INTRA-ANNUAL CAMBIAL ACTIVITY AND CHANGES IN CARBON AVAILABILTY IN STEM OF TWO POPLAR CLONES

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SUMMARY

Cambium 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 focussed 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 clones with different growth performances were considered. Monitoring of stem growth and leaf phenology combined with starch, non-structural soluble sugars and water content in the stem was conducted from February to November 2006 in *Populus* × *canadensis* Moench 'I-214' and *P. deltoides* '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 microdensity analyses. Xylem differentiation at the top of the tree started at the beginning of April for both clone and proceeded down the stem at about 0.5 m day^{-1}, occurring almost at the same time then leaf opening. The rate of growth and wood density was superior in Dvina but this higher productivity could not be explained by differences in number of cambial initial and durations of xylogenesis. However, the most productive clone showed higher glucose, fructose and sucrose contents in the outer wood. The available non-structural soluble sugars in the cambial zone followed the intra-annual pattern of xylem formation, with higher concentration when growth rate was maximum. The accumulations of non structural soluble sugars at a certain time during stem growth corresponded with higher carbon availability to the actively growing meristems in the stem.

Key words: Cambial activity, 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), which 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 are available on intra-annual carbohydrate availability for wood production in broadleaves (Piispanen and Saranpää 2001, Barbaroux and Bréda 2002, Hoch et al. 2003). 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 poplars and eucalyptus, 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 level.

Recently, there have been major steps forward in the comprehension of cambial activity and xylogenesis through anatomical analyses (Fonti et al. 2007, Čufar et al. 2008, Rossi et al. 2006b, Deslauriers et al. 2008), but none included the role of available soluble carbohydrates in the seasonal dynamics of wood formation in stem. Like all processes of cell differentiation, the cambial zone 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 photo-assimilates in the cambium is enhanced during growth in order to support the formation of structural elements like cell walls (Krabel 2000). Even if the involvement of non-structural 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 more or less 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 occurring in the cambium. As the new derivative cells differentiate, other cells are produced, leading to the typical bell-shaped pattern of variation during the year, 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. 2006c, Čufar et al. 2008, Rossi et al. 2008b), but carbohydrate availability during these phases of growth has rarely been taken into consideration in order to better understand the mechanisms of stem growth in trees. Sugar content in wood has been analysed for some phenological stages, such as dormancy (Yoshioka et al. 1988, Magel et al. 2001, Piispanen and Saranpää 2001) and bud break (Kozlowski 1992, Sauter and van Cleve 1994) but has not been directly associated with xylogenesis.

This paper focuses on the relationship between stem growth and non-structural soluble carbohydrates. We tested the hypothesis that an enhanced xylogenesis is linked with higher non-structural soluble carbohydrates in the cambial zone and wood. This hypothesis was verified by considering the non-structural soluble carbohydrates related

to (i) the different growth performances of two poplar clones and (ii) the 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 the intra-annual pattern of wood formation.

MATERIALS AND METHODS

Study site

The study was conducted at the Poplar Research Institute, in Casale Monferrato (AL), Piedmont, Italy (45°07'52'N, 8°30'17'E, 106 m a.s.l.). The climate of the site is temperate with an equinoctial rainfall distribution. Long-term (1926-2005) average annual temperature is 12.1 °C with total rainfall of 765 mm and annual evapotranspiration rate (ET0-FAO) of 879 mm. The plantation consisted of one-year-old coppice of *Populus* × *canadensis* Moench 'I-214' and *P. deltoides* 'Dvina' arranged in 350-m-long rows with an interrow distance of 2 m and 0.5 m within rows, on an alluvial sandy-loam soil. Along four alternating internal rows (two per clone), 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.

Samples collection

From February to November 2006, tree growth and phenology was 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, except for one set of samples collected on DOY 33. 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 1-2). For soluble carbohydrates 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 (figure 1) on 24 (12 per clone) 2-mm-thick sections collected at 0.3 m from the collar (figure 2).

Bud Phenology

Bud phenology and leaf abscission were monitored twice per week (figure 1) and bud break stages were divided in five classes according to Castellani et al. (1967): stage 1, buds swelling with 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; 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 slices of them, were embedded in paraffin and transverse sections 8-12 µm thick were cut with a rotary microtome, dried at 50 °C for 1 hour and cleaned of the residual paraffin with successive immersions in D-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 permanently fixed with histological mounting medium (Eukitt®) to observe the developing tissues. The cambial zone and xylem in radial enlargement showed only primary walls, which did not shine under polarized light (figure 3 a-b, d). Xylem in secondary wall thickening phase shone under polarized light with walls changing from blue at the beginning of the process to red at mature state (figure 3a, 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 respectively in figure 3a); and (ii) the total width of xylem formed (µm), including differentiating and mature cells (zones of enl, wt and mc, figure 3a). The measuring 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 standard techniques (Polge 1978, Mothe et al. 1998). Radiographs were digitalised using a scanner and the acquired grey scale digital images were treated using semi-automatic 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, mean density determined by X-ray transmission was compared with the density directly determined by measuring the mass per volume unit in order to correct the microdensity measurements.

