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BRIEF COMMUNICATION

Wood ontogeny during the first year of hybrid poplar development

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Abstract

During the first year of hybrid poplar development, we assessed radial growth dynamics quantified by the proportion of secondary xylem tissue within the stem area, the vessel area percentage, the content of both lignin and cellulose, the lignin monomeric composition, and the macromolecular properties of cellulose. The intraannual radial growth dynamics in the proportion of secondary xylem tissue was fitted by the Gompertz regression line whereas changes in the vessel area percentage were fitted maximally by a cubic regression line. Under a constant temperature and photoperiod, this study reveals that nonlinear patterns of radial growth dynamics were the result of a developmental programme which drove cambial activity and ageing. The increased proportion of guaiacyl units found might be important for the greater stability of the lignin structure in the first year of hybrid poplar development. The tensile strength of juvenile wood was ensured by the trade-off between a slight increase in the degree of polymerization of cellulose and a slight decrease in the content of cellulose during ageing.

Additional key words: cellulose, guaiacyl, lignin, *Populus tremula*, *Populus* × *canescens*, syringyl, xylem.

Wood formation is a dynamic process which is temporally and spatially controlled by gene expression (Finaev 2007, Goué *et al*. 2008, Pina *et al*. 2012), hormonal signals (Tuominen *et al*. 1997, Uggla *et al*. 2001, Israelsson *et al*. 2005), environmental conditions, such as photoperiod, temperature, water, and macronutrients availability (Antonova and Stasova 1997, Rossi *et al*. 2006, Paiva *et al*. 2008), as well as by interactions among all these factors. Seasonal transition from earlywood to latewood formation is an important developmental switch with differences occurring at both the anatomical and chemical levels. The formation of latewood is the result of slower rates of cambial cell division, decrease in the rate and period of cell expansion, and a longer duration of secondary wall thickening (Wodzicki 1971, Dodd and Fox 1990). Larson *et al*. (2001) reported that a decline in cambial activity during the transition from earlywood to latewood coincides approximately with the cessation of terminal shoot extension and the reduction of soil moisture.

Variation in the chemical composition of cell walls between species (*i.e*., hardwoods or softwoods) has an influence on their commercial utilization (Davison *et al*. 2006). Determination of the chemical attributes of plant cell walls is also of great importance for evaluating both the effects of hybridization and the results of plant breeding. Microfibrils of cellulose constitute the reinforcing rods of the cell wall. Cellulose imparts tensile strength to the wall to resist pressure potential and to allow a growth habit. Lignins also impart strength to cell walls, facilitate water transport, and impede the degradation of cell wall polysaccharides, thus acting as a major line of defence against pathogens, insects, and other herbivores. The composition and cross-linking

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Abbreviations: DP - degree of polymerization; G - guaiacyl; NBO - nitrobenzene oxidation; S - syringyl; SEM - scanning electron microscopy.
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lignin have significant impacts on the lignin degradability (Chen *et al*. 2001, Grabber 2005). One measure of dicotyledonous angiosperm lignin composition is the proportion of guaiacyl (G) and syringyl (S) content. These aromatic units determine the type and number of cross-links.

Very limited information is available concerning the ontogenetic changes in the macromolecular traits of lignin and cellulose biopolymers during the earliest stages of plant development. The objective of this study was to assess trends in wood anatomy parameters and the macromolecular properties of lignin and cellulose due to stem ontogeny under constant environmental conditions in the first year of hybrid poplar development.

Clonally micropropagated plants used in this study were derived from a mature hybrid poplar tree T-14 [*Populus tremula* 70 × (*Populus* × *canescens* 23)] which was more than 30 years old. The procedures of *in vitro* micropropagation, *ex vitro* acclimatization, as well as the initiation of wood formation during the stressful acclimatization phase were described in Kaňuchová and Ďurkovič (2013). On day 0, plantlets fully acclimatized to the *ex vitro* environment were transferred to a controlled room and grown at a 16-h photoperiod, irradiance of 150 μmol m^{-2} s^{-1} (cool white fluorescent tubes), day/night temperatures of 24/20 ± 1 °C, and relative humidity of 50 ± 2 %. Under these conditions, the effects of the main environmental variables on imbalances in cambial cell division and the temporal positioning of the growth rate culmination were thereby minimized.

