Intra-annual cambial activity and carbon availability in stem of poplar

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Summary Cambial activity is influenced by many environmental and physiological factors and among them, carbon acts as a source of energy for the growing meristems. This work has focused on the intra-annual stem growth of poplar compared with the carbon available for xylogenesis processes in cambium and outer wood. The major stages of xylem production and differentiation in two poplar genotypes with different growth performances were considered. Monitoring of stem growth and leaf phenology combined with starch, nonstructural soluble sugars and water content in the stem was conducted from February to November 2006 in Populus canadensis Moench ‘I-214’ and Populus deltoides Marsh. ‘Dvina’. Anatomical analyses of wood formation were performed by measuring the width of the zones with differentiating and mature xylem. At the end of the growing period, wood density was assessed by micro-density analyses. Xylem differentiation at the top of the tree started at the beginning of April for both genotypes and proceeded down the stem at about 0.5 m day⁻¹, occurring almost at the same time as leaf opening. The rate of growth and wood density was superior in Dvina, but this higher productivity could not be explained by differences in the number of cambial initials and the duration of xylogenesis. However, the most productive poplar genotype showed higher glucose, fructose and sucrose content in the outer wood. The nonstructural soluble sugars available in the cambial zone followed the intra-annual pattern of xylem formation, with a higher concentration when the growth rate was maximum. The accumulations of nonstructural soluble sugars at a certain time during stem growth corresponded with a higher carbon availability to the actively growing meristems in the stem.

Keywords: carbohydrates, cell differentiation, phenology, wood density.

Introduction

Poplar hybrids present a wide variability in phenology, productivity and growth rate, leading to differences in biomass production and wood properties (Pellis et al. 2004, Monclus et al. 2005, Giovannelli et al. 2007). The different productivities have mainly been explained by intrinsic leaf features (Ceulemans and Deraedt 1999, Pellis et al. 2004, Monclus et al. 2005) that correspond to the energy source for growth. In the stem, soluble carbohydrates are the main source of energy for cell production and differentiation. However, few studies on intra-annual carbohydrate availability for wood production in broadleaves (Piispanen and Saranpää 2001, Barbaroux and Bréda 2002, Hoch et al. 2003) are available. These studies focused on carbohydrate content in the stem, but did not consider the meristem at the origin of wood production, the cambium. In modelling the influence of assimilates availability on plant growth, Thaler and Pagès (1998) found that root growth depends on assimilate allocation. In selected poplar and eucalyptus trees, the rapid increase in height corresponded to high carbohydrate use in meristems (Kozlowski 1992). However, to our knowledge, no comparative analysis has been done between wood formation and carbon availability at an intra-annual time scale.

Recently, there have been major steps forwards in the comprehension of cambial activity and xylogenesis through anatomical analyses (Rossi et al. 2006b, Fonti et al. 2007, Čufar et al. 2008, Deslauriers et al. 2008). At the plant level, wood formation is controlled by many factors such as hormones and sugars. Auxin has been reported to promote and to control xylem differentiation forming a concentration gradient over the developing tissues that peak around the cambium (Uggla et al. 1996, Tuominen et al. 1997). Similar to all processes of cell differentiation, the cambial zone (CZ) during xylogenesis may be considered as a powerful carbon
sink. The metabolic processes involved in cambial cell division, as well as differentiation of cambial cell derivatives, require energy. In particular, the conversion of photosynthetic assimilates in the cambium is enhanced during growth to support the formation of structural elements like cell walls (Krabel 2000). Even if the involvement of nonstructural carbohydrates in xylogenesis has already been reported (as energy, structural carbon supply and chemical signal), some physiological aspects of their role in the different stages of xylem formation remain unexplored.

Wood formation is a cyclical and gradual proliferation of xylem cells in which phases of cell production and differentiation are separated in space and time. Differentiation follows cell division that occurs in cambium. As the new derivative cells differentiate, other cells are produced, leading to a typical bell-shaped pattern of intra-annual variation, in which growth onset, maximum rate and ending are the crucial phenological stages (Rossi et al. 2006b). Some of these critical stages, such as onset and maximum growth rate, have been related to environmental factors such as temperature and photoperiod (Rossi et al. 2006, 2008), but carbohydrate availability during these phases of growth has to be taken into consideration to better understand the mechanisms of stem growth. Sugar content in wood has been analysed for some phenological stages, such as dormancy (Yoshioka et al. 1994), but has not been directly associated with xylogenesis.

This study focuses on the relationship between stem growth and nonstructural soluble carbohydrates. We tested the hypothesis that an enhanced xylogenesis is linked with higher nonstructural soluble carbohydrates in the CZ and wood. This hypothesis was tested by considering the nonstructural soluble carbohydrates related to (i) different growth performances of two poplar clones and (ii) different phases of the intra-annual pattern of xylogenesis. Several morpho-physiological factors were assessed (phenology, xylogenesis, stem density and stem water content) as they could also play an important role in the growth performance of the clone and in the intra-annual pattern of wood formation.