Stem water content

Discs for water content analysis were immediately wrapped in plastic film and weighed to determine the fresh mass (g) within 15 minutes after harvest. The fresh volume of samples (cm^3) was assessed by water displacement (Borghetti et al. 1991). The dry mass (g) was measured on the sample after it was maintained at 72 °C for 96 hours. Relative stem water content (RWC) was calculated following Domec and Gartner (2001):

$$RWC = \frac{M_f - M_d}{(V_f - V_s)} * 100$$

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 volume of fresh and solid material (cm^3) respectively. 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).

Soluble sugars and starch analysis

The samples were frozen in liquid nitrogen and freeze-dried for ten days. After removing the bark, a white powder containing tissues of the cambial zone (CZ) was collected by scraping the middle part between bark and xylem with a scalpel, except during dormancy when cambial tissues were not separable (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 (figure 2). The dried powders were kept under vacuum at -20 °C until sugar extraction.

For both CZ and MX, 40 mg dried powder were 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 and diluted with 2 ml of distilled water (pH 7). The obtained solution 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 CA, USA) and (ii) a quaternary-amine, strong anion-exchange resin (pre-packed 3ml Bond Elut SAX cartridge, Varian CA, USA). The cartridges were activated by 6 ml MeOH and conditioned by adding 6 ml of distilled water. The elution was performed using an additional 6 ml of distilled water, evaporated to dryness under vacuum and then diluted with 0.5 mm of distilled water. Analyses were conducted using a binary LC pump 250

(Perkin Elmer, USA) equipped with an automatic injection system (ISS101, Perkin Elmer). A Water Column Heater Module (Waters Division, Millipore, Milford, MA, USA) 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 was performed according to Romani et al. (1994) and the identity of soluble carbohydrates was confirmed using authentic carbohydrate standards (Sigma, USA) and adding an internal standard. The recovery was estimated for each carbohydrate. Thus 0.25, 0.50, 0.75, and 1.0 ml 1mg ml^{-1} carbohydrate solutions were fractionated and analysed as previously described, with 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 (Flucka), samples were diluted with distilled water to 5 ml and three 0.25-ml aliquots and each sample were assayed colorimetrically by using glucose oxidase (Sigma). Absorbance was read at 440 nm. For I-214 clone, the amount of pellet remaining after extraction of soluble sugars form cambium on DOY 207 was not sufficient to perform the starch analysis.

Statistical analyses

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

$$y = I + A\exp(-e^{(\beta-\kappa t)})$$

Where the parameters A, β and κ are the growth asymptote, the time-axis placement and 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 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):

$$r = \frac{A\kappa}{4}$$

The seasonal changes in soluble sugars were compared between clones using analysis of variance (ANOVA by GLM procedure, 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 clones in March (DOY 81) when bud swelling (stage 1) was observed (table 1). However, during the next stages, I-214 demonstrated faster bud development with stage 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 bud development lasted 14 days (table 1). For Dvina, stage 5 was reached one week later with a bud development of 21 days. Leaf abscission began on 10 October (DOY 283) for both clones and lasted about 35 days. Lignification of apical buds ended earlier for I-214 than Dvina. Overall, leaves were present for about 200 days.

Despite differences in the initial height of the plants (4.33 m for Dvina versus 3.52 m for I-214), both clones had similar height growth during 2006 (table 2, figure 4), estimated at *ca.* 2 m. Compared with Dvina, I-214 had a higher rate of apical growth (0.036 m day^{-1}), earlier height culmination (about 10-20 days), but reached maximum growth rate later (DOY 159). Dvina concluded apical growth at a total height of 6.45 m, compared with 5.77 m for I-214 (figure 4).

Xylem phenology, radial growth and density

Similar seasonal dynamics of cambial activity were observed in the two clones. In winter, the dormant cambium was composed of two-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 9 April (DOY 99) for Dvina and I-214, respectively (table 3). At the base, xylem differentiation started one week later in both clones. At both sampling heights, the first vessels were mature 10-14 days after the onset of xylem differentiation and were therefore 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 between the clones (figure 4). In July-August, the width of the differentiating xylem decreased with a first minimum achieved on DOY 230. From DOY 230, a new resumption of cambial cells division and differentiation was observed, 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 DOY 256 for both clones, two weeks later than the base (Table 3). Xylogenesis duration at the top was about 160 days, three weeks longer than at the base, with no marked differences between clones (table 3).

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, of 8030 µm, compared with 5656 µm for I-214 (table 2). Both clones reached their maximum growth rates at the end of May, on DOY 144. Stem radial growth at the top decreased slightly from 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 clones, 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 relative stem water content were observed in the clones, with values more or less following the distribution of precipitations (figure 6). During dormancy, I-214 had lower relative stem water content than Dvina (63% versus 80%). After cambial reactivation, relative stem water content decreased around DOY 150, when the width of the zones containing cells in enlargement and wall thickening was at its maximum. Relative stem water content reached its minimum values (40-50%) at the end of ring width formation.

Soluble sugars and starch analysis

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 while raffinose, galactose and mannitol were only detected in small quantities in MX (<8 µmol g^{-1} DW, table 4). In CZ, the total soluble sugars were about ten-fold higher than in MX. For all major soluble sugars, Dvina showed higher quantities, except for sucrose in MX (table 5, figure 7). Significant differences were observed also between sampling dates (DOY, table 5), although no interaction clone×DOY was observed ($p<0.05$, table 5).