Wood anatomy experiments were made on three different and entire stem cross-sections sampled on 12 different sampling dates of *in vivo* plant growth (days 1, 16, 29, 43, 57, 71, 85, 99, 123, 151, 179, and 209). On each date, stem segments were sampled 0.5 cm upwards from the root-stem junction. The segments were fixed in 3.5 % (v/v) glutaraldehyde in a 0.1 M sodium phosphate buffer, pH 7.2, at 4 °C overnight. Deparaffinized cross-sections, 15-μm thick, were double stained with 1 % (m/v) safranin and 1 % (m/v) alcian blue as described in Marjamaa *et al*. (2003) and examined with an *Olympus BX50F* microscope (*Olympus Europa*, Hamburg, Germany). At each sampling date, the proportion of secondary xylem tissue within the stem area was determined for the entire stem cross-sections using *NIS-Elements AR 3.0* image analysis software (*Laboratory Imaging*, Prague, Czech Republic) as described in Ďurkovič and Mišalová (2009). Vessel area percentage was calculated as the ratio of the area occupied by vessels to the area of the secondary xylem multiplied by 100. Vessel wall characteristics (the presence of helical thickenings, intervessel pitting, and simple perforations) were documented by scanning electron microscopy (SEM). Wood sections (transverse, radial, and tangential surfaces) were mounted on specimen stubs, sputter-coated with gold, and observed by high-vacuum SEM using a *VEGA TS 5130* instrument (*Tescan*, Brno, Czech Republic) operating at 15 kV.

Stems of 4-month-old and 1-year-old plants were sampled 0.5 cm upwards from the root-stem junction. The bark was peeled off, and the wood was frozen and stored at -18 ºC prior to chemical analyses. Stem and branchwood samples taken from the mature, 30-year-old donor tree, were used for a comparison to juvenile samples. Lignin content was determined according to the American Society for Testing and Materials (ASTM) standard procedure. Cellulose content was determined by the Seifert (1956) method. Measurements were performed in three replicates. Nitrobenzene oxidation (NBO) was carried out as described in Kačík *et al*. (1995). Degree of polymerization (DP) of cellulose was determined by viscometry as described in Ďurkovič *et al*. (2011). The neutral sugar composition of cellulose samples was determined after acid hydrolysis (Seaman *et al*. 1954). Measurements were performed in four replicates.

The distribution of data pertinent to the proportion of secondary xylem tissue within the stem area was fitted by the Gompertz function. The distribution of the proportion of vessel lumen areas within the sampling interval was fitted by a cubic regression line. Wood chemistry data were subjected to one-way analysis of variance and Duncan's multiple range test was used for the separation of means.

The temporal pattern for radial growth of secondary xylem in the first year of hybrid poplar development following *in vitro* micropropagation and *ex vitro* acclimatization is presented in Fig. 1. On day 1, wood cells were already differentiated sufficiently to distinguish vessels, fibers, and parenchyma ray cells. Lignin deposition was also found in secondary phloem fibers as identified by the red staining reaction. On day 209, at the end of the first growing season, lignification for all the newly formed layers of secondary xylem was completed. The intra-annual distribution of the proportion of secondary xylem tissue within the stem area was fitted by the Gompertz regression line: $y = 58.42 \exp[-e^{(-0.63 - 0.07\,x)}]$ ($R^2 = 0.67$, $P = 0.001$). The proportion of secondary xylem tissue asymptotically approached 58.4 %. By day 36, 95 % of the asymptote value was reached (data not shown). The temporal course of the vessel area percentage within the sampling interval of measurements was fitted by a cubic regression line: $y = 10.67 + 0.40\,x - 0.004\,x^2 + 0.00001\,x^3$ ($R^2 = 0.61$, $P = 0.001$). The highest density of vessels within the secondary xylem area culminated on day 62 suggesting an enhanced formation of vessels in earlywood. The lowest vessel area percentage was found on day 179 when the formation of fibers prevailed in latewood (data not shown). In the first year of hybrid poplar development, solitary vessels and the occasional occurrence of vessel pairs were predominantly found (Fig. 1). Intervessel pitting and simple perforations (Fig. 2*A*-*C*) characterized a fully differentiated and functional water transport system mediated by vessels. In addition, helical thickenings also contributed to the cell wall structure and sculpturing in vessels occurring near the primary xylem (Fig. 2*D*). The seasonal effect (*i.e*., climatic variation) is among the most significant external sources of variation affecting the

intra-annual course of cambial activity and the development of newly derived cells. In this study, the intra-annual radial growth dynamics of hybrid poplar wood was recorded under the constant temperature and photoperiod to minimize the effects of these environmental variables. Here, using this approach, we show that nonlinear patterns of radial growth dynamics pertinent to the proportion of secondary xylem tissue

