffin dispenser (Leica EG 1120). When it cooled down, the paraffin block was taken out of the mould and cut using the rotation microtome (Leica RM 2235) so that a part of the microcore was uncovered. The microcores were then immersed into water overnight for repeated hydration so that they could be cut more easily on the microtome. Subsequently, microsections of 12 μm in thickness were produced using the rotation microtome; they were laid on water surface (40°C). This straightened the microsections, which could be then taken out and mounted on glass slides with glue (egg white and glycerine). The slides with specimens were dried for 5 minutes at the temperature of 60°C and then dried completely in the air. Further, the specimens went through another alcohol series, this time connected with staining. To highlight the non-lignified parts, Astra Blue stain was used and to highlight lignified parts safranin was used. To achieve the better colour of lignified cell walls safranin was used at first separately and then in a solution with Astra Blue. Times of microsection soaking before closing the specimen were 10 min for (ethanol 96%, xylene), 2^{+} h for (safranin), and 5 min for (safranin + Astra Blue).

The specimens were closed with Canadian balsam and a cover slip. Cover slips of the resulting microscopic specimens were loaded down with rubber plugs for 14 days.

The finished microscopic specimens were used to analyse the process of new cell development and their gradual differentiation. In each specimen three radial series of cells in the phloem part were selected and the number of the cells contained was counted. The presence of the following types of phloem cells was examined: early phloem, longitudinal parenchyma and late phloem.

Subsequent evaluation and further processing of results consisted of these steps: description of the phloem formation in relation to temperatures; definition of the trend of phloem cell growth; and the investigation of specific correlations between cell growth and meteorological data.

Moreover, the difference between the individuals from the young and the old stands was studied so that the influence of age could be analyzed.

Biotic environmental factors are measured on these plots regularly, and temperature and humidity aspects are measured every day. To analyse the impact of external environmental factors on the formation of phloem cells we used meteorological measurements of the Department of Forest Ecology. The following factors were measured continually: air temperature at 2 m above the ground, measured in hourly intervals, and soil moisture measured by means of soil moisture sensors CS 616 (Campbell Scientific, USA) at the depth of 30 cm, in hourly intervals (Rhoades et al. 1989). Soil temperature was measured at the depth of 10 cm and 30 cm, in hourly intervals by means of Pt100/8 soil temperature sensors (EMS Brno, Czech Republic).

For each day on which the samples for phloemogenesis analysis were taken (in weekly intervals) the average values of climatic data were established. These values were matched with the found number of cells and the relationship between them was examined by means of non-linear regression. Michailov's growth function was used (Korf et al. 1972).

RESULTS AND DISCUSSION

The starting cambial activity in specimens is manifested by radial expansion of cells, slight narrowing of their tangential cell walls and sometimes by a well visible cell content. After a few days, their number rises

and then the first phloem and xylem cells are differentiated. The interval between the cambium activation and the first phloem tissue cell differentiation varies in different trees. We assign the beginning of cambial activity to the date of April 9 (–7 days +0 days, as samples were taken in regular weekly intervals – it applies to all dates mentioned). The growth of the number of cells was visible in some trees on that very day, however, in others only a week later, i.e. on April 16. In the week before April 9 the temperature did not fall below 4.9°C and in the week before April 16 the temperature did not fall below 6.2°C.

In all trees early phloem cells were found out before the beginning of cambial activity. These cells were formed at the end of the previous vegetation period. As regards the newly formed early phloem cells (EP), there were distinct differences between young and old trees. Whereas in young trees new early phloem cells started to appear on April 16, in old trees they were present on April 9.

The end of early phloem formation is characterized by the beginning of longitudinal axial parenchyma (AP) formation. It was observed on May 14 in young trees. In the week before minimum and maximum temperature was 5.3°C and 23.2°C, respectively. In old trees this activity was seen a week later, after a week with minimum and maximum temperatures being 6.5°C and 20.7°C, respectively. Although the time of EP formation was 14 days longer in old trees, there were no large differences in the numbers of formed cells. On average, there were four cells in the radial direction, both in old and young trees.

