XYLEM AND PHLOEM FORMATION IN SESSILE OAK
FROM SLOVENIA IN 2007

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ABSTRACT

We studied the timing of xylem and phloem formation, and the widths and structure of completed xylem and phloem increments in sessile oak (*Quercus sessiliflora* Salisb.) from Slovenia in the growing season of 2007. Weekly samples of inner phloem, cambium and xylem were analysed using light microscopy. Cambial reactivation and, consequently, xylem and phloem started before our first sampling at the end of March 2007. Weekly measured xylem and phloem increments revealed that phloem production was faster in the first part of the growing season (i.e., from the beginning of April until the third week of May), but thereafter xylem production accelerated. The period of most intense xylem cell production was assessed to be in the period April-May and that of phloem in April. Production of cells in the cambium stopped in the first half of August on xylem and phloem sides. The average width of the xylem increment of the investigated sessile oaks was 1837.55 ± 951.88 µm, phloem was 286.81 ± 232.04 µm and the ratio between phloem and xylem increments was 0.197 ± 0.118. Trees with wider xylem increments also had wider phloem increments, and vice versa. Lower ratios between phloem and xylem growth rings of 2007 were found in trees with wider increments, indicating that cambial cell production favours xylem (specifically late wood) formation in more productive trees. Inter-tree variability in xylem increment was relatively high (± 951.88 µm), whereas intra-tree variability was much lower (± 254.15 µm), but increased with the width of increment. The portion of late wood increased with the width of the xylem growth rings, whereby xylem increments narrower than 1000 µm consisted of more than 50 % of early wood but this portion gradually decreased to 30 % in wider rings.

KEY WORDS: sessile oak (*Quercus sessiliflora*), cambial activity, cell production, phloem, xylem, early wood, late wood, light microscop

INTRODUCTION

Quercus sessiliflora and *Quercus robur* grow in a great variety of regions in the Europe, also in Slovenia, and are thus economically and ecologically important tree species. This provides great potential for dendroclimatological and dendroecological studies (Akkemik et al. 2006, Werf et al.

2007, Čufar et al. 2008 a, b), since not only ring widths, but also intra-annual features, such as ring density and early wood vessel size, may be good climate proxies (Zhang 1997, Garcia-Gonzáles and Eckstein 2003, Fonti and Garcia-Gonzáles 2004, Fonti et al. 2007).

The impact of various climatic parameters on the growth of coniferous trees, with the emphasis on wood formation processes, has been intensively investigated in the recent years (Rossi et al. 2007, 2008, Seo et al. 2008). However, some published papers have also dealt with deciduous trees; e.g., diffuse-porous, such as beech (Schmitt et al. 2000, Werf et al. 2007), birch (Schmitt et al. 2004), London plane, horse chestnut and Norway maple growing in an urban environment (Marion et al. 2007), or ring-porous species, such as black locust (Schmitt et al. 2000), pedunculate oak (Werf et al. 2007), sessile oak (Akkemik et al. 2006) and beech (Čufar et al. 2008a).

In oak, a typical ring-porous species, it is possible to distinguish early and late wood anatomically. Early wood contains large vessels forming a clear ring in cross-section, whereas the diameter of the radially arranged pores is markedly reduced in late wood (Brazier and Franklin 1961, Panshin and de Zeeuw 1980, IAWA 1990). The width of early wood increases a little at first, but tends to be constant (about 1 mm) in xylem growth rings wider than 3 mm. Late wood width, on the other hand, increases almost linearly with increasing ring width (Zhang 1997).

With the exception of recent dendroclimatological studies on *Quercus* spp. in Slovenia (Čufar et al. 2008b), information on its wood and phloem formation dynamics is still lacking. In the current study, therefore, we compared the timing of xylem and phloem formation, and the widths and structure of the completed xylem and phloem increments of 2007 in sessile oak (*Quercus sessiliflora* Salisb.) from Slovenia.