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 (DOY 110) and a further increase in correspondence to the maximum growth rate (DOY 152). Afterwards, a new minimum was reached during decreasing (DOY 207) and ending (DOY 236) of xylem differentiation (figure 7). The same seasonal trend was observed in CZ during xylem differentiation with significant higher concentration in Dvina, especially at maximum growth rate, when glucose and fructose concentration was more than 1.5 times higher than in I-214 (figure 7). In CZ, sucrose showed a different trend with 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 clones, 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 *ca.* 2 in both clones but drastically decreased during maximum growth rate, with values of 0.7 for I-214 and 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, while in CZ, only a small quantity of raffinose was detected (table 4). In MX, these sugars were detected mainly during dormancy (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 higher concentration found in DVINA (figure 8 and table 5). In both MX and CZ, starch decreased after bud break (DOY 107-152) and in MX 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 clones.

DISCUSSION

The clone with the greater radial growth and density showed the higher concentration of non-structural soluble sugars in both cambial zone and xylem. Moreover, during the growing season, the non-structural soluble sugars varied according to xylem growth with much higher concentrations (5 to 10 times higher) found in the cambial zone than in the wood. Sucrose transport from source leaves to sink organs is controlled by sink strength, the ability of a sink to attract sucrose. 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. In this work, we demonstrated that the accumulations of non structural soluble sugars at a certain time during growth corresponded with higher carbon availability to an actively growing meristem. Moreover, as cell division activity is crucial in attracting assimilate (Ho 1988) sugars followed more or less the different stage of wood formation in stem. The observed vigorous xylogenesis, both between clones 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 clones was constituted of two-four cells during dormancy and increased to seven-eight cells in both clones during exponential growth, which may show a similar division potential if rate of division is not accounted for. Although duration of xylogenesis can

influence radial growth (Marion et al. 2007, Rossi et al. 2007, 2008a; Deslauriers et al. 2008), xylem differentiation at both tree heights lasted only 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 amount and density of xylem produced imply that Dvina was faster in producing and maturating cells. Moreover, similar trends of relative stem water content were observed in both clones, meaning 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 cambial zone.

The higher growth and density of Dvina was linked with higher contents 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 clone has a superior flow and availability of carbohydrate to differentiate tissues despite its higher utilisation. 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 content and low concentration of starch in mature xylem, 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). 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. 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

Differences in the onset of vessel enlargement were found between clones and tree heights. 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 clones, wood formation started about one 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*, 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. Therefore, carbohydrate needs for wood formation could not be entirely supplied by newly produced assimilates. This contrasts with the classical view that 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. For stem radial growth however, C-reserves were probably used for the beginning of wood formation as the total soluble sugars in mature xylem halved in respect to dormancy. Very shortly after bud break (less than two 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 (t_p) estimated for both clones on May 24 (DOY 144), about 10-15 days later than that of height growth. The major soluble sugars in the cambial zone 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 in order to

respond to the high demand of cell wall material. During growth, sucrose is the major form of translocated carbon (cf. 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 in outer wood of *Robinia pseudoacacia* 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).

Recent assessments in *Acer platanoides* (Marion et al. 2007) and *Fagus sylvatica* (Čufar et al. 2008) growing in temperate regions positioned the maximum growth rate around the end of May, in agreement with our results. Maximum growth rate in conifers of cold climates occurs around the culmination of day length, in order to safely complete secondary cell wall lignification before winter (Rossi et al. 2006c). However, conifers have sapwood covering tens of tree rings, whereas xylem of broadleaves stops conducting one (ring-porous) or a few years (diffuse-porous) after its production. Plants therefore have to produce and activate the conducting elements rapidly in spring to sustain the water transport and high transpiration of the developing leaves, which could be a reason to anticipate maximum growth rate. Moreover, fibre completed differentiation in less than two weeks (data not shown) ending cell differentiation faster than that in conifers (Rossi et al. 2006b).

Decreasing of cambial activity and end of cell differentiation

Reduction in growth rate and total soluble sugars both 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 non-structural soluble sugar was still higher in the cambium of Dvina while similar contents were observed between clones when xylogenesis was concluded. Cell differentiation was completed one month later, at the end of August, indicating that soluble sugar concentration in xylem and cambium decreases before the end of xylogenesis. In total, xylogenesis lasted about 160 days at the top and between 133 and 141 days at the base, depending of the clone. As leafs were present about 200 days until October 10 (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 previous results on *Populus* × *canadensis* Moench 'robusta' (Witt and Sauter 1994). In this specie, authors estimated the summer starch deposition rate in poplar wood of 0.24 mg starch g^{-1} DW per day. Moreover, wood density increased in the last 15% of the ring width during August, representing a 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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TABLE 1

Stage	I-214	Dvina
1	22 March (81)	22 March (81)
2	28 March (87)	31 March (90)
3	30 March (89)	4 April (94)
4	3 April (93)	7 April (97)
5	5 April (95)	11 April (101)

Table 1. Bud phenology of Dvina and I-214 in 2006 reported as date and DOY in parentheses. Stage 1, buds swelling with 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; stage 5, small leaves completely open but of smaller sizes than mature leaves and shoots elongating.