After one cell of axial parenchyma on average was formed, the formation of late phloem started. In young trees it was first observed on May 14, in old ones on May 21. This means the late phloem started forming very soon after the first cells of axial parenchyma and its production continued till the second half of September, both in young and old trees. On average, 3.3 cells were formed in young trees and 3.6 in old trees. The graph of the process is shown in Figs. 1–3.

When assessing the growth trend, at first the growth of early phloem, axial parenchyma and late phloem were evaluated separately, and then as a whole. Fig. 1 shows that in young trees the formation of new EP cells starts approximately a week later and ends approximately a week earlier than in old trees. However, the increase in the number of cells is much faster and as a result the numbers of cells in this type of tissue are the same on both plots. When AP is formed, the rate of the increase gets balanced. As regards LP, there is a faster decrease in the formation of new cells in young trees. Although the period when LP is formed is longer in young trees (the formation starts sooner), the decrease in the formation of these cells leads to the fact that the resulting number of cells is lower than in old trees.

The curve of the total number of newly formed cells (without distinguishing between axial parenchyma and phloem) is S-shaped for old trees, but not for young trees (Fig. 4) as the growth is faster at the beginning of the vegetation period and then the growth stagnation comes earlier, at around mid-July.

The cell growth is more affected by the temperature of several preceding days, not only by the temperature around the sampling day. Therefore, we did not take into account the average temperature of the previous day only, but also the average temperature of the previous week and the average temperature of the preceding three days.

When the relation between soil moisture and the number of cells was studied, no dependences were confirmed. On the other hand, when investigating the relationship between air temperature and the number of cells, we found the following dependences, where the values show the determination coefficients for Michailov's growth function.

The table shows a positive relationship between air temperature and the formation of cells.

At the beginning of the examined vegetation period, before the start of cambial activity, we found out that 1–2 early phloem cells remained from the