### Materials and methods

#### Study site

This study was conducted at the Poplar Research Institute, Casale Monferrato (AL), Piedmont, Italy (45°07’52” N and 8°30’17” E, 106 m a.s.l.). The climate of this site is temperate with an equinoctial rainfall distribution. Long-term (1926–2005) average annual temperature is 12.1 °C with a total annual rainfall of 765 mm and with an annual evapotranspiration rate (ET0-FAO) of 879 mm. The plantation consisted of 1-year-old coppice of *Populus × canadensis* Moench ‘1-214’ *Populus deltoides* Marsh. × *Populus nigra* L. and *P. deltoides ‘Dvina’*. Clones of both genotypes were arranged in 350-m-long rows with an inter-row distance of 2 and 0.5 m within rows, on an alluvial sandy-loam soil. Along four alternating internal rows (two per genotype), 140 stems of average dimensions, one per stool, were selected and numbered. The average stem diameters at 0.3 m were 25.6 ± 8.9 mm for Dvina and 17.9 ± 8.9 mm for I-214. During most of the experiment, the plantation was left with natural rainfall, being irrigated only during a long dry period.

#### Sample collection

From February to November 2006, tree growth and phenology were monitored, combined with soluble carbohydrates and water content in the stem (Figure 1). Three stems were randomly harvested in each row and measured (height and diameter at 0.3 m) each week from day of the year (DOY) 61 to 319 (2 March–15 November), except for one set of samples collected on DOY 33 (2 February). Sampling was done in the early morning to avoid the period of excessive transpiration. Stem discs of 1–2 cm thickness were cut at 0.3 m from the collar and at 1 m from the top for anatomical measurements of wood formation (Figure 2). For the stem water content analysis, additional stem portions of 5 cm were cut on 14 sampling days (Figures 1 and 2). For soluble carbohydrate content analysis, wood samples of 25 cm in length were collected above the samples of xylem formation (Figure 2) on five sampling days (Figure 1) corresponding to the main stages of cambial activity: (i) dormancy, (ii) onset of xylem differentiation, (iii) maximum growth rate, (iv) decreasing...
and (v) ending of xylem differentiation. Microdensity was assessed at the end of the vegetative period when the tree ring was formed (Figure 1). Two-millimetre-thick sections were collected at 0.3 m from the collar (Figure 2) on 12 individuals per genotype.

Bud phenology
Bud phenology and leaf abscission were monitored twice per week (Figure 1) and bud break stages were divided into five classes according to Castellani et al. (1967): stage 1, buds swelling with a slight separation of the scales; stage 2, bud break initiated at the apical part of the bud with leaves visible; stage 3, bud break with leaves open, but still joined together and scales still present; stage 4, leaves slightly open with or without scales; and stage 5, small leaves completely open, but of smaller sizes than mature leaves and shoots elongating.

Anatomical measurements of radial growth
Stem discs were placed in ethanol (50% in water) and stored at 5 °C. The discs, or their slices, were embedded in paraffin and transverse sections of 8–12 μm thickness were cut using a rotary microtome, dried at 50 °C for 1 h and cleaned off the residual paraffin with successive immersions in n-limonene and ethanol (Rossi et al. 2006a). The sections were stained with a solution of 0.04% safranin, 0.15% astrablue and 2% acetic acid in distilled water (van der Werf et al. 2007), and were permanently fixed with a histological mounting medium (Eukitt®, Biopita, Milan, Italy) to observe the developing tissues. The CZ and xylem in a radial enlargement showed only primary walls, which did not shine under polarized light (Figure 3A, B and D). Xylem in a secondary wall thickening phase shone under polarized light with walls changing from blue at the beginning of the process to red at the mature state (Figure 3A and C). Lignification was characterized by the appearance of red, initiating in the middle lamella and spreading into the secondary walls of the differentiating cells (Figure 3A).

Two types of measurements were taken on the sample: (i) the width of the zone containing both vessels and fibres in differentiation (μm), including cells in radial enlargement and wall thickening (enl and wt in Figure 3A) and (ii) the total width of xylem formed (μm), including differentiating and mature cells (zones of enl, wt and MX in Figure 3A). The measurement was done on photographs taken with a Nikon camera fixed on a microscope at magnifications of 2–50×, according to the width of the measured zones. When the total width of xylem formed exceeded 2000 μm, measurements were taken using a binocular to the nearest 10 μm with a semiautomatic measuring system connected to a computer. In spring, when at least one horizontal row of vessels was observed in the enlarging phase (Figure 3D), xylem formation was considered to have begun. In late summer, when no further cell was observed in wall thickening and lignification, xylem formation was considered complete.

Density measurements
Sections were air-dried at 12% moisture content and X-rayed together with a calibration wedge following the standard techniques (Polge 1978). Radiographs were digitalized using a scanner, and the acquired greyscale digital images were treated using semiautomatic procedures (Mothe et al. 1998). Density values (kg m⁻³) were assigned to each pixel of the wood samples by comparing their grey scales with those of the calibration wedge. Each tree ring was divided into 20 segments of equal width, and the tree-ring density profiles were produced by averaging the values obtained from these segments. For each wood section, the mean density determined by X-ray transmission was compared with the density directly determined by measuring the mass per volume unit to correct the microdensity measurements.

