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Original article

Xylem and phloem formation in chestnut (*Castanea sativa* Mill.) during the 2008 growing season

Katarina Čufar[a], Martina Cherubini[b], Jožica Gričar[c], Peter Prislan[a], Stefano Spina[b], Manuela Romagnoli[b,*]

[a] University of Ljubljana, Biotechnical Faculty, Department of Wood Science & Technology, Cesta VIII/34, SI-1000 Ljubljana, Slovenia
[b] University of Tuscia, Facoltà di Agraria, DAF, Via San Camillo de Lellis, I-01100 Viterbo, Italy
[c] Slovenian Forestry Institute, Department of Yield and Silviculture, Večna pot 2, SI-1000 Ljubljana, Slovenia

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ABSTRACT

Cambial activity and the dynamics of xylem and phloem formation were monitored during the 2008 growth season in five chestnut (*Castanea sativa*) trees growing near Viterbo, Italy. The study was based on microscopic observations of micro-cores taken from stems at weekly intervals from mid-April until mid-October 2008. These observations allowed us to identify the timing of xylem and phloem formation. Cambial divisions, xylem and phloem formation had already started before the first sampling. By the end of April, the first earlywood vessels were already lignified and in early phloem the first formed sieve cells had finished postcambial growth. Formation of earlywood was completed by the end of May, which was about 2–3 weeks earlier than the completion of early phloem. The highest production of xylem cells was recorded between mid-May and mid-June and of phloem ones from mid-June until mid-July. Wood and phloem production mainly terminated in the middle of August while differentiation of xylem cells lasted until mid-October. The phloem ring was completed by the beginning of October 2008. Xylem growth rings were on average 2027 ± 635 μm wide and phloem rings 265 ± 68 μm. The proportion of latewood was 73 ± 8% and of late-phloem 49 ± 11%.

Introduction

Ecologically relevant information stored in annual wood rings has been for long time employed by dendrochronology. However, if we want to understand better which information is recorded in the wood structure we need to understand how the cambium captures environmental information during its formation. Studying wood formation on a cellular level can be very helpful here since it allows to define the timing of cell development and to relate it with the registered environmental conditions.

Most wood formation studies have been done in conifer species (e.g., Antonova and Stasova, 1993; Horacek et al., 1999; Schmitt et al., 2004; Deslauriers et al., 2003; Rossi et al., 2006a; 2008; Gričar, 2007; De Luis et al., 2007; Mäkinen et al., 2008) and rarely on dicotyledons (Schmitt et al., 2000; Marion et al., 2007; van der Werf et al., 2007; Čufar et al., 2008a,b). These studies have mainly described the duration of cambial activity and the seasonal dynamics of wood formation, which proved to vary among different sites and tree species. Long term observations of wood formation performed at the same site are still rare (e.g., Deslauriers et al., 2008; Mäkinen et al., 2008). They have shown that in the same tree the dynamic of wood formation varies from year to year depending on current year's conditions.

The cambium produces wood and also secondary phloem. Despite its great importance for conducting assimilates, studies of phloem formation are very rare and were mainly performed several decades ago (e.g., Evert and Murmanis, 1965; Derr and Evert, 1967; Davis and Evert, 1968; Tucker and Evert, 1969; Alfieri and Evert, 1973; Lawton, 1976). In normal conditions, the amount of phloem produced by the cambium is much lower than that of xylem (Gričar et al., 2009). As in xylem, annual growth increments can also be distinguished in phloem, in its non-collapsed part, although growth ring boundaries are sometimes less distinctive. Since the sieve cells are functional for only 1–2 years after their formation, they subsequently collapse and the phloem undergoes secondary changes, which make recognizing and studying longer series of phloem rings very difficult (Cheadle, 1956; Gričar and Čufar, 2008).

The few recent investigations of seasonal dynamics of phloem formation have been mainly done in conifers (e.g., Antonova and Stasova, 2006; Gričar and Čufar, 2008). Such studies, together with

* Corresponding author. Tel.: +39 761 357 399; fax: +39 761 357 250.
E-mail address: mroma@unitus.it (M. Romagnoli).

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studies of the structure and width of phloem increments, proved to be particularly useful in combination with wood formation data because they can reveal additional information on physiological conditions of trees (Gričar et al., 2009).

The aim of this work was to investigate the seasonal dynamics of cambial activity, and the formation of xylem and phloem growth rings in ring-porous chestnut (*Castanea sativa* Mill.), a common and widespread dicotyledon tree species mainly growing on sites influenced by Mediterranean climate. In particular, we intended to present the dynamics of xylem and phloem formation at a selected coppice site in central Italy during the 2008 growing season.

Materials and methods

The study was carried out at a chestnut (*C. sativa* Mill.) coppice stand in the locality of Monti Cimini, part of the Comune di Soriano, Viterbo, Italy (approx. 42°17′N, 12°12′E, 850 m a.s.l.). The area is on volcanic soil and the climate is Mediterranean. The amount of annual precipitation in nearby Soriano nel Cimino is 1180 mm (Servizio Idrografico, 1916–2000). The precipitation maximum is recorded from October until December, and the driest period occurs in summer, although there is no significant evidence of drought according to the Bagnouls–Gaussen diagram (Piovesan et al., 2008). The mean annual temperature is 14 °C, with a maximum in August (up to 24.2 °C) and minimum in December (6.2 °C) (Servizio idrografico 1997–2001) (Fig. 1).

