ABSTRACT

This study focused on the wood formation process on cellular basis, comparing seasonal cambium dynamics and differentiation phases between two age-different (45- and 130-year-old) European beech (Fagus sylvatica L.) stands, during 2013, and thereafter, to infer the influence of weather conditions (temperature, precipitation). Microcores were taken from 12 trees at weekly intervals from April till November, by using Trephor tool. The average temperature during the growth period was 12.7°C and the warmest month of the year was July (18.8°C). According the Standardized Precipitation Index, the year was classified as near normal (SPI = −0.19). The results showed that onset, cessation and duration of the phenological phases of wood formation (i.e. cambial cell production, cell enlargement, secondary wall thickening and lignification) occurred almost simultaneously in both European beech stands. The final tree-ring width was measured 1655 μm in the 45-year-old and 1571 μm in the 130-year-old European beech stand. The timing of wood formation phases and the width of xylem increment was influenced by temperature and precipitation.

KEYWORDS: Fagus sylvatica L., cambium dynamics, temperature, precipitation, Gompertz function.

INTRODUCTION

As trees grow seasonally in temperate climatic regions, wood formation occurs periodically, starting with cell division in vascular cambium and proceeding with differentiating cambial derivatives in four steps: cell expansion, secondary cell wall deposition, lignification and ultimately, cell death (Plomion et al. 2001). The duration of cambial cell production (CCP) depends on internal factors combined with numerous environmental conditions (Larson 1994). The result of this environmental effect is permanently registered in the tree-ring structure (Fonti et al. 2010) and chronologically archived in the wood as a signal (Fonti and Jansen 2012). As trees
grow older and bigger, a general decline of tree-ring width is observed, showing a trend which is commonly found in ring-width chronologies (Esper et al. 2008, Campelo et al. 2015). Several studies have reported that the climate-growth relationships differ with tree age (Eckstein and Krause 1989, Linderholm and Linderholm 2004, Mencuccini et al. 2005, Yu et al. 2008, Rossi et al. 2008, De Luis et al. 2009, Rozas et al. 2009, Wang et al. 2009, Li et al. 2012). Certain studies demonstrated a stronger climatic signal in older trees (Carrer and Urbinati 2004) and others in younger (Vieira et al. 2009). There are also studies where no difference between age classes was found (Esper et al. 2008, Dorado Liñánet al. 2012). Copenheaver et al. (2011) found that growth in old trees is more sensitive to drought than in young trees, while Haavik et al. (2011) underlined that younger trees exhibited greater growth rates as well as stronger climate-growth relationships than the older.

European beech (Fagus sylvatica L.) longevity and adaptivity renders the species suitable for evaluating the climate-growth relationships (Eckstein et al. 1984; Čufar et al. 2008). European beech is a diffuse porous hardwood, consisting of vessels uniformly distributed throughout growth rings, not exceeding 100 μm in diameter (Tsoumis 1991). Wood structure and quality depend on cambial activity and wood formation process (Vavrčík et al. 2013). Hence, the predicted climatic changes (IPCC 2013) with frequent and intense events (e.g., drought, heat waves) are expected to negatively affect the species (Stojnic et al. 2013). Since European beech stands are planned to be expanded by new stands in the future in the Czech Republic (Ministry report 2014), detecting the potential age-dependency of tree growth under the perspective of the upcoming climate change scenario becomes important.

This study tested the hypothesis that timing and duration of wood formation changes with tree age. The aim of the research was to monitor CCP during one year (2013) in two age-different (45- and 130-year-old) European beech (Fagus sylvatica L.) stands at weekly intervals in the Czech Republic in order to compare age-related intra-annual dynamics of tree-ring formation.

**MATERIAL AND METHODS**

**Site characteristics**

The research was conducted in 2013, in two age-different European beech stands, located in Rájec-Němčice research site in the Czech Republic (Fig. 1). The younger stand was 45 years old (49° 26´ 29.946“ N, 16° 42´ 06.237“ E, altitude 600 m a.s.l.) and the older stand was 130 years old (49°28’2.977“N and 16°41’18.131“E, 630 m a. s. l.), both even-aged and composed entirely of European beech (Urban et al. 2014).

Area’s soil type was classified as modal oligotrophic Cambisol with “Moder” humus form (Fabiánek et al. 2009) and acid granodiorite bedrock. The plot was ranked as 5S1 forest type – oligo-mesotrophic silver beech forests with Oxalis acetosella L. (Pliva 1987).

The weather data (air temperature, precipitation) were obtained from Protivanov weather station, approximately 10 km away from the research site (Czech Hydrometeorological Institute). The long-term mean air temperature and annual precipitation for the period 1961-2013 were 6.6 °C and 602 mm, respectively. The mean air temperature in the study year (2013) was 7.0°C and the annual precipitation was 592 mm (Fig. 2). The highest mean annual air temperature and precipitation were recorded during the growth period (in July: 18.8°C and in June: 106.3 mm respectively).
Sample preparation

Six healthy dominant trees were selected per stand. In total, microcores were taken from 12 trees at weekly intervals from April to November. The microcores (1.8 mm in diameter) were collected by using Trephor tool (Rossi et al. 2006), at 1.3–2.0 m above ground following a spiral up the trunk. Each microcore contained phloem, cambium and at least two of the last formed xylem rings.