Stem water content
Discs for water content analysis were immediately wrapped in a plastic film and weighed within 15 min after harvest to determine the fresh mass (g). The fresh volume of samples (cm³) was assessed by water displacement (Borghetti et al.
The dry mass (g) was measured after the samples were maintained at 72 °C for 96 h. Relative stem water content (RWC) was calculated following Domec and Gartner (2001):

\[
RWC = \frac{M_f - M_d}{(V_f - V_s)} \times 100,
\]

where \(M_f\) and \(M_d\) are the fresh and dry mass of the wood (g), respectively, and \(V_f\) and \(V_s\) are the volumes of fresh and solid material (cm³), respectively. The \(V_s\) was estimated by dividing \(M_d\) by 1.53 assuming a density of 1.53 g cm⁻³ for dry cell wall material (Skaar 1988).

**Analysis of soluble sugars and starch**

The samples were frozen in liquid nitrogen and freeze dried for 10 days. After removing the bark, a white powder containing tissues of the CZ was collected by scraping the middle part between bark and xylem using a scalpel, except during dormancy when cambial tissues were not separable (2 February, DOY 33). The mature xylem (MX) of the current year (Figure 2) was collected and reduced to a fine powder with a rotor mill. During dormancy (DOY 33), MX corresponded to the tree ring of the previous year of growth. The dried powders were kept under vacuum at −20 °C until sugar extraction.

For both CZ and MX, 40 mg of dried powder was repetitively extracted three times in 5 ml of 80% EtOH, adjusted to pH 7, at room temperature. The 15-ml solution was evaporated to dryness at room temperature with a Savant Speedvac Plus SC210A system (ThermoQuest, SVPT, Rome, Italy) and diluted with 2 ml of distilled water (pH 7). The solution obtained was then fractionated using
liquid–solid extractions carried out by eluting samples through (i) a reverse-phase cyclohexyl resin (pre-packed 3 ml Bond Elut CH cartridge, Varian, Harbor City, CA) and (ii) a quaternary-amine, strong anion-exchange resin (pre-packed 3 ml Bond Elut SAX cartridge, Varian). The cartridges were activated by adding 6 ml MeOH and conditioned by adding 6 ml distilled water. Analyses were conducted using a binary LC pump 250 (Perkin Elmer) equipped with an automatic injection system (ISS101, Perkin Elmer). A Water Column Heater Module (Waters Division, Millipore, Milford, MA) controlled by a Temperature Control Module (Waters) maintained the column at 80 °C. The column was 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 was water, Milli-Q grade, at 0.5 ml min⁻¹.

Identification and quantification of soluble carbohydrates were performed according to Romani et al. (1994), and the identity of soluble carbohydrates was confirmed using authentic carbohydrate standards (Sigma) and adding an internal standard. The recovery was estimated for each carbohydrate. Thus, 0.25, 0.50, 0.75 and 1.0 ml of 1 mg ml⁻¹ carbohydrate solutions were fractionated and analysed as previously described, with the recovery ranging from 92% to 99%. Calibration curves were performed for raffinose, sucrose, glucose, galactose, fructose, mannitol and sorbitol. Total soluble sugar content was obtained as the sum of the detected sugars (> 0.1 μmol g⁻¹ DW).

Starch was measured in the pellet remaining after extraction with 80% ethanol according to Gucci et al. (1991). After incubation at 55 °C for 16 h with amyloglucosidase (Fluka), the samples were diluted with distilled water to 5 ml, and three 0.25-ml aliquots of each sample were assayed colorimetrically using glucose oxidase (Sigma). Absorbance was read at 440 nm. For I-214 clone, the amount of material remaining after the extraction of soluble sugars from cambium on 26 July (DOY 207) was not sufficient to perform the starch analysis.

Statistical analyses

Gompertz functions were fitted by nonlinear regressions (NLIN procedure in SAS) to estimate the pattern of radial and height growth (y) against time (t, computed in DOY) (Deslauriers et al. 2003):

\[ y = I + A \exp \left( -e^{(\beta - ct)} \right), \]

where the parameters A, β and κ are the growth asymptotes, the time-axis placement and the rate of change of the curve, respectively, and I represents the initial growth, which is set to zero for radial growth. The estimated tree height at the end of growth is obtained by summing the parameters I and A. In the Gompertz function, the inflection point \( t_p \) corresponds to the culmination of growth rate (Rossi et al. 2006c), defined as

\[ t_p = \frac{\beta}{\kappa}. \]

From the estimated parameters, the weighted mean absolute rate of growth (r) was calculated according to Deslauriers et al. (2003):

\[ r = \frac{AK}{4}. \]

The seasonal changes in soluble sugars were compared between poplar genotypes using the analysis of variance (ANOVA by the general linear model procedure (GLM), SAS system). A factorial model was used to test the effects of clone and sampling date. For each sampling date, differences between clones were found using LS means (Quinn and Keough 2002).

Results

Leaf phenology and height growth

Bud development started in both poplar genotypes in March (DOY 81) when bud swelling (stage 1) was observed (Table 1). However, during the next stages, I-214 demonstrated a faster bud development with stages 2 and 3 (bud opening but leaves still joined together) realized within 6 days. The completion of leaf opening was observed earlier for I-214, on 5 April (DOY 95), indicating that the bud development lasted for 14 days. For Dvina, stage 5 was reached 1 week later with a bud development of 21 days. Leaf abscission began on 10 October (DOY 283) for both genotypes and lasted for about 35 days. Lignification of apical buds ended earlier for I-214 than for Dvina. Overall, leaves were present for about 200 days.