MATERIAL AND METHODS

The experiment was performed in a forest site, Roznik, in Ljubljana (46°03'N, 14°28'E, 323 m a.s.l.), which belongs to the Blechno fagetum forest association. The predominant tree species in the site are *Fagus sylvatica* and *Quercus sessiliflora*. The privately owned forest is devoted to its natural development. Its main function is predominately recreational and social, so only basic forest management, such as sanitary felling, is performed.

The climate is humid continental. Based on the climate record from the nearby meteorological station of Ljubljana for the period 1900 - 2007, the mean annual temperature at the site is 9.8 °C, with January being the coolest (-1.0 °C) and July the warmest month (20.0 °C). The period May-September is well supplied with precipitation since 47 % of 1409 mm annual precipitation falls in this period.

We selected for the study five dominant, healthy sessile oaks (*Quercus sessiliflora* Salisb.) at the beginning of the 2007 vegetation period. The trees were about 150-years old with DBH of 55-80 cm and heights of 25-30 m. The selected trees grow on the southern slope of Rožnik (about 20°), in deep, slightly acidic, brown soils on sandstone. We carried out weekly sampling of blocks of intact tissue (10 x 10 x 30 mm) containing inner phloem, cambium and outer xylem from living trees, 1.3 m above the ground; from the end of April until the end of September 2007. The distance between neighbouring samples was at least 10 cm in a horizontal direction to avoid the influence of wounds on tissues of the next sampling locations (Gričar 2007). After removal from the trees, blocks of tissue were immediately fixed in FAA (formaldehyde-ethanol-acetic acid solution), dehydrated after one week in a graded series of ethanol (30 %, 50 % and 70 %), and embedded in paraffin (for detail see Rossi et al. 2006b). For light microscopy, permanent cross sections of 13 μm thickness

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were prepared on a Leica RM 2245 rotary microtome, using Leica 819 disposable blades, stained with safranin and astra blue and mounted in Euparal.

An Olympus BX51 light microscope and a Nikon NIS-Elements Basic Research v.2.3 image analysis system was used for anatomical observations. We counted the number of cambial cells along three radial files and averaged those. We additionally measured the width of the xylem and phloem increment formed from the onset of cambial growth to the time of sampling, along three radial files. The Gompertz function was applied to describe the seasonal dynamics of wood formation of the sampled trees in 2007.

RESULTS AND DISCUSSION

Cambial activity and xylem and phloem formation in 2007

When we started sampling, on 30 March 2007, cell divisions in the cambium had already started; the number of cambial cells had increased to 9-11 layers and the walls of cells were very thin (Figs. 1, 2a). We observed 1-2 layers of sieve tubes in the early phloem adjacent to the cambium at that time; however, we cannot say whether these cells were formed from overwintered cambial cells without previous cell divisions, which has been reported for various species (Alfieri and Evert 1968, 1973, Gričar et al. 2006), or after cambial reactivation. At the beginning of April, newly formed xylem (early wood vessels and fibres) and phloem cells (early phloem sieve tubes) adjacent to the cambium slowly began to expand (Fig. 2a). The development of early wood and early phloem cells occurred during April and May. The differentiation phases (i.e., post-cambial growth, secondary cell wall formation and lignification) of the vessels and ground tissue in the xylem could be followed by observation of cross-sections under a light microscope, while it was not feasible to study the development of sieve tubes, companion cells and parenchyma cells (having only thin, mainly cellulosic walls) in the phloem using only this technique. It has already been reported that oak sieve tube members are difficult to study in detail because they are usually crushed outside the narrow conducting zone and the delicate and extremely thin cell walls appear almost transparent in macerations (Howard 1977). In addition, sieve tubes in cross-section can be distinguished from parenchyma cells only if the sieve areas at the end are in view.
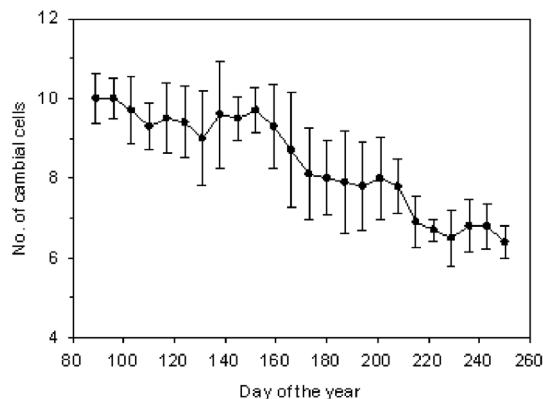

Fig. 1: Average number of cambial cells in sessile oak from Slovenia in the 2007 growing season.