We selected five isolated dominant healthy chestnut trees with mean diameters of 17 cm, heights of 15 m, and ages of 30 years. Each of the selected trees was a standard, i.e., a single shoot that has been left after the last coppicing in 2006.

Samples of tissues containing the bark, cambium and the last formed wood were collected by taking micro-cores with Trephor (Rossi et al., 2006b). The micro-cores (diameter 1.8 mm, length 15 mm) were extracted at the basal part of the stems, at weekly intervals from April until October.

Immediately after extraction from the tree, the samples were put in 70% ethanol for fixation and conservation. They were then embedded in paraffin using a Leica TP 1020-1 tissue processor for dehydration in a graded series of ethanol (70%, 90%, 95% and 100%) and bio-clear (D-limonene) for paraffin infiltration (Rossi et al., 2006b). Cross-sections of 10 μm thickness were prepared on a Leica RM 2245 rotary microtome, using disposable Feather N35H blades. For better adhesion of the sections, slides were pre-treated with albumin. Sections were dried at 70 °C for half an hour and cleaned of residual paraffin by immersing the slides in bio-clear and ethanol. Micro-sections were finally stained with a mixture of safranine and astra blue according to van der Werf et al. (2007) and mounted on glass slides in Euparal. A Nikon Eclipse 800 light microscope (bright field and polarized light) and the NIS Elements image analysis system were used for observations and analyses. The measurements were done in the cambium and in the developing xylem and phloem growth rings of 2008. We selected three radial rows in the cambium in order to count the number of cells and to measure the width of the cambium (in μm). Additionally we measured the width of the increment in the current xylem and phloem growth rings along three radial rows.

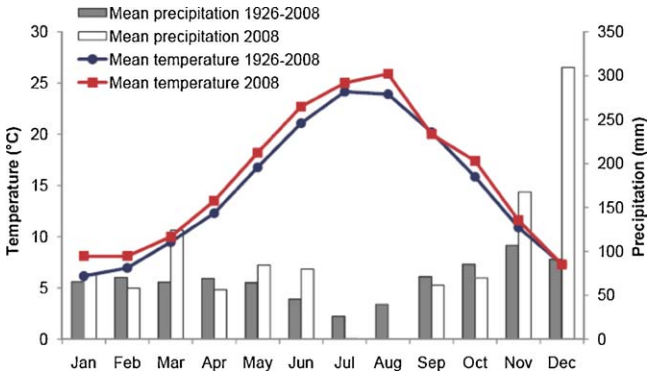

Fig. 1. Climatic diagram of Viterbo meteorological station. Monthly mean temperature and monthly precipitation for the long period 1926–2008, and monthly mean temperature and monthly precipitation for 2008.

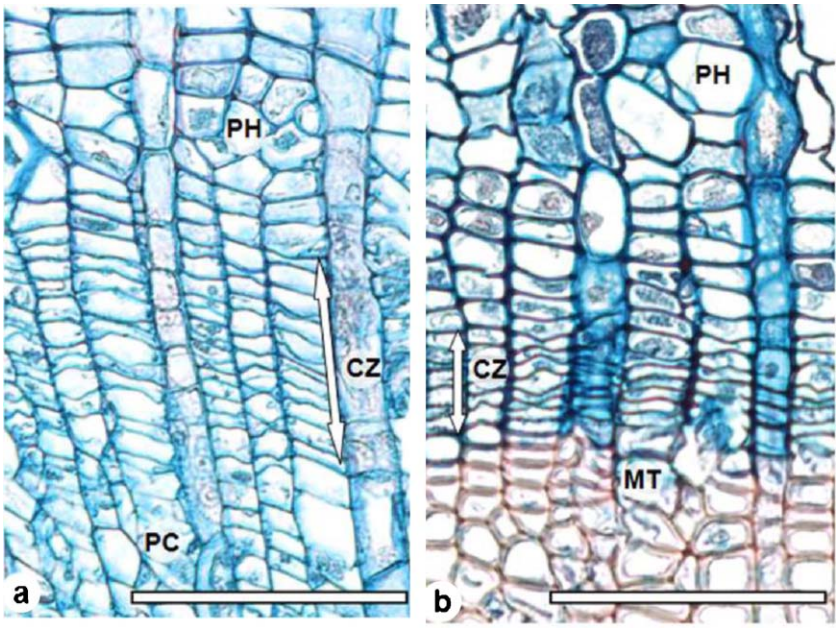

Fig. 2. Cambial zone (CZ) in chestnut (*Castanea sativa*): (a) active CZ with approx. 12 layers of cells with thinner cell walls. Below the CZ are xylem cells in postcambial growth (PC), above is secondary phloem (PH); date 25 June 2008. (b) Dormant CZ, consisting of approx. 6 layers of cells with small radial dimensions and slightly thickened cell walls. Below the CZ is part of the fully formed latewood (MT), above is PH; date 17 October. Scale bars – 100 μm.