

**SAMPLING CAMBIAL REGION AND MATURE XYLEM FOR  
CARBOHYDRATE ANALYSES**

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## SUMMARY

15 This paper describes a procedure to analyse soluble carbohydrates and starch in xylem and cambium extracted by the scraping method. An application on woody logs of 2-years-old *Populus × canadensis* Moench ‘I-214’ during different phases of cambium activity is reported. Samples are freeze-dried in liquid nitrogen and, successively, the bark is removed. The cambium and the differentiating xylem are gently scrapped with a  
20 razor blade from the inner side of the bark and the outermost side of the stem, respectively. The xylem is milled until obtaining a powder. After extraction of the powder in a EtOH solution and centrifugation, the supernatant and the resulting pellet are used for assessing the contents of soluble sugars and starch, respectively. Soluble sugars are determined using the High-Performance Liquid Chromatography (HPLC).

25 The amount of the cambial region obtained by scraping changed in respect to cambium phenology with higher amount of dry matter obtained during tree ring production. The HPLC method was particularly suitable to study the intra-annual sugars dynamic in woody forming tissues and mature xylem. In both tissues (cambial region and xylem), the major soluble sugars detected were glucose and fructose which represented together  
30 more 80% of the total soluble sugars during wood formation. However, the total soluble sugars was higher in cambium compared with xylem, especially with the trees were actively growing. The scraping technique provided the possibility to sampling different tissues (mature and developing xylem, cambium and phloem) during the year, allowing the metabolic changes during tree-ring formation to be investigated.

35 **Keywords:** xylogenesis, cell differentiation, annual ring, soluble sugars, starch

## INTRODUCTION

Tree rings are the results of a complex process involving the division of the cambial tissues and the differentiation of the newly formed cells (Rossi et al., 2006). This growth process requires a great amount of carbon and energy translocated from the source, the crown, to branches, stem and roots in form of sugars. Sugars in the growing tissues are utilised by the carbon sinks or converted in starch for storage. Although seasonal dynamics of wood formation has been analyzed thoroughly in the last decade both directly and indirectly (Giovannelli et al., 2007; Thibeault-Martel et al., 2008; Moser et al., in press; Rossi et al., 2009), the internal factors affecting tree-ring formation, such as cambium age, hormone distribution and carbohydrate availability (Rossi et al., 2008; Ugglä et al., 1998; Schrader and Sauter, 2002), still remain to be clearly identified and precisely quantified. In particular, since the 1960s biologists have been trying to explain the influence of carbohydrates on cambial activity and early-latewood formation (Gordon and Larson, 1968; Wargo, 1976; Saranpää and Höll, 1989; Geisler-Lee et al., 2006). However, only a few studies on intra-annual carbohydrate availability for wood production in broadleaves are available (Piispanen and Saranpää, 2001, Barbaroux et al., 2003; Hoch and Körner, 2003). These studies were focused on carbohydrate content in the stem, but did not consider the meristem at the origin of wood: the cambium.

The main limitation in performing biochemical and molecular investigations in the cambial cells is that this tissue is usually difficult to collect. The cambial region consists of a cell layer in different developmental stages as the cambial zone (the true meristem) and the derivatives, which undergo expansion and secondary wall formation towards the

mature xylem and the phloem (Uggla and Sundberg, 2002). Even during the maximum  
60 expansion, cambium is constituted by 4-15 cells lacking in secondary wall (Rossi et al.,  
2007; Deslauriers et al., 2009), a very thin and soft tissue. Surprisingly, all variations in  
biochemistry, metabolic activity and gene expression across the cambial region occur  
within a very narrow region. Nevertheless, few details have been given about the  
available techniques for sampling and preparing cambium for biochemical analyses  
65 (Uggla and Sundberg, 2002; Magel et al., 1994; Micheli et al., 2002), leading to a  
number of difficulties in the accomplishment of such important experiments (Chaffey,  
2002).

The arising question concerns how to extract suitably the cambial region in living trees  
during the year. In spring, the bark slippage is a well known marker of cambial  
70 reactivation. In this period, the bark can be easily removed from the stem and the  
cambial region is visible as a translucent film on the inner part of the bark or on the  
outermost mature xylem side. Although apparently easy to do, this sampling procedure  
involves difficulties connected to the sample size (radial thickness of cambial region),  
which depends on the cambium phenology and the whole plant water status. On the  
75 contrary, during dormancy, cambium is composed of few cells, which are strictly  
attached to the inner part of the bark. As a result, the cambial region is very difficult to  
sample when the vigorous growth is not occurring. This represents one of the major  
limitations when investigations should be performed in the time and should involve the  
different phases of cambium phenology.