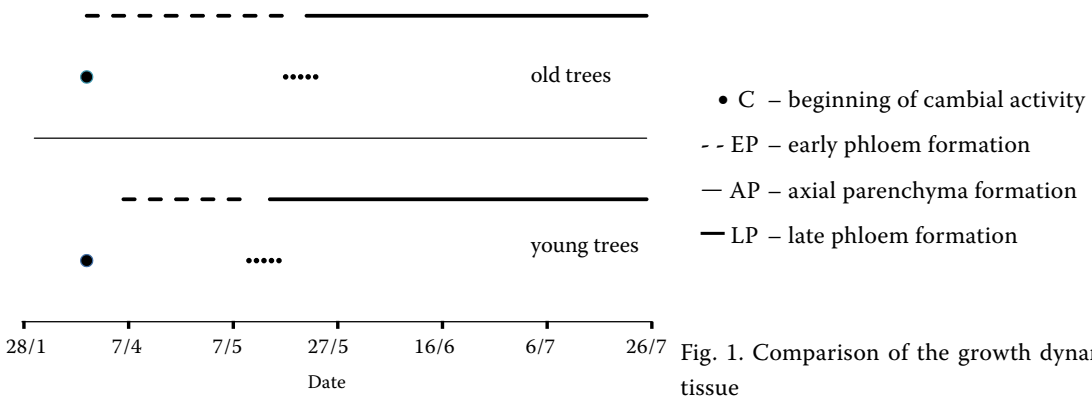

Fig. 1. Comparison of the growth dynamics of phloem tissue

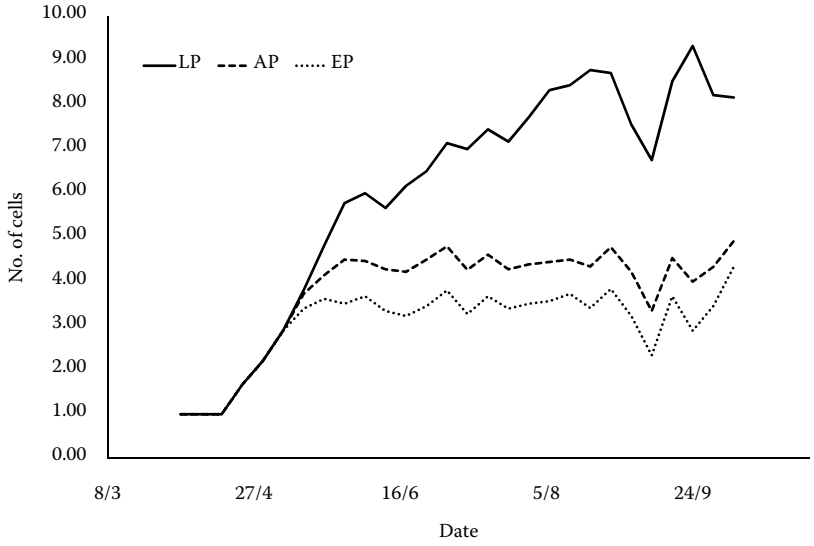

Fig. 2 The growth of phloem tissue in young trees.
EP – early phloem formation,
AP – axial parenchyma formation,
LP – late phloem formation

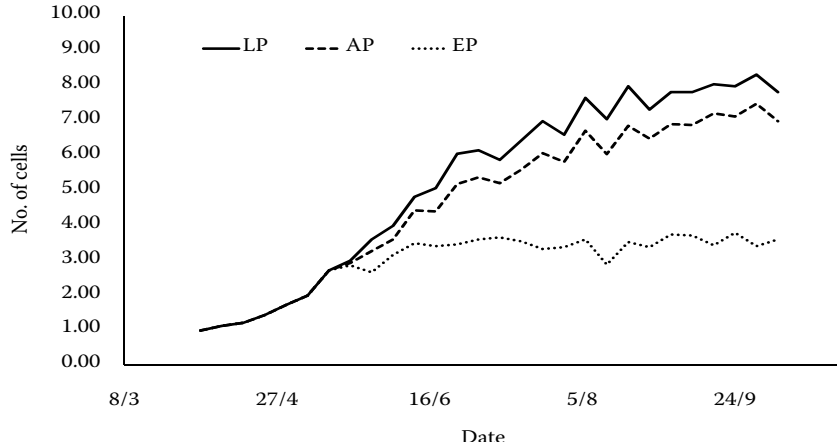

Fig. 3. The growth of phloem tissue in old trees
EP – early phloem formation,
AP – axial parenchyma formation,
LP – late phloem formation

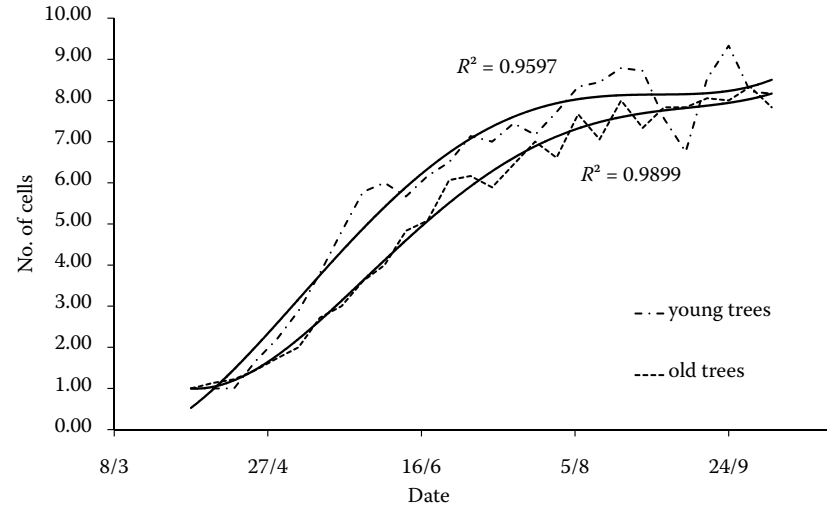

Fig. 4. The overall growth trend of young and old trees with the determination coefficients of fitted curves

previous vegetation period. This is in agreement with the results of research conducted in Slovenia (Gričar 2007). One of the Slovenian research plots was located in an area where spruce is not autochthonous – Pokljuka; the second research plot was in an area where spruce grows naturally – Sorško polje. The dendrometric parameters of the trees from both these plots correspond to the values of the older plot of Rájec-Němčice research site. That is why we compared the results of Gričar (2007) with the results from our older plot only.