By the second half of April, the first ring of the developing early wood vessels had been created and the second ring of early wood vessels was forming in wider xylem growth rings. Late wood formation began in the third week of May and was very distinct due to the great differences in the diameter of the early and late wood vessels in ring-porous oak (Fig. 2b). Late wood vessels were no longer arranged in characteristic rings. Transition from early to late phloem was determined on the basis of the formation of a late phloem band of fibres with secondary, lignified cell walls. Using polarized light, we observed biosynthesis of the secondary cell wall layers of fibres at the end of May (Fig. 2b). The fibres were in small groups arranged as discontinuous tangential bands, usually 2-5 cell layers wide. Development of the phloem fibres continued in June.

The measured xylem and phloem weekly increments revealed that relative phloem production was faster in the first part of the growing season (i.e., from April to the third week of May), but thereafter xylem production accelerated (Fig. 3). The period of most intense xylem cell production was assessed to be in the period April-May and phloem in April. About half of the annual increment (143.41 µm) of 2007 was formed in the first month of the growing season and about 75 % (215.11 µm) till the beginning of June in the case of phloem, but phloem production was thereafter very slow. Half of the xylem increment of 2007 (857.49 µm) was formed by the end of May and three-quarters of it by the beginning of July, indicating that xylem production was more uniform than phloem.

The average number of cambial cells decreased below 8 layers after the summer solstice (21 June). The number of cells then slowly continued to diminish to 6-7 layers by mid-August, when cell division stopped on the xylem and phloem sides simultaneously (Figs. 1, 2c). This is in accordance with our previous investigation in silver fir and Norway spruce (Gričar and Čufar 2008). On the xylem side, production and formation of latewood, consisting of vessels, axial parenchyma and ground tissue, took place in June, July and the first half of August, when cambial activity ceased. At that time, xylem cells below the cambium were in the final stages of maturation and the 2007 xylem growth ring was fully developed by the end of September. On the phloem side, late phloem, which contained fibres, sieve tubes and axial parenchyma, was creating from June until August.
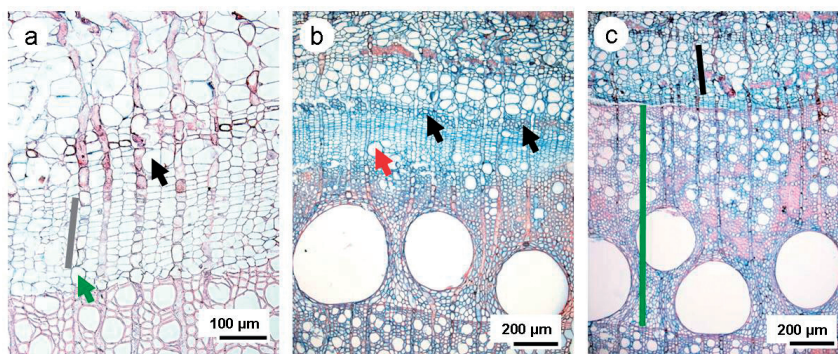

Fig. 2: a – Newly formed phloem (black line) and xylem (green arrow) cells and active cambium (gray line) at the beginning of April; b – late wood formation (red line) and development of phloem fibres (black line); c – phloem (black line) and xylem (green line) increment in 2007.
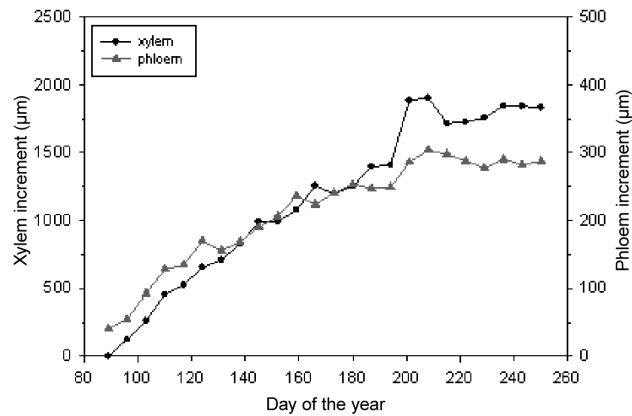
Fig. 3: Weekly xylem and phloem increments in sessile oak.