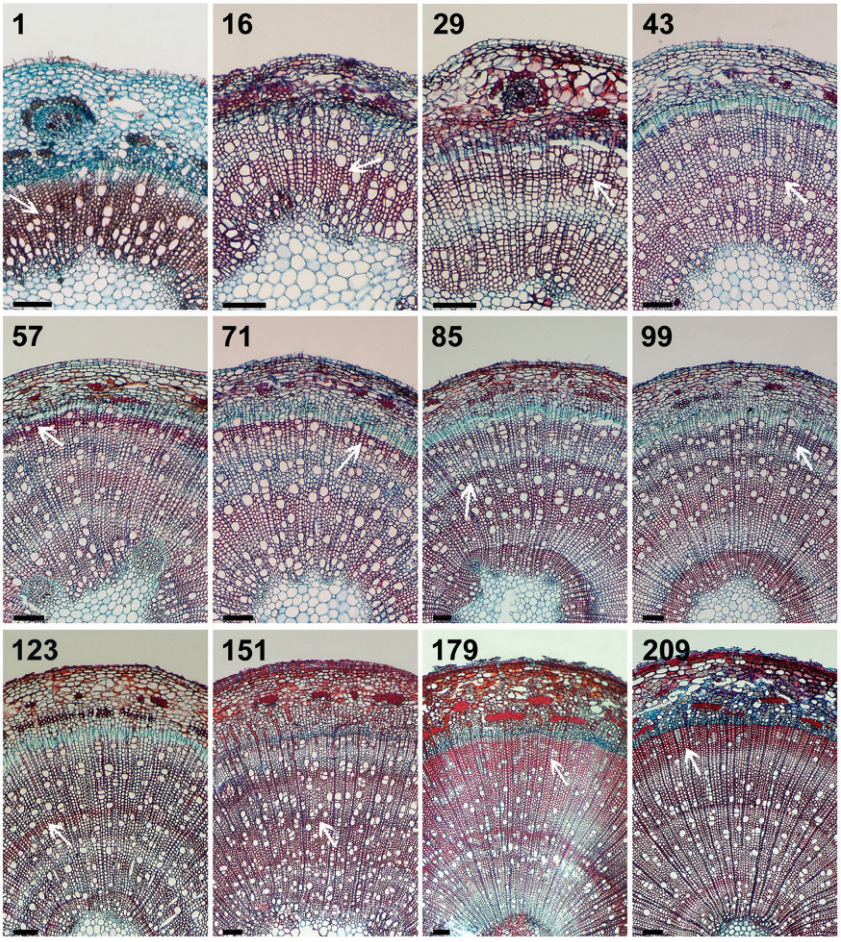

Fig. 1. Temporal pattern of intra-annual radial growth dynamics of wood during the first growing season of the T-14 hybrid poplar development. Numbers indicate sampling days, *white arrows* show thick-walled fibers. Cross-sections, *scale bars* = 100 μm.