TABLE 2

Growth	Clone	I	A	β	κ (10^{-2})	r	t_p (DOY)	R^2
Height	Dvina	4.33(0.07)	2.12(0.10)	4.41(0.6)	2.85(0.4)	0.031	154	0.81
	I-214	3.52(0.07)	2.25(0.11)	6.68(1.1)	4.18(0.7)	0.036	159	0.78
Radial	Dvina	0	8030.8(95.9)	6.33(0.4)	4.39(0.3)	88	144	0.95
	I-214	0	5656.3(76.0)	7.34(0.5)	5.09(0.3)	83	144	0.94

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, β and κ are the parameters of the Gompertz function. Numbers in parenthesis are the standard deviations of the estimated parameters. Rates (r) are expressed in $m.day^{-1}$ for height growth and in $\mu m.day^{-1}$ for radial growth and t_p values correspond to the times of the inflection point of the Gompertz function.

TABLE 3

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

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). Numbers in parentheses represent the standard deviations of the mean.

TABLE 4

Zone	DOY	Raffinose (µmol g^{-1} DW)		Galactose (µmol g^{-1} DW)		Mannitol (µmol g^{-1} DW)	
		Dvina	I-214	Dvina	I-214	Dvina	I-214
CZ	110	nd	0.77±0.89	nd	nd	nd	nd
	152	0.14±0.28	nd	nd	nd	nd	nd
	207	nd	nd	nd	nd	nd	nd
	236	1.05±0.99	2.06±1.17	nd	nd	nd	nd
	33	3.44±0.76	2.55±0.60	4.02±1.50	6.52±1.29	0.79±1.59	4.88±1.89
MX	110	nd	nd	nd	nd	nd	nd
	152	nd	nd	1.20±0.23	1.03±0.18	nd	nd
	207	0.22±0.21	0.28±0.05	nd	nd	nd	nd
	236	0.18±0.16	0.25±0.11	nd	nd	nd	nd

Table 4. Seasonal changes in raffinose, galactose and mannitol (µmol g^{-1} DW) reported as average ± standard deviation detected in the cambial zone (CZ) and in mature xylem (MX) of *Populus* × *canadensis* 'I-214' and *P. deltoides* 'Dvina'. The sampling days correspond 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. Amounts of soluble sugar equal to zero are expressed as not detected (nd).

TABLE 5

Sugars	Source	CZ		MX	
		F	***p*-value**	***F***	***p*-value**
Total	Clone	50.6	<0.0001	25.4	<0.0001
	DOY	36.9	<0.001	33.5	<0.0001
	Clone×DOY	8.93	<0.001	2.3	ns
Glucose	Clone	30.4	<0.0001	40.4	<0.0001
	DOY	41.1	<0.0001	29.6	<0.0001
	Clone×DOY	9.1	<0.001	3.9	<0.05
Fructose	Clone	25.2	<0.0001	18.1	<0.001
	DOY	16.1	<0.0001	29.0	<0.0001
	Clone×DOY	4.5	<0.05	0.8	ns
Sucrose	Clone	20.2	<0.001	1.6	ns
	DOY	31.6	<0.0001	34.5	<0.0001
	Clone×DOY	0.2	ns	0.4	ns
Starch	Clone	13.2	0.0017	27.2	<0.0001
	DOY	9.65	<0.0001	81.4	<0.0001
	Clone×DOY	1066	0.0012	3.82	0.0130

Table 5. ANOVA comparisons of contents in soluble sugars and starch detected in the cambial zone (CZ) and in mature xylem (MX) among clones, sampling dates (DOY) and their interaction (clone×DOY). ns, not significant (p>0.05).

FIGURE LEGENDS

Figure 1. Sampling timetable of Dvina and I-214 during 2006.

Figure 2. Sampling design from stems of Dvina and I-214. On all sampling days (see figure 1 for dates), discs of 1 or 2 cm were cut at 0.3 m above the collar and at 1 m from the top for xylem formation analysis (XF). These discs contained primary and mature xylem of the 1st (2005) and current year of study (2006). Additional discs 5 and 20 cm were cut above those of xylem formation on fewer sampling days (figure 1) for the determination of stem water content (WC) and soluble sugars analysis (SS). After the end of the growing period (figure 1), other discs were taken for density measurement (1 cm discs).

Figure 3. Sections of developing stem tissues collected on DOY 200 (a-c) and 103 (d) from hybrid poplars. a-b: developing xylem under visible (a) and polarized light (b) showing cambial zone (cz), enlarging xylem cells (enl), cell wall thickening (wt) and mature xylem (mx) of I-214 (20×). c: developing xylem of Dvina under phase contrast showing the width of developing xylem (enl and wt) compared with mature xylem (mx) (4×). d: first enlarging vessel at the beginning of xylem formation under visible light (100×).

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 line); 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); 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 6 individual plants. The intra-annual trends were calculated by the Gompertz function (see table 2).

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 show standard deviation. B: Distribution frequency of the density measurements (kg m^{-3}) for Dvina (black bars) and I-214 (grey bars).

Figure 6. A: Microclimatic conditions expressed as daily precipitation (mm) and mean temperature (°C). B: Variation of the relative stem water content (%), measured on stem section at 0.3 m from the base, in Dvina (black dots) and I-214 (grey dots).