Widths and ratio of the phloem and xylem increments

Fully formed xylem growth rings of 2007 were on average 1837.55 μm wide. Inter-tree variability of the xylem increment was relatively high (± 951.88 μm), whereas intra-tree variability was much lower (± 254.15 μm), but increased with the width of increment. Namely, the standard deviation of the tree with the narrowest xylem ring (955.28 μm) was 150.43 μm and that with widest one (3201.40 μm) 380.92 μm. The portion of late wood increased with the width of xylem growth rings, which is in agreement with previous investigations in ring porous species (e.g., Panshin and de Zeeuw 1980). In xylem increments narrower than 1000 μm, early wood occupied more than 50 % of the ring, but its proportion gradually decreased to 30 % in wide rings. Although the width of early wood tends to be more or less constant in ring-porous trees, its width slowly increased with the width of xylem ring; from 487.28 to 992.28 μm, which is inconsistent with the observations of other authors (Zhang 1997). Zhang (1997) reported that the width of early wood increases a little at first, but tends to be constant (about 1 mm) in xylem growth rings wider than 3 mm. Late wood width, on the other hand, increases almost linearly with increasing ring width. We found that only 1, occasionally 2 rings of vessels were observed in narrower rings (less than 1000 μm), but in rings wider than 1000 μm also 3 rings were clearly visible.

The average width of the xylem increment of the investigated sessile oaks was 1714.98 ± 883.04 μm, that of phloem 286.81±232.04 μm and the ratio between phloem and xylem increments was 0.197 ± 0.118. Trees with wider xylem increments also had wider phloem increments, and vice versa. Lower ratios between the phloem and xylem growth rings of 2007 were found in trees with wider increments, indicating that cambial cell production favours xylem (specifically in late wood) formation in more productive trees.

In favourable growing season, xylem increment is considerably wider than that of phloem (Panshin and de Zeeuw 1980, Kozlowsky and Pallardy 1997). The structure of phloem growth rings is in general more complex than that of xylem and is highly dependent on its width (Gričar and Čufar 2008, Gričar et al. 2009). In a recent study on silver firs, we demonstrated that the use of width and structure of phloem and the ratios between the phloem and xylem increments and dormant cambium, can provide information on tree health conditions (Gričar et al. 2009).

Growth ring boundaries between annual phloem increments are generally not as distinctive as in xylem, because anatomical differences among cells in early and late phloem are not so

characteristic and vary a lot among different species. Since phloem cells collapse within a few years after their formation, cellular structure can only be studied in a narrow zone close to the cambium, the so-called conducting zone (Alfieri and Evert 1968, 1973, Gričar and Čufar 2008). Older tissues of bark are usually crushed and, eventually, the external, distorted and collapsed phloem tissues are sloughed off. For all these reasons, phloem is fairly unsuitable for dendrochronological or dendroecological studies.

CONLUSIONS

- Cambial reactivation and, consequently, xylem and phloem started before our first sampling at the end of March 2007 and cell divisions stopped in the first half of August on xylem and phloem sides.
- Relative phloem production was faster in the first part of the growing season, but xylem production then accelerated.
- The period of most intense xylem cell production was assessed to be in the period April-May and that of phloem in April.
- Trees with wider xylem increments also had wider phloem increments, and vice versa.
- Lower ratios between the phloem and xylem growth rings of 2007 were found in trees with wider increments, indicating that cambial cell production favours xylem (specifically in late wood) formation in more productive trees.
- Inter-tree variability in the xylem increment was relatively high, whereas intra-tree variability was much lower, but increased with the width of increment.
- The portion of latewood increased with the width of the xylem growth rings.

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