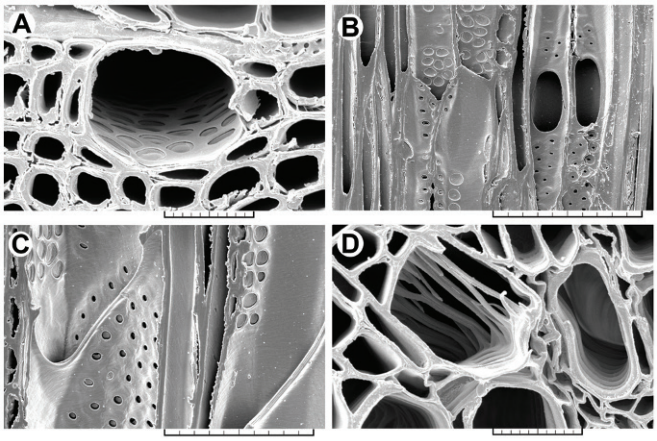

Fig. 2. Vessel wall features in the first year of the T-14 hybrid poplar development. *A* - SEM image of solitary latewood vessel pitting. Cross-section, *scale bar* = 20 μm. *B* - SEM image of vessel pairs with simple perforations. Radial section, *scale bar* = 100 μm. *C* - SEM image of perforated vessel surrounded by uniseriate parenchyma ray (*left*) and libriform fiber (*right*). Tangential section, *scale bar* = 50 μm. *D* - SEM image of helical vessel wall thickenings near the primary xylem. Cross-section, *scale bar* = 20 μm.

Table 1. The content of Klason lignin in the T-14 hybrid poplar wood (expressed as a percentage of its content prior to extraction), the products of nitrobenzene oxidation [%], and the syringyl/guaiacyl (S/G) ratio in lignin. Data represent means ± SD. Means followed by the same letters within the row are not significantly different at the 0.05 level of significance (Duncan's multiple range test).

Content	4-month-old (earlywood)	1-year-old	Donor tree (branchwood)	Donor tree (stem)
Klason lignin	21.41 ± 0.08 a	21.48 ± 0.09 a	18.56 ± 0.07 b	17.37 ± 0.13 c
p-Hydroxybenzoic acid	0.12 ± 0.00 a	0.09 ± 0.01 b	0.05 ± 0.01 c	0.06 ± 0.01 c
p-Hydroxybenzaldehyde	0.07 ± 0.01 a	0.07 ± 0.01 a	0.06 ± 0.01 a	0.06 ± 0.01 a
Vanillic acid	0.33 ± 0.03 a	0.33 ± 0.03 a	0.23 ± 0.03 b	0.17 ± 0.02 c
Vanillin	2.96 ± 0.22 a	3.02 ± 0.07 a	2.17 ± 0.28 b	1.98 ± 0.12 b
Syringic acid	0.76 ± 0.15 a	0.47 ± 0.04 b	0.60 ± 0.07 b	0.48 ± 0.03 b
Syringaldehyde	4.89 ± 0.16 a	4.80 ± 0.13 a	4.18 ± 0.13 b	4.40 ± 0.27 b
Total yield on wood	9.12 ± 0.54 a	8.78 ± 0.18 a	7.29 ± 0.49 b	7.15 ± 0.44 b
S/G ratio	1.72 ± 0.05 c	1.58 ± 0.05 c	1.99 ± 0.19 b	2.26 ± 0.03 a

Table 2. The content of cellulose in the T-14 hybrid poplar wood (expressed as a percentage of its content prior to extraction), the proportions of saccharides in the extracted cellulose [%], and degree of polymerization of cellulose (DP). Data represent means ± SD. Mean values followed by the same letters within the row are not significantly different at the 0.05 level of significance (Duncan's multiple range test).

Content	4-month-old (earlywood)	1-year-old	Donor tree (branchwood)	Donor tree (stem)
Cellulose	41.61 ± 0.09 c	41.28 ± 0.08 d	44.27 ± 0.16 b	47.33 ± 0.19 a
L-rhamnose	0.05 ± 0.03 b	0.04 ± 0.01 b	0.08 ± 0.01 a	0.05 ± 0.01 b
L-arabinose	0.30 ± 0.09 b	0.24 ± 0.02 bc	0.22 ± 0.01 c	0.39 ± 0.01 a
D-xylose	0.08 ± 0.08 a	0.02 ± 0.00 ab	0.02 ± 0.00 b	0.02 ± 0.00 ab
D-mannose	1.75 ± 0.08 b	1.82 ± 0.01 ab	1.88 ± 0.05 a	1.55 ± 0.03 c
D-glucose	97.59 ± 0.22 b	97.77 ± 0.03 ab	97.60 ± 0.07 b	97.90 ± 0.05 a
D-galactose	0.23 ± 0.02 a	0.11 ± 0.01 b	0.21 ± 0.01 a	0.10 ± 0.02 b
DP	706 ± 7 a	710 ± 6 a	687 ± 10 b	558 ± 8 c

within the stem area and the vessel area percentage were the result of a strict developmental programme. Thus, these internally-driven growth patterns are fixed in ontogeny, as outlined by Klingenberg (1998), and may further be complemented by environmental variables, such as the photoperiod, which drives the temporal positioning the maximum growth rate culmination, and the temperature, which allows metabolic activities to be maintained during cell production and differentiation (Rossi *et al*. 2006).