80 According to the literature, the cambial region in trees is mainly extract from stem  
portions (consisting of bark, phloem, cambial region and mature xylem) through two

methods: (i) by scraping the macro-fractions (Micheli et al., 2002), or (ii) by cryo-sectioning the micro-fraction (Uggla and Sundberg, 2002) of individual tissues. The scraping method uses the unlignified enlarging xylem as a breaking zone to separate the

85 mature xylem from the cambium, phloem and bark. The scraping method is generally used when a large amount of differentiating cambial tissues are required for biochemical and molecular analyses. Even if this method is less time consuming and expensive than cryo-sectioning, some limitation may occur as: (i) the sample is not homogeneous but composed of a multiplicity of different tissues (cambium, developing xylem and

90 phloem), (ii) the amount of scraped tissue depends on the phenological stage of cambium (growth or dormancy), (iii) the ratio between the amount of cambium and differentiating tissues changes during in time (season) and space (along the stem), and (iv) the chemical composition of the extracted heterogeneous tissues is often unknown. Therefore, there is a need to test the scraping method to verify the collected material.

95 The aim of this paper is to describe a suitable procedure to analyse soluble carbohydrates and starch in xylem and cambium extracted by the scraping method and to present an application on broadleaves.

## SAMPLING CAMBIUM AND XYLEM

100 Woody samples are extracted from the stem in form of logs of 10-15 cm in length,  
immediately immersed in liquid nitrogen and freeze-dried at a constant temperature of -  
50 °C under vacuum (0.15 mbar or less). During drying, the loss of intra and  
intercellular water in the cambial region causes the shrinkage of the elastic developing  
tissues leading to the appearance of many wide empty spaces within the growing tree  
105 ring, as observed at the scanning electron microscope (Figure 1A). Occasionally, breaks  
between xylem and cork can take place in correspondence to the differentiating cells  
(Figure 1B). Before handling the samples, the temperature of the logs is gradually  
increased to 22 °C in an environment with a relative humidity of 40% (Magel, 2002).  
Before dissection, the dried logs have to be maintained into sealed bags under vacuum  
110 and stored at room temperature. For collecting the cambial zone, bark is removed from  
the logs and cambium and the differentiating xylem are gently scrapped with a razor  
blade from the inner side of the bark and the outermost side of the stem, respectively  
(Figure 1C-D). The mature xylem are cut off from the log using a chisel (Figure 2A),  
reduced to a fine powder with a rotor mill (Figure 2B) and stored in falcon tubes under  
115 vacuum up to biochemical analyses.

## BIOCHEMICAL ANALYSES

For both cambial region and xylem, 40 mg of dried powder are repetitively extracted three times in 5 ml of 80% EtOH solution, adjusted to pH 7, at room temperature. In  
120 each extraction, the homogenates are gently vortexed and, after 30 min, centrifuged at 10.000 rpm for 5 min. The supernatant and the resulting pellet are used for assessing the contents of soluble sugars [High-Performance Liquid Chromatography (HPLC)] and starch, respectively.

### HPLC analysis

125 The supernatant is evaporated to dryness at room temperature with a Savant Speedvac Plus SC210A system and diluted with 2 ml of distilled water (pH 7). The solution obtained is then fractionated using liquid-solid extractions carried out by eluting samples through a reverse-phase cyclohexyl resin (pre-packed 3 ml Bond Elut CH cartridge, Varian CA, USA) and a quaternary-amine, strong anion-exchange resin (pre-  
130 packed 3ml Bond Elut SAX cartridge, Varian CA, USA). The cartridges are activated by 6 ml MeOH and conditioned by adding 6 ml of distilled water. The elution is performed using an additional 6 ml of distilled water, evaporated to dryness under vacuum and then diluted with 0.5 ml of distilled water. Analyses are conducted using a binary LC pump 250 (Perkin Elmer, USA) equipped with an automatic injection system  
135 (ISS101, Perkin Elmer). A Water Column Heater Module (Waters Division, Millipore, Milford, MA, USA), controlled by a Temperature Control Module (Waters), maintains the column at 80 °C. The column is an 8×300 mm Shodex Sugar SC 1011 (Showa Denko Europe GmbH, Germany) equipped with a Guard Pak Insert Sugar Pak II

(Waters). The mobile phase is water, Milli Q grade, at 0.5 ml min<sup>-1</sup>. Identification and  
140 quantification of soluble carbohydrates is performed according to Romani et al. (1994)  
and the identity of soluble carbohydrates is confirmed using authentic carbohydrate  
standards (Sigma, USA) and adding an internal standard. In figure 3A, a typical  
chromatogram of 25 µl injection of 0.1% standard solution of raffinose, sucrose,  
glucose, galactose, fructose, mannitol and sorbitol is reported. Sorbitol, a sugar not  
145 detected in woody poplar tissues is used as internal standard and added to the crush  
material. The recovery is estimated for each carbohydrate. Thus 0.25, 0.50, 0.75, and  
1.0 ml 1mg ml<sup>-1</sup> carbohydrate solutions are fractionated and analysed as previously  
described, with recovery ranging from 92 to 99%. Calibration curves are performed for  
raffinose, sucrose, glucose, galactose, fructose, mannitol and sorbitol. Total soluble  
150 sugar content is obtained as the sum of the detected sugars (>0.1 µmol g<sup>-1</sup>DW).