Table 1. Bud phenology of Dvina and I-214 in 2006 reported as date and DOY in parentheses. Stage 1, buds swelling with a slight separation of the scales; stage 2, bud break initiated at the apical part of the bud with leaves visible; stage 3, bud break with leaves open but still joined together and scales still present; stage 4, leaves slightly open with or without scales; and stage 5, small leaves completely open but of smaller size than mature leaves and shoots elongating.

<table>
<thead>
<tr>
<th>Stage</th>
<th>I-214</th>
<th>Dvina</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>22 March (81)</td>
<td>22 March (81)</td>
</tr>
<tr>
<td>2</td>
<td>28 March (87)</td>
<td>31 March (90)</td>
</tr>
<tr>
<td>3</td>
<td>30 March (89)</td>
<td>4 April (94)</td>
</tr>
<tr>
<td>4</td>
<td>3 April (93)</td>
<td>7 April (97)</td>
</tr>
<tr>
<td>5</td>
<td>5 April (95)</td>
<td>11 April (101)</td>
</tr>
</tbody>
</table>
Despite differences in the initial height of the plants (4.33 m for Dvina versus 3.52 m for I-214), both I-214 and Dvina had a similar height growth during 2006 (Table 2; Figure 4), which is estimated at ca. 2 m. Compared with Dvina, I-214 had a higher rate of apical growth (0.036 m day$^{-1}$) and an earlier height culmination (about 10–20 days).

Table 2. Growth response curves for Dvina and I-214, fitted to the cumulative height growth (m) and stem radial growth (µm) at 0.3 m. Parameter $I$ represents the initial estimated tree height, and $A$, $\beta$ and $\kappa$ are the parameters of the Gompertz function. The numbers in parentheses are the standard deviations of the estimated parameters. Rates ($r$) are expressed in m day$^{-1}$ for height growth and in µm day$^{-1}$ for radial growth and $t_p$ values correspond to the times of the inflection point of the Gompertz function.

<table>
<thead>
<tr>
<th>Growth</th>
<th>Genotype</th>
<th>$I$</th>
<th>$A$</th>
<th>$\beta$</th>
<th>$\kappa$ (10$^{-2}$)</th>
<th>$r$</th>
<th>$t_p$ (DOY)</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Height</td>
<td>Dvina</td>
<td>4.33 (0.07)</td>
<td>2.12 (0.10)</td>
<td>4.41 (0.6)</td>
<td>2.85 (0.4)</td>
<td>0.031</td>
<td>154</td>
<td>0.81</td>
</tr>
<tr>
<td></td>
<td>I-214</td>
<td>3.52 (0.07)</td>
<td>2.25 (0.11)</td>
<td>6.68 (1.1)</td>
<td>4.18 (0.7)</td>
<td>0.036</td>
<td>159</td>
<td>0.78</td>
</tr>
<tr>
<td>Radial</td>
<td>Dvina</td>
<td>0</td>
<td>8030.8 (95.9)</td>
<td>6.33 (0.4)</td>
<td>4.39 (0.3)</td>
<td>88</td>
<td>144</td>
<td>0.95</td>
</tr>
<tr>
<td></td>
<td>I-214</td>
<td>0</td>
<td>5656.3 (76.0)</td>
<td>7.34 (0.5)</td>
<td>5.09 (0.3)</td>
<td>83</td>
<td>144</td>
<td>0.94</td>
</tr>
</tbody>
</table>

Figure 4. Intra-annual tree growth in Dvina (left) and I-214 (right) in 2006. (A) Tree height growth (m) and its intra-annual trend (black lines); (B) stem growth (µm) at 1 m from the top of the tree (grey dots) and at 0.3 m from the base (black dots) and its intra-annual trend (black lines); and (C) width of the developing cell zones (µm) at 1 m from the top of the tree (grey dots) and at 0.3 m from the base (black dots). Dots represent average growth and bars standard deviation among six individual plants. The intra-annual trends were calculated by the Gompertz function (see Table 2).
but reached maximum growth rate later on 8 June (DOY 159). Dvina concluded the apical growth at a total height of 6.45 m, compared with that of 5.77 m for I-214.

**Xylem phenology, radial growth and density**

Similar seasonal dynamics of cambial activity were observed in the two poplar genotypes. In winter, the dormant cambium was composed of two to four rows of cells close together. During exponential radial growth, the number of rows of cambial cells increased to eight (data not shown). At the top, xylem differentiation started on 5 April (DOY 95) and on 9 April (DOY 99) for Dvina and I-214, respectively (Table 3). At the base, xylem differentiation started 1 week later in both clones. The time of vessel formation, calculated as difference between the onset of xylem differentiation and the date when the first mature vessels were observed, was estimated at 10–14 days for both sampling heights. As vessel formation started earlier at the top, mature vessels were observed earlier at the top of the stem.

In April, the width of differentiating xylem increased rapidly, reaching a maximum of 300 and 220 μm at the base and at the top of the stem, respectively, at the beginning of June. Similar amplitudes of differentiating xylem were observed in I-214 and Dvina (Figure 4). In July–August, the width of the differentiating xylem decreased with a first minimum achieved on 18 August (DOY 230). However, a new resumption of cambial cell division and differentiation was observed afterwards, which appeared as a small false ring in several stems and led to higher variations in the end of xylem differentiation. At the top, xylem formation ended on 13 September (DOY 256) for both genotypes, 2 weeks later than at the base (Table 3). Xylogenesis duration at the top was about 160 days, 3 weeks longer than at the base, with no marked differences between genotypes.