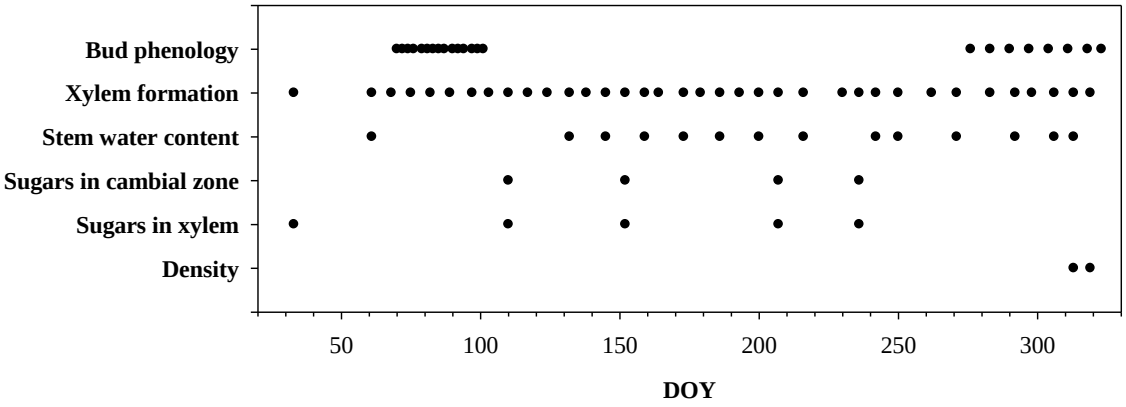
Figure 7. Seasonal changes in total soluble sugars, glucose, fructose and sucrose (µmol g^{-1} DW) detected in the cambial zone (CZ, dots) and mature xylem (MX, squares) of *Populus* × *canadensis* 'I-214' (gray) and *Populus deltoides* 'Dvina' (black). Total soluble sugars content was obtained as the sum of the amount of glucose, fructose, sucrose, raffinose, galactose and mannitol. The sampling days corresponded 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. Vertical bars and asterisks indicate standard deviations among trees and significant differences between clones, respectively.

Figure 8. Seasonal changes in starch (mg g^{-1} DW) detected in the cambial zone (CZ, dots) and mature xylem (MX, squares) of *Populus* × *canadensis* 'I-214' (gray) and *Populus deltoides* 'Dvina' (black). The sampling days corresponded to the main stages of cambial activity: (i) dormancy, (ii) onset of xylem differentiation, (iii) maximum

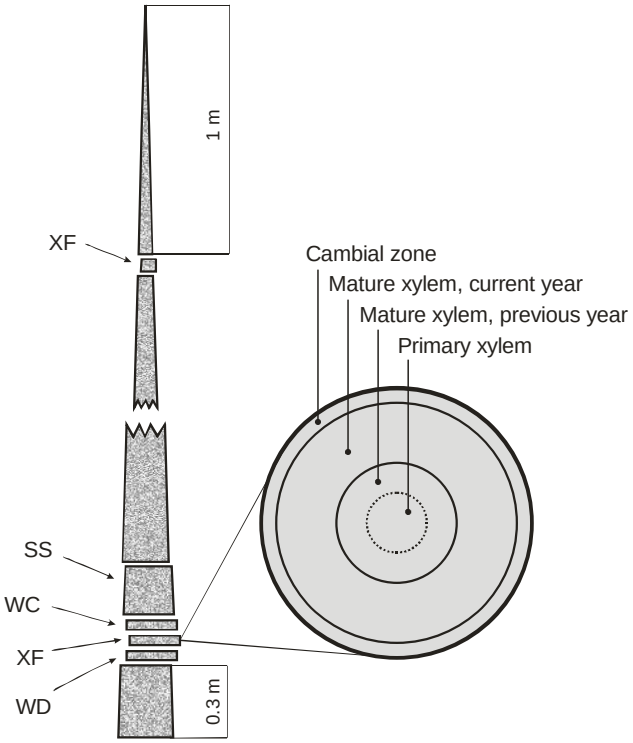
1 growth rate, (iv) decreasing and (v) ending of xylem differentiation. Vertical bars and
2 asterisks indicate standard deviations among trees and significant differences between
3 clones, respectively.