### **Starch content**

Starch content is measured in the pellet remaining after extraction with 80% ethanol  
according to Gucci et al. (1991). The ethanol-insoluble residue is suspended in 1.5 ml  
acetate buffer (pH 5), boiled at 100°C for 1 hour in a sand bath and cooled at room  
155 temperature. After incubation at 55°C for 16 h with 150 µl amyloglucosidase from  
*Aspergillus niger* (Fluka), samples are diluted with distilled water to 5 ml and three  
0.25-ml aliquots and each sample are assayed colorimetrically by using glucose oxidase  
(Sigma-Aldrich, Italy).



### Material and methods

Woody samples of *Populus × canadensis* Moench ‘I-214’ were collected in 2007 in Casale Monferrato (AL, Italy). Plants were growing on an alluvial sandy-loam soil in one-year-old coppice with stumps arranged in 350-m-long rows with a distance of 2 m  
165 between rows and 0.5 m within rows. The average stem diameter at 0.30 cm from the collar was 17 mm.

Cambial phenology was studied by intra-annual analyses of wood formation on stem disks collected weekly according to Deslauriers et al. (2009). For the analysis of soluble sugars and starch, only the main phases of xylem growth were considered and  
170 corresponded to (i) dormancy [day of the year (DOY) 33], (ii) onset of xylem differentiation (DOY 110), (iii) maximum growth rate (DOY 152), (iv) decreasing (DOY 207) and (v) ending (DOY 236) of xylem differentiation.

### Results

After freeze-drying, the bark was easily separated from the mature xylem except for the  
175 samples collected during dormancy (DOY 33). The amount of the cambial region obtained by scraping changed in respect to cambium phenology (table 1). Very low amounts of scrapped tissues were obtained during dormancy (0.05 g), due to the state of the cambium (a row of 3-4 cells). In most of the samples, this quantity was too low to perform HPLC analysis. Instead, during wood formation (DOY 110, 152 and 207), the  
180 scrapped tissues considerably increased with amounts varying between 0.16 and 0.23 g

of dry weight. These quantities represented about 1.8, 3.4 and 1.5 % of the collected dry material for onset, maximum growth rate and decreasing of cambium activity respectively. The high stem water content during onset and maximum growth rate also facilitated the extraction of the material by scrapping. At the end of wood formation, the quantity of scrapped tissues decreased at 0.2 g, representing less than 1 % of the collected dry material (20.3 g).

In figure 3B, a typical chromatogram of 20 µl woody tissue extracted of poplar, with sorbitol as internal standard is reported. In this example, it was possible to detect raffinose, sucrose, glucose, galactose, fructose and mannitol. In both tissues (cambial region and xylem), the major soluble sugars detected were glucose and fructose which represented together more 80% of the total soluble sugars during wood formation (figure 4). However, the variability of the soluble sugars found was greater in xylem, especially in winter. So, mannitol, raffinose and galactose accounted for about 15% of the total sugars within the xylem during winter (DOY 33). However, these carbohydrates disappeared completely during growth except for the galactose which was present only in small traces in the xylem.

Compared with the xylem, the cambial region had quantities of soluble sugars about 6-10 times higher (figure 5). Higher quantity of soluble sugars was found in cambium during the maximum growth rate (DOY 152), twice that observed at the onset or the end of wood formation, forming 16% of dry matter compared with about 8 % of dry matter respectively. In xylem, the total soluble sugar showed a high level in winter, a decrease during the onset of xylem differentiation (DOY 110) and a further increase in correspondence to the maximum growth rate (DOY 152). Afterwards, new minimums

were reached during decreasing and ending of xylem differentiation with values of less  
205 than 1% of dry matter. Starch was detected at different amount depending on the tissues  
in both the species (figure 5). As for the soluble sugars, cambial region had higher level  
of starch content than xylem. In cambial region of poplar, more than 80% of the total  
starch was detected at the end of wood formation (DOY 236) while within the xylem  
the highest level of starch was detected during dormancy (DOY 33) and at the onset of  
210 growth (DOY 110).

## **Discussion**

Understanding of tree-ring formation involve knowledge on the growth processes in  
trees and investigations on the cambial meristems, which require specific technical  
procedures for sampling and preparing this thin and soft tissue. In this paper, we  
215 describe a suitable and rapid method to extract the cambial region and mature xylem  
from the stem for biochemical and molecular analyses. This technique provides the  
possibility to sampling different tissues (mature and developing xylem, cambium and  
phloem) along the stem and the circumference, allowing the metabolic changes during  
growth to be investigated.