The seasonal trend of xylem production at the base was characterized by a sharp increase, followed by a plateau indicating the end of radial growth (Figure 4). Dvina had higher rates of radial growth and produced wider tree rings, 8030 μm, than I-214, 5656 μm (Table 2). Both poplar genotypes reached their maximum growth rates at the end of May, on DOY 144. Stem radial growth at the top decreased slightly from 14 June (DOY 165) and remained constant. On that date, height growth had exceeded 1 m, and the collected discs were located in the 1-year-old stem, at a constant distance from the growing buds (Figure 4).

The density profiles were similar in the two genotypes, but with lower values for I-214 (Figure 5). In Dvina, wood density gradually increased from 350 to 400 kg m⁻³ along the tree ring. In I-214, the density remained at values between 275 and 300 kg m⁻³, except for the last 10% of the ring width, showing an abrupt increase in density.

**Stem water status**

Similar trends of RWC were observed in the genotypes, with values more or less following the distribution of precipitations (Figure 6). During dormancy, I-214 had a lower RWC than Dvina (63% versus 80%). After cambial reactivation, RWC decreased around the end of May (DOY 150), when the width of the zones containing cells in enlargement and wall thickening was at its maximum. The RWC reached its minimum values (40–50%) at the end of ring width formation.

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**Table 3. Timing of xylem formation in Dvina and I-214 expressed at 0.3 m from the collar (base) and 1 m from the top of the stem (top). The numbers in parentheses represent the standard deviations of the mean values.**

<table>
<thead>
<tr>
<th>Stem height</th>
<th>Genotype</th>
<th>Onset of xylem differentiation (DOY)</th>
<th>First mature vessel (DOY)</th>
<th>End of xylem differentiation (DOY)</th>
<th>Duration of differentiation (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Top</td>
<td>Dvina</td>
<td>94.5 (3.7)</td>
<td>109.0 (2.5)</td>
<td>256.0 (14.5)</td>
<td>161.5</td>
</tr>
<tr>
<td></td>
<td>I-214</td>
<td>98.6 (4.7)</td>
<td>108.6 (2.8)</td>
<td>256.2 (13.8)</td>
<td>157.6</td>
</tr>
<tr>
<td>Base</td>
<td>Dvina</td>
<td>101.8 (2.4)</td>
<td>113.9 (3.5)</td>
<td>242.6 (13.5)</td>
<td>140.8</td>
</tr>
<tr>
<td></td>
<td>I-214</td>
<td>105.7 (4.8)</td>
<td>115.1 (3.1)</td>
<td>238.5 (10.4)</td>
<td>132.8</td>
</tr>
</tbody>
</table>

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**Figure 5. (A) Variation in wood density (kg m⁻³) along the growth ring for Dvina (black dots) and I-214 (grey dots). Vertical bars indicate standard deviation. (B) Distribution frequency of the density measurements (kg m⁻³) for Dvina (black bars) and I-214 (grey bars).**
Soluble sugars and starch

The major soluble sugars detected were glucose and fructose, together representing 70% and 90% of the total soluble sugars during dormancy and the growing period, respectively (Figure 7). Sucrose represented 12% in MX and 6% in CZ of the total soluble sugars, whereas raffinose, galactose and mannitol were detected only in small quantities in MX (< 8 μmol g⁻¹ DW, Table 4). In CZ, the total soluble sugars were about 10-fold higher than those in MX. For all major soluble sugars, Dvina showed higher quantities, except for sucrose in MX (Table 5; Figure 7). Significant differences were also observed between sampling dates, although no interaction genotype × DOY was observed (P < 0.05).

In MX, the total amounts of soluble sugars were mainly related to glucose and sucrose with high concentrations in winter, a decrease during the onset of xylem differentiation (20 April, DOY 110) and a further increase in correspondence to the maximum growth rate (1 June, DOY 152). Afterwards, a new minimum was reached during decreasing (26 July, DOY 207) and ending (24 August, DOY 236) of xylem differentiation (Figure 7). The same seasonal trend was observed in CZ during xylem differentiation with a significantly higher concentration in Dvina, especially at maximum growth rate, when glucose and fructose concentrations were more than 1.5 times higher than in I-214 (Figure 7). In CZ, sucrose showed a different trend with the highest amounts recorded at decreasing of xylem differentiation (DOY 207). The glucose–fructose ratio changed during the season in both CZ and MX. For both genotypes, this ratio in CZ was about 1.2 at the onset of xylem differentiation and at maximum growth rate and decreased to 0.7–0.9 during the successive stage. During cambium dormancy, the glucose–fructose ratio in MX reached about 2 in both genotypes, but drastically decreased during maximum growth rate, with values of 0.7 for I-214 and of 1.1 for Dvina. This decrease was mainly due to a doubling of fructose concentration (from 16.5 to 35.1 μmol g⁻¹ DW in I-214 and from 24.3 to 49.2 μmol g⁻¹ DW in Dvina).