4

FIGURE 1

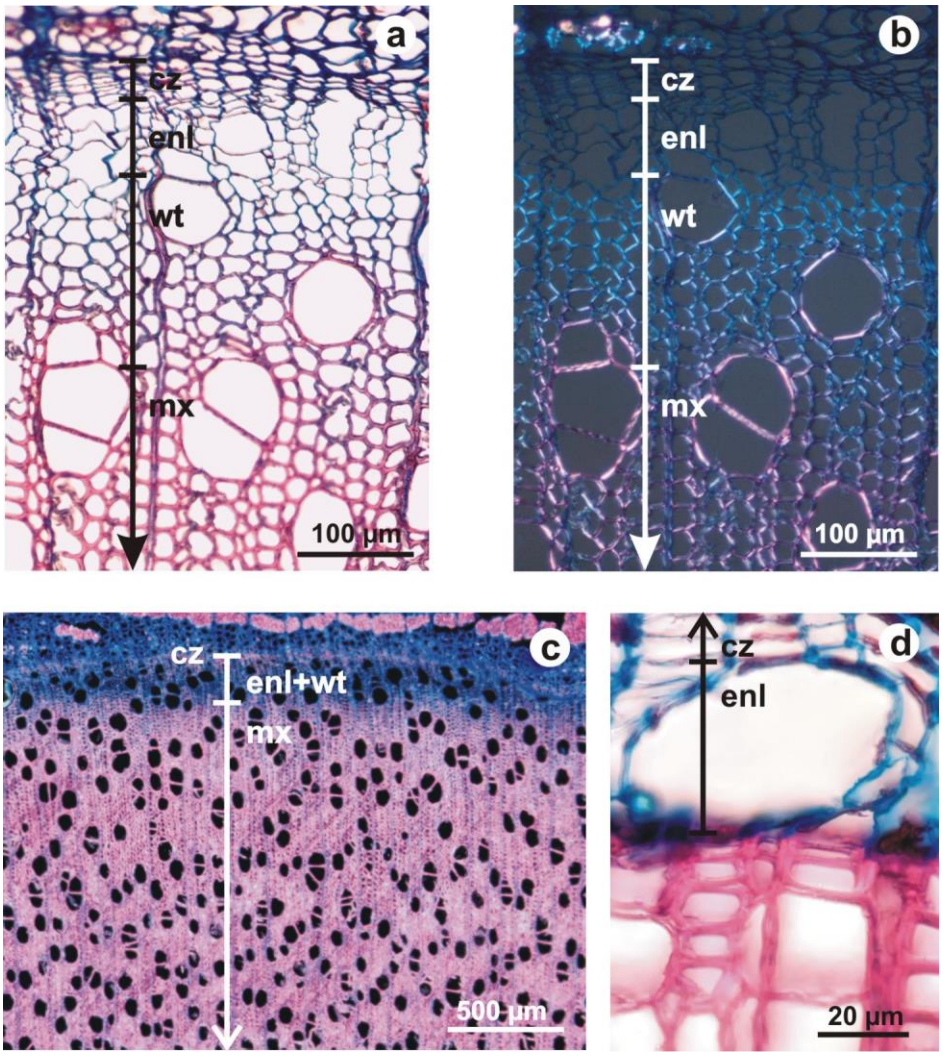

Bud phenology
Xylem formation
Stem water content
Sugars in cambial zone
Sugars in xylem
Density
50
100
150
200
250
300
DOY