220 The amount of cambial region scraped from the inner side of the bark and the outermost  
side of the xylem varied between the phases of dormancy and growth. Higher quantities  
were recuperated when the width of the developing zone and the degree of hydration of  
the stem were superior. On DOY 152 for example, the developing area was fully  
hydrated and, due to the lack of lignification and the apposition of cellulosic matrix, it  
225 was highly elastic and easy to scrap with a razor blade. Similar results was reported for

*Picea abies*, where the maximum width of the developing zone was recorded during early spring and it was associated to the high stem water content (Rosner et al., 2001). On the contrary, during winter (DOY 33), the cambial zone of poplar consisted of 4-5 narrow initial cells without differentiating xylem and phloem cells (Deslauriers et al., 230 2009). In these conditions, it was possible to scrap a very little amount of cambial region (< 50 mg), and only on the phloem side. Therefore, an analysis of soluble sugars in cambium with the scraping technique is easier only when the tissue is actively producing cells and with differentiating xylem. Otherwise, larger amount of fresh material have to be collected.

235 The rapid HPLC method used was particularly suitable for studies involving synthesis of sugars in woody forming tissues and mature xylem. The use of a single column enabled the quantification of five soluble sugars, namely, the sucrose pool (sucrose and raffinose), glucose, galactose, fructose and mannitol. These sugars represented more than 95% of total soluble carbohydrates in the woody tissues of poplar. With such a 240 precise determination of sugars, it was possible to observe that the oligosaccharides concentration changed with respect to the tissue and cambium phenology. The period of wood formation was characterized by a high concentration of fructose and glucose both in cambial region and in xylem while an increase of sucrose was recorded at the end of xylem differentiation. Sucrose represents the major substrate for the synthesis of sugar 245 polymers forming the microfibrillar phase of the cell. In the cytosol, sucrose is converted by sucrose synthase to fructose (used in the glycolysis) and the activated sugar uridine diphosphate-(UDP)-glucose which represent the substrate for the cellulose biosynthesis (Kock, 2004). The high level of glucose in the cambial region could be linked to the high  $\beta$ -1,4-glucan unit requirement during secondary wall formation.

250 In this work, more than 75% of the total starch measured in cambial region and wood  
ray parenchyma was detected during dormancy (DOY 33) and after the end of xylem  
differentiation (DOY 236). These results support the hypothesis that starch is directly  
used in differentiating tissues during wood formation as source of glucose for cellulose  
biosynthesis. Elle and Sauter (2000) found that the increased endoamylase activity,  
255 related with the starch degradation, leads to higher concentrations of malto-  
oligosaccharides, maltose and finally glucose.

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commerciale.

## REFERENCES

- 265 Barbaroux, C., Breda N., Dufrene E., 2003. Distribution of above-ground and below-ground carbohydrate reserves in adult trees of two contrasting broad-leaved species (*Quercus petraea* and *Fagus sylvatica*). *New Phytologist* 157, 605-615.
- Chaffey, N., 2002. An introduction to the problems of working with trees, in Chaffey N.J. (Ed), *Wood formation in trees. Cell and molecular biology techniques*. Taylor & Francis, New York, pp. 9-16.
- 270 Deslauriers, A., Giovannelli, A., Rossi, S., Castro, G., Fragnelli, G., Traversi, M.L., 2009. Intra-annual cambial activity and carbon availability in stem of poplar. *Tree Physiology* 29:1223-1235.
- Elle, D., Sauter, JJ., 2000. Seasonal changes of activity of a starch granule bound endoamylase and of a starch phosphorylase in poplar wood (*Populus × canadensis* Möench ‘robusta’) and their possible regulation by temperature and phytohormones. *Journal of Plant Physiology* 156, 731-740
- 275
- Geisler-Lee, J., Geisler, M., Coutinho, P.M., Segerman, B., Nishikubo, N., Takahashi, J., Aspeborg, H., Djerbi, S., Master, E., Andersson-Gunnerås, S., Sundberg, B., Karpinski, S., Teeri, T.T., Kleczkowski, L.A., Henrissat, B., Mellerowicz, E.J., 2006. Poplar carbohydrate-active enzymes. Gene identification and expression analyses. *Plant Physiology* 140, 946-962.
- 280
- Giovannelli, A., Deslauriers, A., Fragnelli, G., Scaletti, L., Castro, G., Rossi, S., Crivellaro, A., 2007. Evaluation of drought response of two poplar clones (*Populus × canadensis* Möench ‘I-214’ and *P. deltoides* Marsh. ‘Dvina’) through high resolution analysis of stem growth. *Journal of Experimental Botany* 58, 2673-268
- 285