In comparison with the other major soluble sugars, no seasonal trend of raffinose, galactose and mannitol was observed in MX, whereas in CZ, only a small quantity of raffinose was detected (Table 4). In MX, these sugars were detected mainly during dormancy (2 February, DOY 33), with higher amounts of galactose and mannitol found in I-214. At the onset of xylem differentiation (DOY 110), galactose, raffinose and mannitol disappeared, and only galactose was detected during maximum growth rate (ca. 1 μmol g⁻¹ DW). Traces of raffinose were recorded during both decreasing and ending of xylem differentiation in MX.

During dormancy, starch concentration ranged between 1 and 1.5 mg g⁻¹ DW in MX, with a higher concentration found in Dvina (Figure 8; Table 5). In both MX and CZ, starch decreased after bud break (DOY 107–152), and in MX it remained at low concentrations for the rest of wood formation. In CZ, high concentrations of starch were observed during the ending of xylem differentiation (DOY 236) (from 43 to 45 mg g⁻¹ DW) with no significant difference between poplar genotypes.

Discussion

The poplar genotype with the greater radial growth and density showed a higher concentration of nonstructural soluble sugars in both CZ and xylem. Moreover, during the growing season, the nonstructural soluble sugars varied according to xylem growth with much higher concentrations (5–10 times higher) found in the CZ than in the wood. Sucrose transport from source leaves to sink organs is controlled by sink strength or by leaf maturation and photosynthesis (Marchi et al. 2007). The translocation and use of sugar is not fixed in time and depends directly on the state of the meristem (Thaler and Pagès 1998), represented in this work by
cambial division and cell differentiation, which varied greatly during the growing period. We demonstrated that the accumulations of nonstructural soluble sugars at a certain time during growth corresponded with a higher carbon availability to an actively growing meristem. Moreover, as cell division activity is crucial in attracting assimilates (Ho 1988), sugars followed more or less the different stages of wood formation in the stem. The observed vigorous xylogenesis, both between the genotypes and within the growing period, showed a close link between the amount of carbon and the activity of cambium.

**Growth performance**

According to our results, the higher productivity of Dvina could not be explained by the different phenology of stem growth or cambial cell development. Cambium of both I-214 and Dvina was constituted of two to four cells during dormancy and increased to seven to eight cells during exponential growth, which may show a similar division potential if rate of division is not accounted for. Although the duration of xylogenesis can influence the radial growth (Marion et al. 2007, Rossi et al. 2007, 2008a, Deslauriers et al. 2008), xylem differentiation at both tree heights lasted only for a few days more in Dvina than in I-214. At the observed rates of growth, ca. 50 days more would be required for I-214 to produce the 2300 μm lacking to attain the ring width of Dvina. The similar width of the differentiating zones associated to different amounts and density of xylem produced implies that Dvina was faster in producing and maturing cells. Moreover, similar trends of RWC were observed in both poplar genotypes, which means that the developing cells had a comparable water supply for their expansion and maturation. The higher wood production in Dvina was, therefore, caused by a higher rate of xylem production and differentiation and not by differences in phenology or cells constituting the CZ.

The higher growth and density of Dvina was linked with the higher content of soluble sugar measured in the outer wood, especially when growth rate was maximum. The higher content of soluble sugars measured in Dvina demonstrated that this genotype has a superior flow and availability of carbohydrate to differentiate tissues, despite its higher use. In a previous work, Giovannelli et al. (2007) found that leaf CO₂ assimilation was similar in both genotypes showing that the photosynthetic rate was probably not involved in the higher carbohydrate availability of Dvina. Even at the onset of wood formation, growth potential of Dvina was already greater because of the higher soluble sugars content and starch concentration in the cambium and xylem, as a high amount of available carbon influences how trees start growth (Barbaroux and Bréda 2002, Skomarkova et al. 2006). The increased demand for assimilates may also cause more assimilates to be directed to the metabolism (Kuiper 1993), illustrating feedback relationships in the allocation to growth (Lacointe 2000). Therefore, the vigorous xylogenesis of Dvina could be linked with higher soluble carbohydrates caused by its higher sink strength.
**Stages of wood formation**

**Cambial dormancy** Dormancy was characterized by high levels of soluble sugars and low concentration of starch in MX, confirming the results from other broadleaved species (Yoshioka et al. 1988, Piispanen and Saranpää 2001) and was due to winter starch-to-sugar conversion in woody tissues driven by low temperatures (Sauter 1988, Schrader and Sauter 2002). Dormant cambium of poplar showed a high prevalence of carbohydrate-active enzyme transcripts as starch synthase and β-amylase, which are related to starch metabolism (Geisler-Lee et al. 2006). This conversion increases the concentration of soluble carbohydrates, which act as effective cryoprotectants (Magel et al. 1994, Sauter et al. 1996) and could be used as energy source at the onset of the growing period (Magel et al. 2001). In this study, glucose, fructose and sucrose were major soluble sugars, while small amounts of galactose, raffinose and mannitol were detected. Raffinose is supposed to be derived from starch breakdown induced by low temperature, and it is considered a major winter cryoprotectant in poplar (Druart et al. 2007). Mannitol, the only soluble sugar that does not derive from the starch-to-sugar conversion, has never been reported in poplar. It is a direct product of the photosynthetic carbon fixation, and may participate in a wide range of physiological processes (Noiraud et al. 2001) as a winter cryoprotectant.