In the first year of hybrid poplar development, differences in the amounts of acid-insoluble Klason lignin were negligible (Table 1). Based on the analysis of NBO products, S units were the main constituent of lignin. Less abundant were G units whereas the representation of *p*-hydroxyphenyl units was very low. The S/G ratio in earlywood lignin of 4-month-old plants was similar to that found in 1-year old plants but definitely lower than that found in the mature donor tree (Table 1). This finding may indicate the preferential condensation of G units in the lignin macromolecules of the juvenile plants. Total lignin content and composition have significant influences on wood pulping, hydrolysis, combustion, *etc*. Here, we showed that the content of Klason lignin remained similar during the first growing season of hybrid poplar development. In the study of 4-, 6-, and 8-year-old *Eucalyptus camaldulensis* plants, the lignin content remained mostly unchanged as the trees aged (Pisuttipiched 2004). The lignin content in the stem of the donor tree (17.37 %) is in good accordance with other results for mature wood of poplar hybrids: 18.1, 18.6, and 19.0 % (Francis *et al*. 2006). Recent findings show that there is a negative correlation between plant growth and lignin content (Novaes *et al*. 2010). The volume of above-ground woody biomass in mature trees is higher than that in juvenile trees due to fast growth rates pertinent to cambial cell division and cell wall thickness. The thick secondary cell walls of wood in mature trees are composed mostly of poly-saccharides whereas lignin content is substantially lower than that in the tiny middle lamella. There may be a reason for a lower proportion of lignin content in mature trees than that in juvenile trees. In addition, high S/G ratios are advantageous for pulping (Bose *et al*. 2009). Lignin isolated from primary walls of hybrid aspen cell cultures consisted exclusively of G units and had a more condensed structure than the lignin of mature aspen (Christiernin *et al*. 2005). The S/G ratio determined in our study had a tendency to

decrease slightly during the first year of hybrid poplar development. In the donor tree, however, S/G ratio was considerably higher. These results can be explained by the preferential condensation of G units in the early stages of lignification and the prevalent deposition of S units in mature wood.

In 1-year-old plants, the amounts of cellulose were lower than those in 4-month-old plants (Table 2). In the first year of hybrid poplar development, DP of cellulose was characterized by a steady-state. D-glucose was the main constituent of the extracted cellulose biopolymer. The proportion of this saccharide was stable enough when the means varied between 97.59 % (4-month-old plants) and 97.90 % (stem of the donor tree). The steady-state proportion pattern was also found for D-mannose, the second most abundant saccharide in the extracted macromolecule. The proportions of other saccharides were far lower than 1 % (Table 2). Cellulose yield in wood and its macromolecular properties (crystallinity and degree of polymerization) tend to influence biomass utilization in various areas of industry. In the study of Jahan and Mun (2005), wood of *Trema orientalis* was sampled at the age of 12, 18, 24, and 30 months. These authors reported that the proportions of crystallinity of cellulose and DP increased with age in the juvenile wood samples of *Trema orientalis*. In our study, DP increased slightly during the first year of hybrid poplar development but the donor tree showed the lowest values of DP. The observations, with respect to the slight increase in DP of cellulose and the slight decrease in the content of cellulose with age, suggest that there was a trade-off between these two traits during wood ontogeny. In addition, our results are in close agreement with the opinion that DP of cellulose in wood is reduced during the ageing of a mature tree (Fengel and Wegener 1984).

Under the constant temperature and photoperiod, this study revealed that nonlinear patterns of radial growth dynamics are the result of a developmental programme which drives cambial activity and ageing. In addition, the increased proportion of G units found may have provided the greater stability of the lignin macromolecular structure in the first year of hybrid poplar development. The tensile strength of juvenile wood was ensured by the trade-off between a slight increase in the degree of polymerization of cellulose and a slight decrease in the content of cellulose during ageing.

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