- Gordon, J.C., Larson, P.R., 1968. Seasonal course of photosynthesis, respiration, and distribution of  $^{14}\text{C}$  in young *Pinus resinosa* trees as related to wood formation. *Plant Physiology* 43, 1617-1624
- 290 Gucci, R., Xiloyannis C., Flore, J.A., 1991. Gas exchange parameters, water relations and carbohydrate partitioning in leaves of field-grown *Prunus domestica* following fruit removal. *Physiologia Plantarum* 83, 497-505.
- Hoch, G., Körner, C., 2003. The carbon charging of pines at the climatic treeline: a global comparison *Oecologia* 135, 10-21.
- 295 Kock, K., 2004. Sucrose metabolism: regulatory mechanisms and pivotal roles in sugar sensing and plant development. *Current Opinion in Plant Biology* 7, 235-246.
- Magel, E., 2002. Biochemistry and quantitative histochemistry of wood, in Chaffey N.J. (Ed), *Wood formation in trees. Cell and molecular biology techniques*. Taylor & Francis, New York, pp. 229-244.
- 300 Magel, E., Jay-Allemand, C., Ziegler, H., 1994. Formation of heartwood substances in the stem of *Robinia pseudoacacia* L. II. Distribution of non-structural carbohydrates and wood extractives across the trunk. *Trees* 8, 165-171.
- Micheli, F., Ermel-Fontaine, F., Bordenave, M., Richard, L., Goldberg, R., 2002. Cell walls of woody tissues: cytochemical, biochemical and molecular analysis of pectins and pectin methylesterases, in Chaffey N.J. (Ed), *Wood formation in trees. Cell and molecular biology techniques*. Taylor & Francis, New York, pp. 179-200.
- 305 Moser, L., Fonti, P., Büntgen, U., Esper, J., Luterbacher J., Franzen, J., Frank, D. Timing and duration of European larch growing season along an altitudinal gradient in the Swiss Alps. *Tree Physiology* in press



- 310 Piispanen, R., Saranpää, P., 2001. Variation of non-structural carbohydrates in silver birch (*Betula pendula* Roth) wood. *Trees* 15, 444-451.
- Romani, A., Baldi, A., Tattini, M., Vincieri, F.F., 1994. Extraction, purification procedures and HPLC-RI analysis of carbohydrates in olive (*Olea europaea* L.) plants. *Chromatographia* 2, 35-39.
- 315 Rosner S., Baier, P., Kikuta, S.B., 2001. Osmotic potential of Norway spruce [*Picea abies* (L.) Karst.] secondary phloem in relation to anatomy. *Trees* 15, 472-482.
- Rossi, S., Deslauriers, A., Anfodillo, T., 2006. Assessment of cambial activity and xylogenesis by microsampling tree species: an example at the alpine timberline. *IAWA Journal* 24, 383-394.
- 320 Rossi, S., Deslauriers, A., Anfodillo, T., Carraro, V., 2007. Evidence of threshold temperatures for xylogenesis in conifers at high altitude. *Oecologia* 152, 1-12
- Rossi S., Deslauriers, A., Anfodillo, T., Carrer, M., 2008. Age dependent xylogenesis in timberline conifers. *New Phytologist* 177, 199-208.
- Rossi, S., Rathgeber, C.B.K., Deslauriers, A. 2009. Comparing needle and shoot  
325 phenology with xylem development on three conifer species in Italy. *Annals of Forest Science* 66, 206
- Saranpää, P., Höll, W., 1989. Soluble carbohydrates of *Pinus sylvestris* L. sapwood and heartwood. *Trees* 3, 138-143.
- Schrader, S., Sauter, J.J., 2002. Seasonal changes of sucrose-phosphate synthase and  
330 sucrose synthase activities in poplar wood (*Populus x canadensis* Möench 'robusta') and their possible role in carbohydrate metabolism. *Journal of Plant Physiology* 159, 833-843.

- Thibeault-Martel, M., Krause, C., Morin, H., Rossi, S., 2008. Cambial activity and  
intra-annual xylem formation in roots and stems of *Abies balsamea* and *Picea*  
335 *mariana*. *Annals of Botany* 102, 667-674
- Ugla, C., Mellerowicz, E.J., Sundberg, B., 1998. Indole-3-acetic acid controls cambial  
growth in Scots pine by positional signaling. *Plant Physiology* 117, 113-121.
- Ugla, C., Sundberg, B., 2002. Sampling of cambial region tissues for high resolution  
analysis, in Chaffey N.J. (Ed), *Wood formation in trees. Cell and molecular biology*  
340 *techniques*. Taylor & Francis, New York, pp. 215-228.
- Wargo, P.M., 1976. Variation of starch content among and within roots of red and white  
oak trees. *Forest Science* 22, 468-471.