**Onset of growth** Higher temperature in spring leads to the onset of tree growth by influencing cambial sensitivity to auxin and by determining when the newly produced

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**Table 4.** Seasonal changes in raffinose, galactose and mannitol (µmol g⁻¹ DW) reported as mean ± standard deviation detected in the CZ and in the MX of *Populus × canadensis* ‘I-214’ and *P. deltoides* ‘Dvina’. The sampling days correspond to the main stages of cambial activity: (i) dormancy (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. Amounts of soluble sugar equal to zero are expressed as not detected (nd).

<table>
<thead>
<tr>
<th>Zone</th>
<th>DOY</th>
<th>Raffinose (µmol g⁻¹ DW)</th>
<th>Galactose (µmol g⁻¹ DW)</th>
<th>Mannitol (µmol g⁻¹ DW)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Dvina</td>
<td>I-214</td>
<td>Dvina</td>
</tr>
<tr>
<td>CZ</td>
<td>110</td>
<td>nd</td>
<td>0.77 ± 0.89</td>
<td>nd</td>
</tr>
<tr>
<td></td>
<td>152</td>
<td>0.14 ± 0.28</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td></td>
<td>207</td>
<td>nd</td>
<td>1.05 ± 0.99</td>
<td>2.06 ± 1.17</td>
</tr>
<tr>
<td></td>
<td>236</td>
<td>3.44 ± 0.76</td>
<td>2.55 ± 0.60</td>
<td>4.02 ± 1.50</td>
</tr>
<tr>
<td>MX</td>
<td>33</td>
<td>3.44 ± 0.76</td>
<td>2.55 ± 0.60</td>
<td>4.02 ± 1.50</td>
</tr>
<tr>
<td></td>
<td>110</td>
<td>nd</td>
<td>1.20 ± 0.23</td>
<td>1.03 ± 0.18</td>
</tr>
<tr>
<td></td>
<td>152</td>
<td>0.22 ± 0.21</td>
<td>0.28 ± 0.05</td>
<td>nd</td>
</tr>
<tr>
<td></td>
<td>207</td>
<td>0.18 ± 0.16</td>
<td>0.25 ± 0.11</td>
<td>nd</td>
</tr>
<tr>
<td></td>
<td>236</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
</tbody>
</table>

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**Table 5.** The ANOVA comparisons of content in soluble sugars and starch detected in the CZ and in the MX among poplar genotypes, sampling dates (DOY) and their interaction (genotype × DOY). ns, not significant (P > 0.05).

<table>
<thead>
<tr>
<th>Sugars</th>
<th>Source</th>
<th>CZ F</th>
<th>P value</th>
<th>MX F</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>Genotype</td>
<td>50.6</td>
<td>&lt; 0.0001</td>
<td>25.4</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td></td>
<td>DOY</td>
<td>36.9</td>
<td>&lt; 0.001</td>
<td>33.5</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td></td>
<td>Genotype × DOY</td>
<td>8.93</td>
<td>&lt; 0.001</td>
<td>2.3</td>
<td>ns</td>
</tr>
<tr>
<td>Glucose</td>
<td>Genotype</td>
<td>30.4</td>
<td>&lt; 0.0001</td>
<td>40.4</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td></td>
<td>DOY</td>
<td>41.1</td>
<td>&lt; 0.0001</td>
<td>29.6</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td></td>
<td>Genotype × DOY</td>
<td>9.1</td>
<td>&lt; 0.001</td>
<td>3.9</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Fructose</td>
<td>Genotype</td>
<td>25.2</td>
<td>&lt; 0.0001</td>
<td>18.1</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>DOY</td>
<td>16.1</td>
<td>&lt; 0.0001</td>
<td>29.0</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td></td>
<td>Genotype × DOY</td>
<td>4.5</td>
<td>&lt; 0.05</td>
<td>0.8</td>
<td>ns</td>
</tr>
<tr>
<td>Sucrose</td>
<td>Genotype</td>
<td>20.2</td>
<td>&lt; 0.001</td>
<td>1.6</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td>DOY</td>
<td>31.6</td>
<td>&lt; 0.0001</td>
<td>34.5</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td></td>
<td>Genotype × DOY</td>
<td>0.2</td>
<td>ns</td>
<td>0.4</td>
<td>ns</td>
</tr>
<tr>
<td>Starch</td>
<td>Genotype</td>
<td>13.2</td>
<td>0.0017</td>
<td>27.2</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td></td>
<td>DOY</td>
<td>9.65</td>
<td>&lt; 0.0001</td>
<td>81.4</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td></td>
<td>Genotype × DOY</td>
<td>1066</td>
<td>0.0012</td>
<td>3.82</td>
<td>0.0130</td>
</tr>
</tbody>
</table>
cells start to differentiate (Fonti et al. 2007). At the top, onset of cell differentiation occurred at the same time as (in I-214) or a week earlier than (in Dvina) complete leaf opening. In both genotypes, wood formation started about 1 week later at the base than at the top, indicating that the onset of vessel enlargement proceeded down the stem at about 0.5 m day$^{-1}$. In *Fagus sylvatica* L., leaf unfolding and cambium reactivation at the stem base occurred almost at the same time (Čufar et al. 2008), corresponding with our results in Dvina but not in I-214. However, in hybrid poplars, cambial reactivation occurred before the development of new leaves in heating portion of the stem, indicating that there was no need for a supply of photosynthate from leaves in this stage (Begum et al. 2007). Therefore, energy and carbon skeleton required for wood formation could not be supplied by the newly produced assimilates, but they should entirely be derived by the enzyme-mediated conversion of the stored starch. This contrasts with the classical view that the onset of stem radial growth does not depend on carbon reserve in diffuse-porous species (Kozlowski 1992, Barbaroux and Bréda 2002, Barbaroux et al. 2003).