Gričar (2007) stated that the number of cells in the cambial zone doubled at the very beginning of the vegetation period, which was considered the beginning of cambial activity. The trees in the Czech Republic manifested this increase only in the second

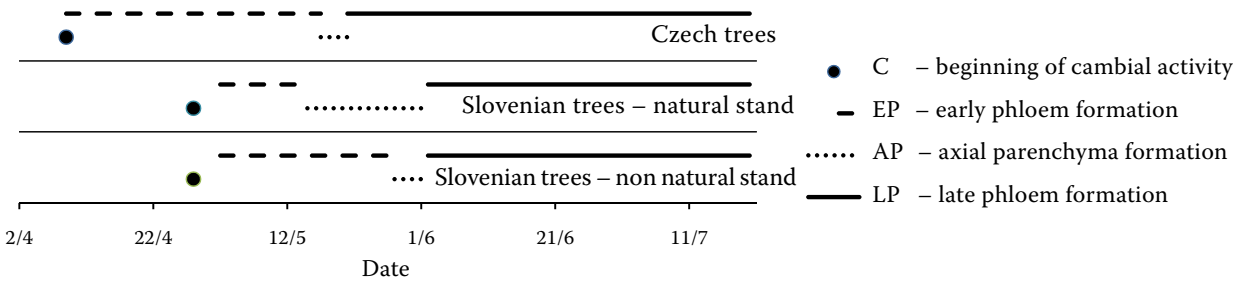

Fig. 5. Comparison of the growth dynamics of phloem tissue on Slovenian plots (Gričar 2007) and on the Czech older plot

part of the vegetation period (around mid-May); therefore, it could not be considered as the beginning of cambial activity. We can conclude that the increase in the number of cambial zone cells is highly variable. Because the transition of cambium from the dormant state to the active one is a matter of several days (Wilson 1964), it is very difficult to identify this moment; especially due to the fact that the periodical sampling was performed in weekly intervals.

In our research, the start of the formation of new early phloem cells was observed at the beginning of April. In Slovenia, new cells started to appear in the first week of May on both plots (Gričar 2007). What was similar to our findings – it happened very soon after the cambial activity was observed.

Axial parenchyma started to appear in Czech trees in the second half of April as well as the first cells of late phloem. In Slovenian trees axial parenchyma was observed to start appearing in mid-May on the plot with autochthonous spruce, and at the end of May on the plot where spruce is not autochthonous. Late parenchyma started to appear in Slovenian trees of both plots as late as at the beginning of June (Gričar 2007).

In spite of large differences in the growth of phloem cells in Czech and Slovenian spruce trees, the number of cells in the particular types of tissue is the same for all three plots. The described differences are presented in Fig. 5.

We can assume that the difference in the growth dynamics of young and old trees is caused by the thickness of bark which has a heat-insulating function. Young trees have a thinner bark, that is why living tissues respond more to the fluctuation of external conditions. A possible cause of the faster growth of phloem cells may be the smaller number of formed cells within the entire stem.

CONCLUSION

When assessing the influence of three selected factors (average daily temperature and soil moisture as external factors, and age as an internal factor) on the formation of cells, some differences were observed. No correlation was found for the relation with soil moisture at the depth of 30 cm. We found a medium up to strong correlation for the relation with average air temperature at 2 m above the ground. As regards the factor of age, the final numbers of cells of particular tissues (EP, AP, LP) did not differ considerably, however, there were differences of weeks in the timing of the formation of the tissues. It means that the factor which describes the variability of phloem tissue growth when comparing young and old trees is not the number of cells but the timing of their formation. When the results obtained in the old stand were compared with the results of other authors, it was found out again that the differences in timing were more significant than the differences in the number of formed cells.

To conclude, the number of cells is not the only important aspect for the description of the phloem cell formation. The timing of their formation, it means the time when the transport of assimilates can start or when storage materials start to arise in the phloem in the form of axial parenchyma, is of the same or even higher importance.