## CAPTION LIST

345 **Table 1.** Weight (g) and water content of the sampled log and amount of scrapped tissue of the cambial region (g) collected in *Populus × canadensis*. The sampling days correspond to the main stages of cambial activity: dormancy (DOY 33), onset of xylem differentiation (DOY 110), maximum growth rate (DOY 152), decreasing (DOY 207) and ending (DOY 236) of xylem differentiation.

350 **Figure 1.** Analyses of a dried stem section of poplar collected on DOY 164 under a scanning electron microscope. A: Intact section of phloem (ph) developing xylem (dx) and mature xylem (mx) showing collapsed cambial regions (asterisk) due to the water extraction by vacuum. B: section with a break between phloem (ph) and mature xylem (mx) in correspondence of the developing xylem (dx). C: outermost part of mature  
355 xylem (mx) with scrapped (arrowhead) and intact (dx) developing xylem. D: inner part of the bark after separation from the xylem with cambial zone (cz) and phloem (ph).

**Figure 2.** Sampling (A) and milling (B) of the xylem of *Populus × canadensis*.

**Figure 3.** Chromatograms of (A) 25 µl injection of 0.1% standard solution of raffinose, sucrose, glucose, galactose, fructose, mannitol and sorbitol and (B) 20 µl of xylem of  
360 *Populus × canadensis*, with sorbitol as internal standard.

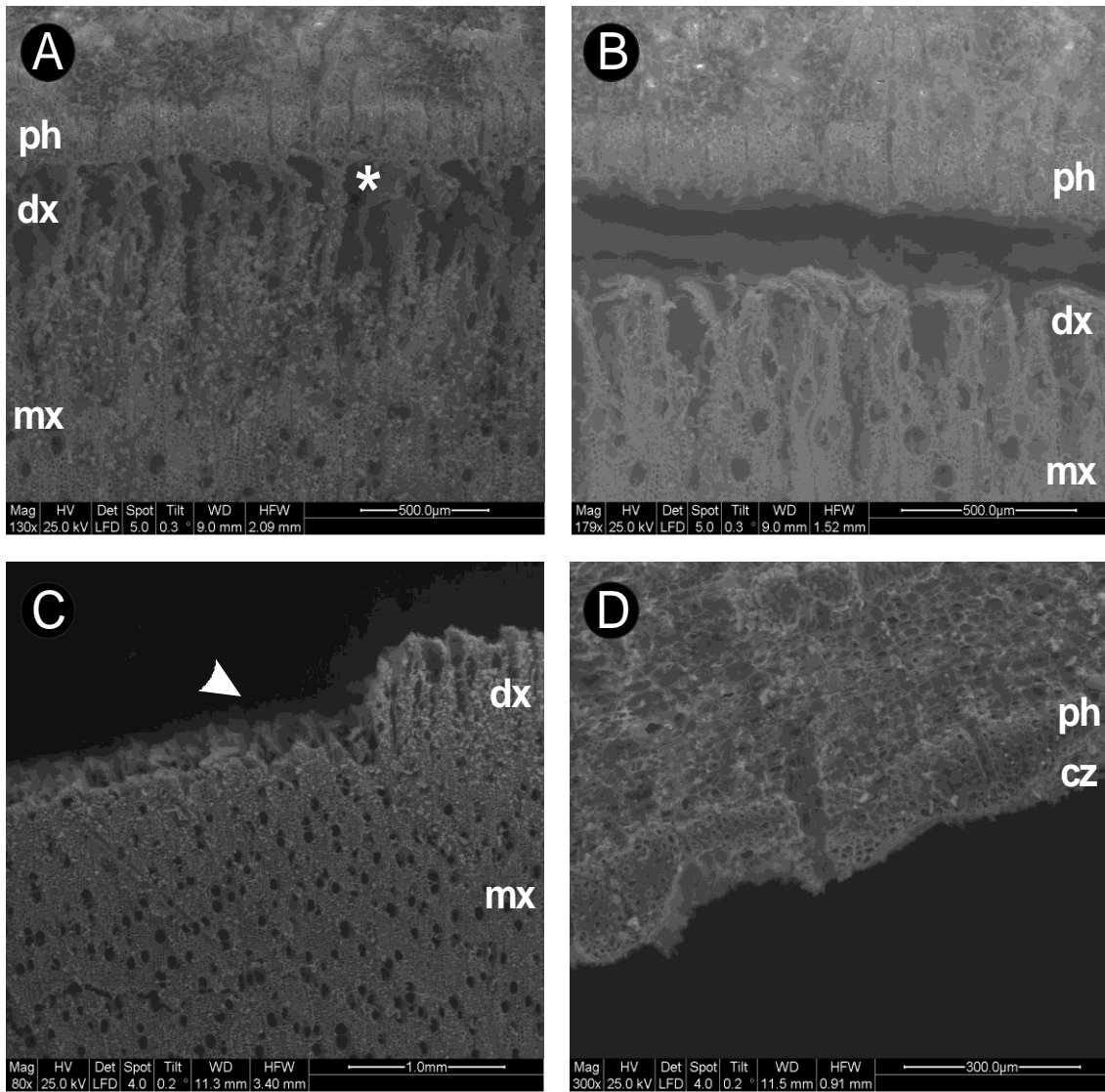
**Figure 4.** Proportion of soluble sugars in cambium and xylem of *Populus × canadensis* during the year. The sampling days correspond to the main stages of cambial activity: dormancy (DOY 33), onset of xylem differentiation (DOY 110), maximum growth rate (DOY 152), decreasing (DOY 207) and ending (DOY 236) of xylem differentiation.