According to Hoch et al. (2003), newly formed leaves of deciduous trees become autonomous from C-reserves at an early stage of their development. Photosynthetic rates are generally low in young expanding leaves and increase after leaf expansion (Marchi et al. 2007) and leaves of dicotyledonous plants change from sink to source when reaching 30–60% of their final area (Turgeon 1989). For stem radial growth, however, C-reserves were probably used at the beginning of wood formation as the total soluble sugars in MX halved in respect to dormancy. Shortly after bud break (< 2 weeks), the differentiating zones of both clones had already achieved about half of their maximum amplitude, rapidly becoming a considerable sink and explaining the abrupt decrease in soluble sugars in xylem. In *Populus × canadensis*, the amount of total soluble sugars in wood of 3-year-old branches was at its minimum during bud break in late April–early May (Sauter and van Cleve 1994), corresponding with our results.

**Maximum growth rate** High rates of stem radial growth were observed between mid-May and mid-June, with a maximum growth rate estimated for I-214 and Dvina on 24 May (DOY 144), about 10–15 days later than that of height growth. The major soluble sugars in the CZ also peaked in correspondence to maximum growth rate except for sucrose that peaked later, when cambial activity was decreasing. These high concentrations of sugars were probably linked with sucrose metabolism in the actively growing tissues to respond to the high demand of cell wall material. During growth, sucrose is the major form of translocated carbon (Krabel 2000), which is cleaved near sink tissues to form fructose and glucose by either enzymes (such as sucrose synthase) or invertase (Johansson 2003). Detailed studies on outer wood of *Robinia pseudacacia* L. and *Populus × canadensis* confirmed the increasing activities of sucrose synthase and invertase from April to July, with a peak in May–June for sucrose synthase, in correspondence with cell differentiation (Hauch and Magel 1998, Schrader and Sauter 2002). In tissues with secondary wall-forming cells, the starch metabolic genes are normally underexpressed, and this suggests that the carbon from photosynthates arriving at these tissues from the leaves is immediately shifted to secondary wall cellulose synthesis or consumed for energy (Geisler-Lee et al. 2006).

Recent assessments on *Acer platanoides* L. (Marion et al. 2007) and *F. sylvatica* (Čufar et al. 2008) growing in temperate regions positioned the maximum growth rate around the end of May, which is in agreement with our results. Maximum growth rate in conifers of cold climates occurs around the culmination of day length, to safely complete secondary cell wall lignification before winter (Rossi et al. 2006c). Broadleaves have to produce and activate the conducting elements rapidly in spring to sustain the water transport and the high transpiration of the developing leaves, which could be a reason to anticipate maximum growth rate. Moreover, fibres completed differentiation in < 2 weeks (data not shown) ending cell differentiation faster than tracheids in conifers (up to 40 days, Rossi et al. 2006b).

**Decreasing of cambial activity and end of cell differentiation** Reduction in both growth rate and in total soluble sugars occurred at the end of July. The reduction in glucose and fructose could be linked to sucrose metabolism, as sucrose synthase and invertase activity decline in August (Hauch and Magel 1998). At the decreasing of xylem differentiation, the total nonstructural soluble sugar was still higher in the cambium of Dvina, while
similar contents were observed between poplar genotypes when xylogenesis was concluded. Cell differentiation was completed 1 month later, at the end of August, indicating that soluble sugar concentration in xylem and cambium decreases before the end of xylogenesis. At the same time, raffinose content increased. The expression of genes involved in raffinose biosynthesis was strongly increased during the decreasing of cambial activity in Populus tremula L., and it could be related to acquisition of cold hardiness (Druart et al. 2007) in concomitance with the decline of photosynthesis (Keskitalo et al. 2005). In a separate experiment, a slight decrease in CO₂ assimilation was recorded on both clones during this phase (Giovannelli et al. 2007). In total, xylogenesis lasted for about 160 days at the top and between 133 and 141 days at the base, depending on the genotype. As leaves were present about 200 days until 10 October (DOY 283), cambium phenology in the stem ended much before, about 40 days earlier than leaf phenology, despite a similar onset.

At the end of cell differentiation, a pronounced starch accumulation took place in the differentiating xylem, confirming the previous results on Populus × canadensis Moench ‘robusta’ (Witt and Sauter 1994). In this species, authors estimated the summer starch deposition rate in poplar wood to be 0.24 mg starch g⁻¹ DW day⁻¹. Moreover, wood density increased in the last 15% of the ring width during August, reaching a mean maximum density of about 350 and 400 kg m⁻³ for ‘I-214’ and Dvina, respectively. These differences in density were related to wider cell walls and to the higher ratio between wall and lumen area in fibres of Dvina (data not shown). The higher density represents a stronger carbon demand at the end of the growing season, at a time when carbon availability in cambium is decreasing. The asynchronous reduction of both xylem production and sugars leads to the unresolved question of whether the conclusion of wood formation is cause or effect of the reduction in sugar availability.

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