References

Alfieri F.J., Evert R.F. (1973): Structure and seasonal development of the secondary phloem in the Pinaceae. Botanical Gazette, ***1***: 17.

Evert R.F. (2006): Esau's Plant Anatomy, Meristems, Cells, and Tissues of the Plant Body: Their Structure, Function, and Development. New Jersey, John Wiley & Sons: 601.

Fabiánek T., Menšík L., Tomášková I., Kulhavý J. (2009): Effects of spruce, beech and mixed commercial stand on humus conditions of forest soils. Journal of Forest Science, ***55***: 119–126.

Fujii T., Salang A., Fujiwara T. (1999): Growth periodicity in relation to the xylem development in three shorea spp. (Dipterocarpaceae) growing in Sarawak. In: Wimmer R., Vetter R. (eds): Tree-Ring Analysis. Biological,

Methodological and Environmental Aspects. Oxford, CAB Inernational: 169–183.

Gričar J. (2007): Xylo-and Phloemogenesis in Silver Fir (*Abies alba* Mill.) and Norway Spruce (*Picea abies* [L.] Karst.). Ljubljana, Slovenian Forestry Institute: 106.

Gričar J., Čufar K. (2008): Seasonal dynamics of phloem and xylem formation in silver fir and Norway spruce as affected by drought. Russian Journal of Plant Physiology, ***4***: 538–543.

Hadaš P. (2002): Temperature and precipitation conditions in the high elevation spruce stands of the Drahanska vrchovina upland. Ekológia, ***21***: 69–87.

Isebrands J., Larson P. (1973): Some observations on the cambial zone in cottonwood. International Association of Wood Anatomists Bulletin, ***3***: 3–11.

Kaennel M., Schweingruber F.H. (1995): Multilingual Glossary of Dendrochronology. Bern, Paul Haupt: 467.

Klimo E., Maršálek J. (1992): Manmade Spruce Ecosystem (Structure, Functions, Production, Processes). Brno, Agriculture University Brno: 177.

Korf V., Hubač K., Šmelko Š., Wolf J. (1972): Dendrometry. Praha, SZN: 376. (in Czech)

Larson P. (1994): The Vascular Cambium: Development and Structure. Berlin, Heidelberg, New York, Springer-Verlag: 725.

Menšík L., Fabiánek T., Tesař V., Kulhavý J. (2009): Humus conditions and stand characteristics of artificially established young stands in the process of the transformation of spruce monocultures. Journal of Forest Science, ***32***: 215–223.

Murmanis L. (1970): Locating the initial in the vascular cambium of *Pinus strobus* L. by electron microscopy. Wood Science and Technology, ***1***: 1–14.

Panshin A.J., De Zeeuw C. (1980): Textbook of Wood Technology. New York, McGraw-Hill: 722.

Plíva K. (1987): Typological Classification System "ÚHUL". Brandýs nad Labem, ÚHUL: 52. (in Czech)

Procházka S., Macháčková I., Krekule J., Šebánek J. (1998): Plant Physiology. Praha, Academia: 484. (in Czech)

Quitt E. (1971): Climatic Areas of Czechoslovakia. Brno, Geografický ústav ČSAV: 80. (in Czech)

Rhoades J.D., Manteghi N.A., Shouse P.J., Alves W.J. (1989): Soil electrical conductivity and soil salinity: new formulations and calibrations. Soil Science Society of America Journal, ***2***: 433.

Rossi S., Anfodillo T., Menardi R. (2006): Trephor: a new tool for sampling microcores from tree stems. IAWA Journal, ***1***: 89.

Schweingruber F.H. (1990): Mikroskopische Holzanatomie, Formenspektren mitteleuropäischer Stamm- und Zweighölzer zur Bestimmung von rezentem und subfossilem Material. Birmensdorf, Eidgenössische Anstalt für das forstliche Versuchswesen: 226.

Wilson B. (1964): A model for cell production by the cambium of conifers. In: Zimmermann M. (ed.): The Formation of Wood in Forest Trees. New York, Academic Press: 19–36.

Zimmermann M., Brown C. (1971): Trees: Structure and Function. New York, Springer-Verlag: 336.

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