1 **FIGURE 2**

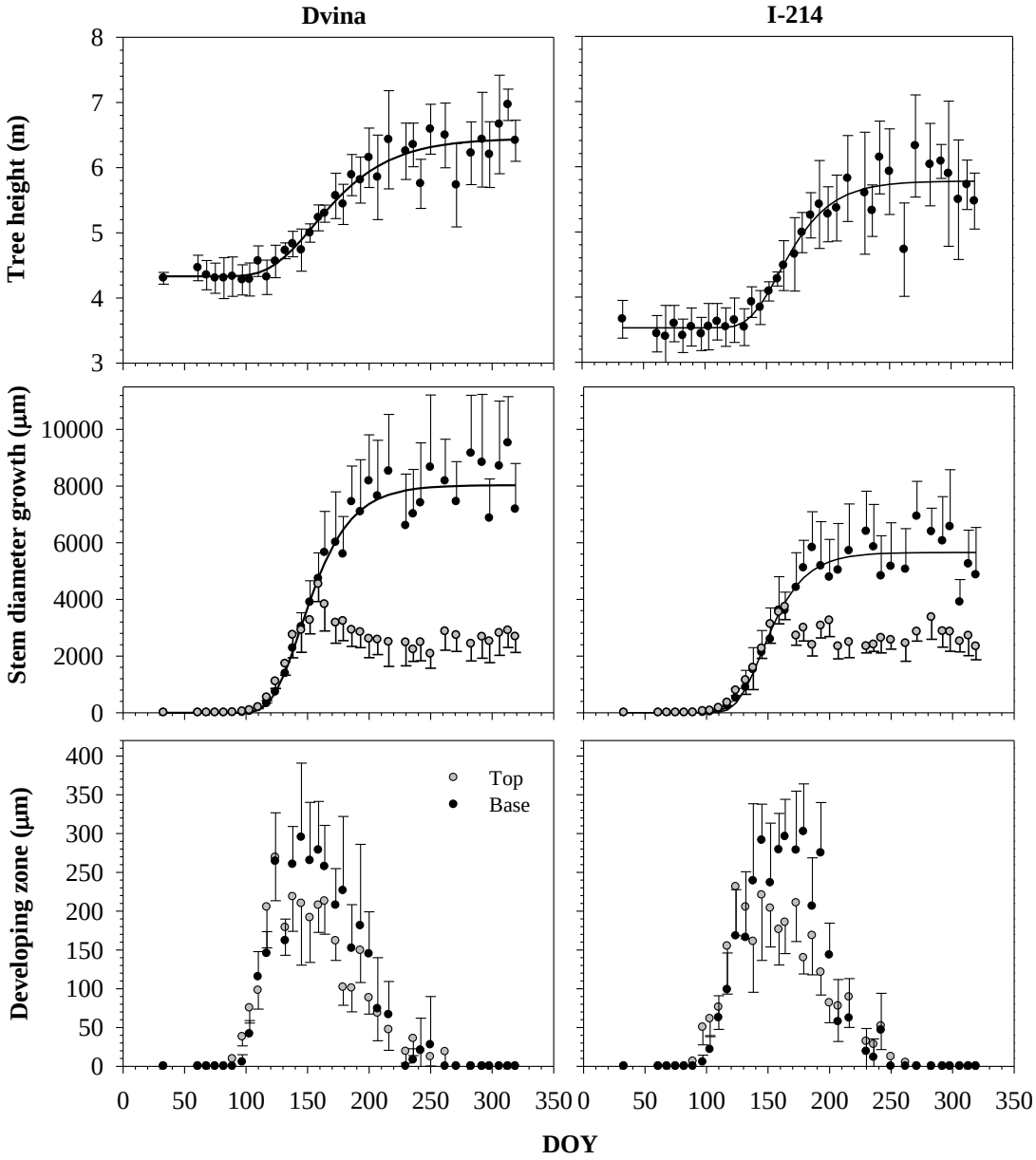

1 m
XF
Cambial zone
Mature xylem, current year
Mature xylem, previous year
Primary xylem
SS
WC
XF
WD
0.3 m

2

FIGURE 3

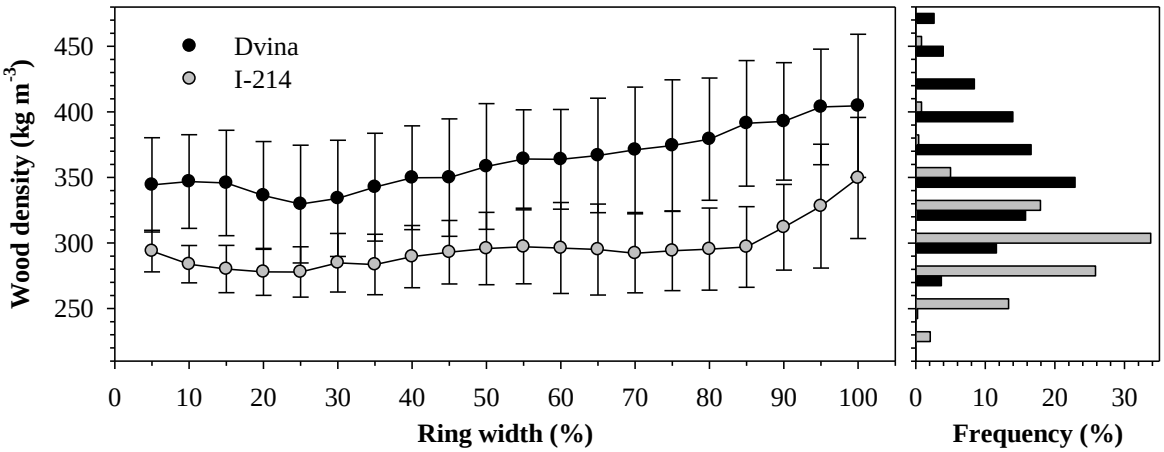

a
cz
enl
wt
mx
100 µm
b
cz
enl
wt
mx
100 µm
c
cz
enl+wt
mx
500 µm
d
cz
enl
20 µm

1 **FIGURE 4**


Dvina
I-214
Tree height (m)
Stem diameter growth (µm)
Developing zone (µm)
Top
Base
DOY

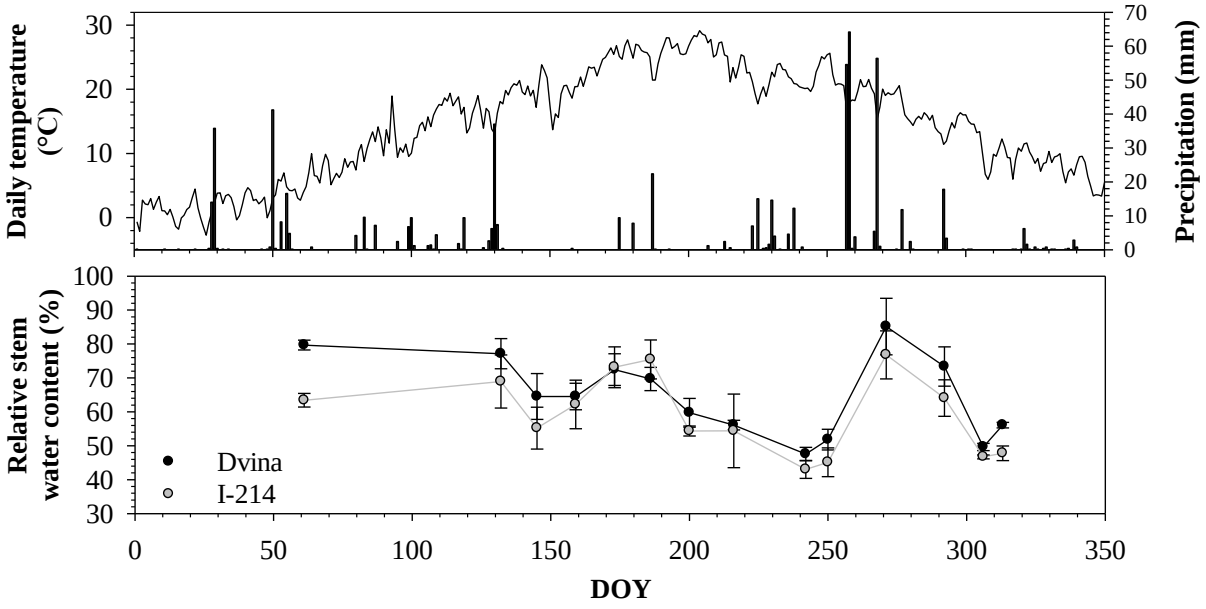
2

1 **FIGURE 5**


Dvina
I-214
Wood density (kg m-3)
450
400
350
300
250
0
10
20
30
40
50
60
70
80
90
100
Ring width (%)
0
10
20
30
Frequency (%)