Immediately after collecting from the trees, the microcores were immersed in FAA (formalin-alcohol-acetic acid) solution (Gričar 2007). One week later, the microcores were dehydrated in ethanol series (70, 90, 95, and 100 %), washed away in xylene and finally embedded in paraffin blocks (Paraplast plus, ROTH, Karlsruhe, Germany). Transverse sections of 12 μm thickness were cut with a Leica RM2235 rotary microtome (Leica Microsystems, Wetzlar, Germany), using Leica 819 low profile microtome blades (Leica Microsystems, Wetzlar, Germany). The sections were transferred to an object glass and stained with a safranin (Merck, Darmstadt, Germany) (0.04 %) and astra blue (Sigma-Aldrich, Steinheim, Germany) (0.15 %) water mixture (van der Werf et al. 2007) and then permanently mounted by Euparal. The observations and histometrical analysis were performed with a Leica DM 2000 microscope (Leica Microsystems, Wetzlar, Germany) connected with a Leica DFC 295 digital camera (Leica Microsystems, Wetzlar, Germany) and a public-domain image processing program ImageJ (Abramoff et al. 2004).

Measurements and data processing

The number of cell layers in the cambium was counted and the widths of the newly formed xylem increment were measured along three radial rows, in the transverse sections (Prislan et al. 2013). The phenological phases of wood formation were assessed as follows: (1) onset of CCP, (2) onset of the differentiation process, (3) maximum rate of xylem cell production, (3) cessation of CCP and (4) cessation of the differentiation process. The phenological phases were assessed for each tree and were computed in days of the year (DOYs). The duration of the CCP was estimated (in DOYs) and the final tree-ring width was measured (μm).

The xylem formation of the tree-ring was analysed by Gompertz function (Rossi et al. 2003):

\[ y = A \cdot e^{-e^{B-k \cdot t}} \]

where:
- \( y \) – weekly cumulative cells,
- \( t \) – day of year,
- \( A \) – upper asymptote, representing the maximum number of cells,
B – place on x axis, estimating the beginning of cambial activity,
k – inflection point on the curve.

One-way ANOVA and Student’s t-tests were performed (SPSS Statistics).

The Standardized Precipitation Index (SPI) was used in order to estimate the shortage of water (McKee et al. 1993). Drought indices monitor the severity, the duration and the frequency of dry conditions, by simplifying the environmental interactions and by quantifying extreme weather conditions. SPI relies on the fact that every parameter involved in the water resources system is actually defined by the deficit of the precipitation rate over different time scales. The SPI\textsubscript{annual} was produced based on water years (WY), starting from the previous October to the current September (each WY included one growth period). Additionally, both SPI\textsubscript{Jan–Apr} and SPI\textsubscript{May–Aug} were also adjusted and calculated for 4-month time interval before and during the growth period. The SPI was calculated using a Drought Indices Calculator (Drin C) public-domain software package (Tsakiris and Vangelis 2004).

The weather conditions (average air temperature, sum of precipitation) and the average number of cambial cells (CC) were calculated at weekly 7-days and 14-days intervals and correlated by Pearson’s correlations (SPSS Statistics).

Logistic regressions (R environment) were used to calculate the probability of wood formation being active at a certain temperature where binary responses were coded as non-active (value zero) and active (value 1). The logistic regression was formed as:

\[
\text{Logit}(\pi_x) = \ln\left(\frac{\pi_x}{1-\pi_x}\right) = \beta_0 + \beta_1 x_j
\]

where: 
\(\pi_x\) – the probability of wood formation being active, 
\(x_j\) – the temperature on a given day \(j\),
\(\beta_0\) and \(\beta_1\) – the intercept and slope of the logit regression (Quinn and Keough 2002, Rossi et al. 2007, Deslauriers et al. 2008).

The temperature thresholds (\(\chi\)) were calculated when the probability of xylogenesis being active was 0.5, i.e. when Logit (\(\pi\)) = 0 and \(\chi = -\beta_0/\beta_1\). Wood formation was more likely to be active for temperatures above \(\chi\), than non-active. In order to verify the model, the goodness of fit was tested by using 1) likelihood ratio, 2) Wald’s \(\chi^2\) (i.e. Z-tests) for regression parameters and 3) Hosmer-Lemeshow test (Quinn and Keough 2002, Rossi et al. 2007, Deslauriers et al. 2008). For each tree and site, the model was fitted with the respective temperature series (mean, minimum and maximum air temperatures). Temperature thresholds were then compared between sites using univariate ANOVA models (Stats of Statistica).