365 **Figure 5.** Total soluble sugar and starch expressed as dry matter percent in cambium  
and xylem of *Populus × canadensis* during the year. The sampling days correspond to  
the main stages of cambial activity: dormancy (DOY 33), onset of xylem differentiation  
(DOY 110), maximum growth rate (DOY 152), decreasing (DOY 207) and ending  
(DOY 236) of xylem differentiation. Vertical bars indicate the standard deviation  
370 between six measurements. Asterisk represent missing data.

**TABLE 1**

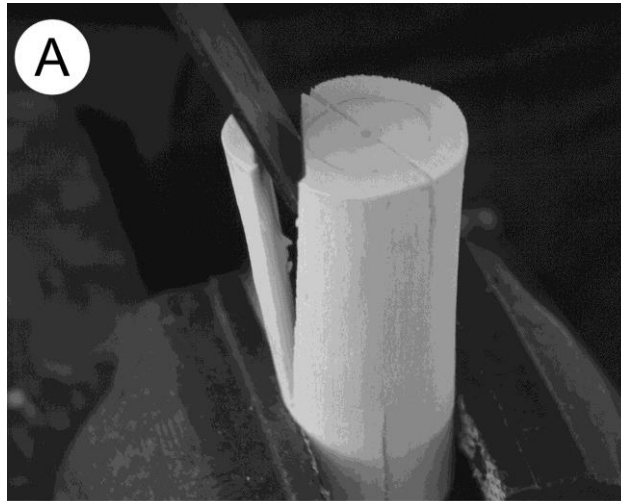
DOY	Sampled log			Scrapped cambial region
	Fresh weight (g)	Dry weight (g)	Water content (%)	Dry weight (g)
33	27.2±2.5	9.2±1.2	180	0.05±0.001
110	25.6±2.8	8.6±1.2	195	0.16±0.005
152	26.0±1.3	9.1±1.0	186	0.31±0.004
207	36.4±1.3	15.2±1.6	138	0.23±0.002
236	42.7±10.0	20.3±4.5	109	0.20±0.006