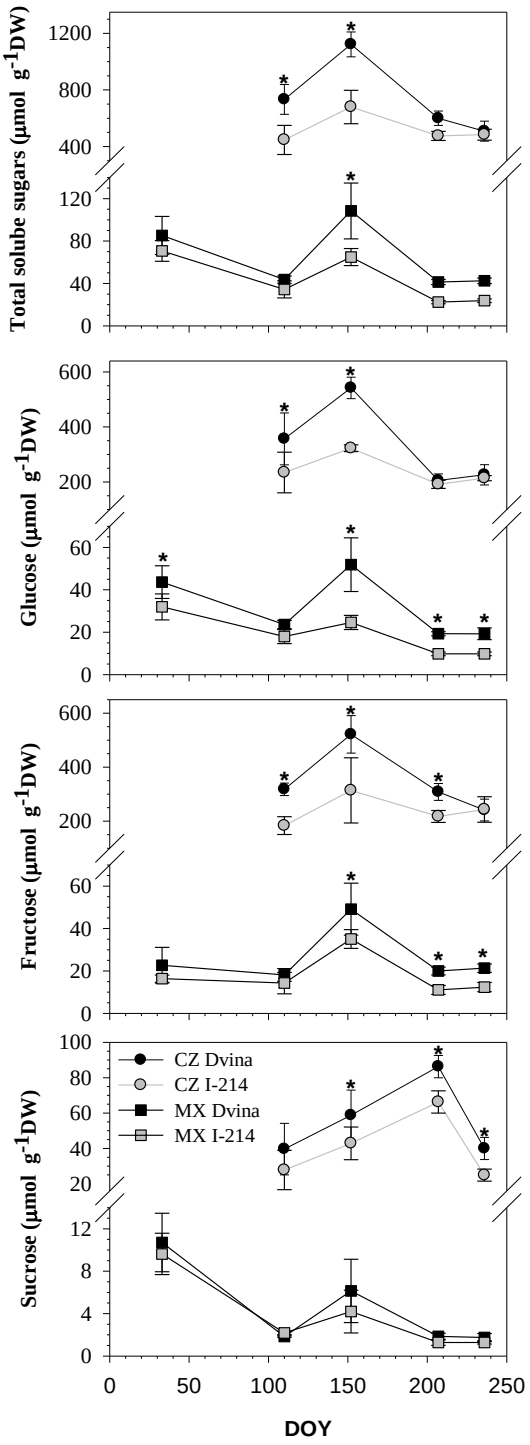
2

1 **FIGURE 6**


Daily temperature (ºC)
Precipitation (mm)
Relative stem water content (%)
Dvina
I-214
DOY

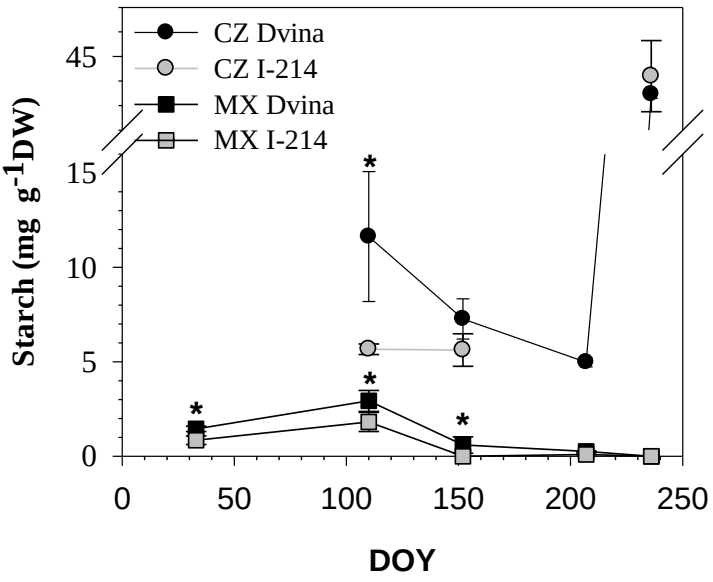
2

1 **FIGURE 7**


Total solube sugars (µmol g-1DW)
Glucose (µmol g-1DW)
Fructose (µmol g-1DW)
Sucrose (µmol g-1DW)
CZ Dvina
CZ I-214
MX Dvina
MX I-214
DOY

2

FIGURE 8


CZ Dvina
CZ I-214
MX Dvina
MX I-214
Starch (mg g-1DW)
DOY
0
5
10
15
45
0
50
100
150
200
250
*
*
*
*