FIGURE 1



375

**FIGURE 2**



**FIGURE 3**

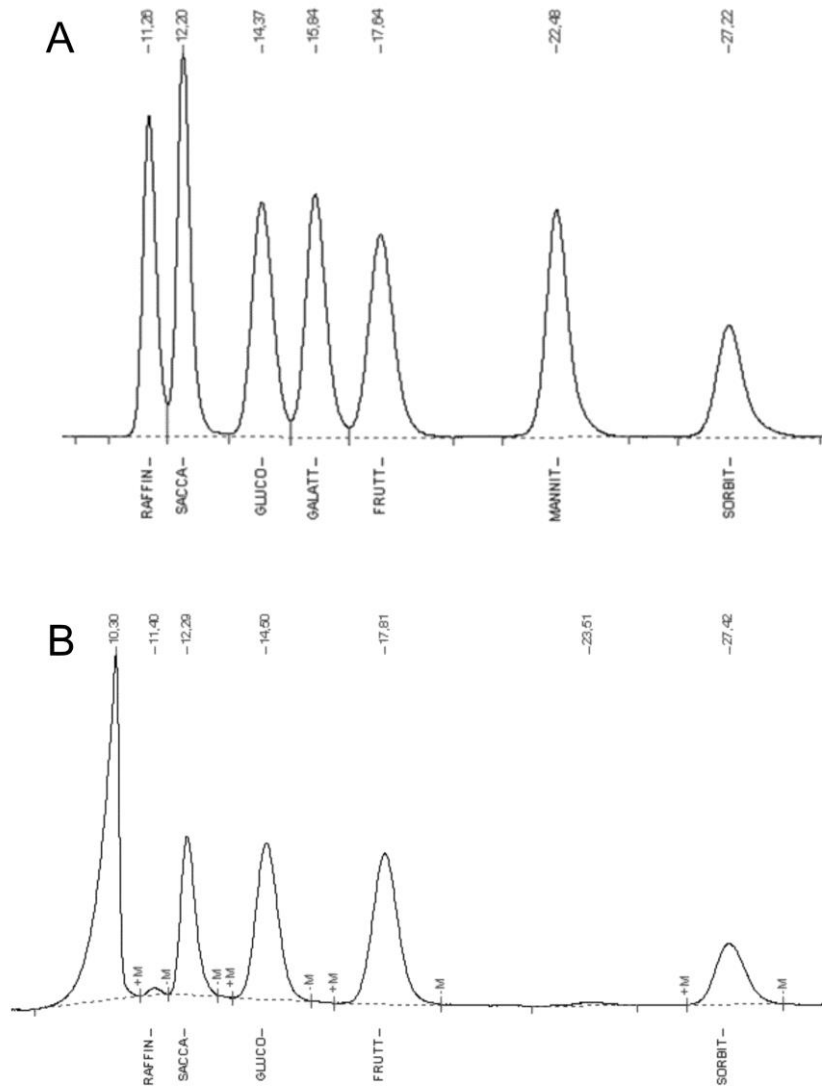
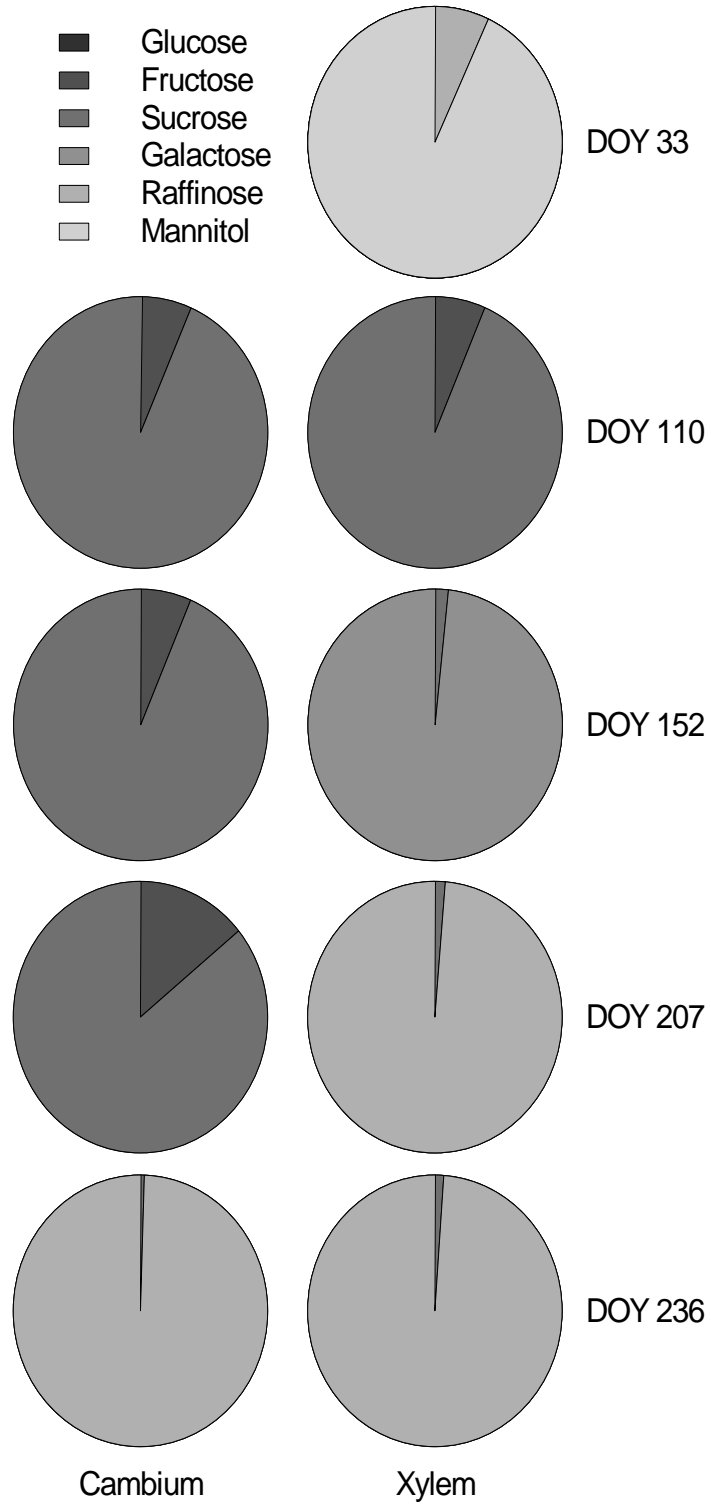




FIGURE 4



**FIGURE 5**