**RESULTS AND DISCUSSION**

**Weather conditions**

The drought index showed that the examined year (2013) was classified as near normal (SPI\textsubscript{annual} = -0.19), tending to be drier during the growth period (Fig. 3), though without exceeding from the normal levels (SPI\textsubscript{May–Aug} = -0.75).
Fig. 3: Standardized precipitation indices \( \text{SPI}_{\text{annual}}, \text{SPI}_{\text{Jan-Apr}}, \text{SPI}_{\text{May-Aug}} \) in 2013 (Protivanov weather station, Czech Hydrometeorological Institute).

Wood formation

Dormant cambium consisted of 4–5 layers of cells (Fig. 4). CCP started with the enlargement of radial dimensions of CC and the increase in the number of cells during the 1st week of May 2013. The number of CC gradually increased reaching the peak (8 cell layers) during the 2nd week of June 2013. No significant timing difference was observed between the examined stands. In July, the average number of CC started to descent and reached again the minimum value of 4–5 layers of cells at the cessation of CCP in both stands.

Fig. 4: Mean number of cambial cells produced during 2013 (standard deviation in error bars, DOY: Day of the Year).

Fig. 5: Daily increment first derivatives of Gompertz function during 2013 growth period (Day of the Year: DOY).

The 45-year-old European beech stand exhibited a higher but not significantly different daily xylem increment (ANOVA, \( F = 0.245; p = 0.62 \)), (Fig 5.). The maximum daily xylem increment was found 24.5 μm in the 45-year-old European beech stand and 21.5 μm in the 130-year-old stand and occurred simultaneously in both stands (around 27 of June 2013).

In 2013, no significant differences were monitored in wood formation seasonal dynamics, between the 130-year-old and the 45-year-old European beech stand. All wood phenological phases occurred concurrently and lasted the same (Tab. 1).

The final tree-ring width was measured 1655 ± 437 μm in the 45-year-old and 1571 ± 419 μm in the 130-year-old stand. The difference between the final tree-ring widths was not found to be significant (ANOVA, \( F = 0.115 ; p = 0.742 \)).
Either old trees or older parts of the stem, form thinner tree-rings than the younger trees or the younger parts of the stem, resulting in a radially declining trend of tree-ring width from pith to bark (Cook et al. 1990, Panyushkina et al. 2003, Campelo et al. 2015). In this study, the 45-year-old and the 130-year-old European beech trees formed tree-rings of a similar width formed during 2013 growing period. Furthermore, the number of CC and the daily xylem increment were found also similar during 2013, between the two examined stands. This is in line with other studies which also found no differences in tree growth between age classes (Esper et al. 2008, Dorado Liñán et al. 2012). A slightly higher growth rate was observed in the 45-year-old European beech stand, but not significant in this study.

Weather- xylem growth relationships

In both examined stands, the increase of the number of CC, observed in 7-days intervals, was significantly but not highly correlated (Pearson’s correlation; 0.05 level) with the respective temperature (Tab. 2). Similar result was also found when temperature of 14-days interval was correlated with the number of CC. Neither 7-days nor 14-days precipitation influenced wood phenology.

In the 130-year-old European beech stand, the duration of CCP was highly and significantly correlated with both temperature (Pearson’s correlation; 0.01 level) and precipitation (Pearson’s correlation; 0.05 level) of the respective period (Tab. 3). On the contrary, in the 45-year-old European beech stand, no similar results were found. Precipitation was also highly and significantly correlated to CCP duration, when both stands were examined together. Copenheaver et al. (2011) reported that older (149–312 years old Quercus alba) trees were more sensitive to dry conditions than the younger (29–126 years old) trees. In this study, although weather conditions were classified as normal (no extreme dry conditions), the 130-year-old European beech stand was found to be influenced more pronouncedly than the 45-year-old European beech stand.

The threshold temperature (Tab. 4) when wood formation had a 0.5 probability of occurrence was calculated for each European beech tree and reported as a mean, minimum and maximum for the examined year (2013). In the 130-year old European beech stand, the calculated thresholds

<table>
<thead>
<tr>
<th>Wood phenological phases</th>
<th>130-year-old</th>
<th>45-year-old</th>
<th>T-test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Onset of cambial activity (DOY)</td>
<td>120 ± 6</td>
<td>126 ± 8</td>
<td>NS</td>
</tr>
<tr>
<td>End of cambial activity (DOY)</td>
<td>210 ± 12</td>
<td>217 ± 9</td>
<td>NS</td>
</tr>
<tr>
<td>Onset of enlargement (DOY)</td>
<td>124 ± 3</td>
<td>127 ± 4</td>
<td>NS</td>
</tr>
<tr>
<td>End of enlargement (DOY)</td>
<td>214 ± 14</td>
<td>219 ± 10</td>
<td>NS</td>
</tr>
<tr>
<td>Onset of secondary wall formation (DOY)</td>
<td>144 ± 10</td>
<td>158 ± 12</td>
<td>NS</td>
</tr>
<tr>
<td>Onset of maturation process (DOY)</td>
<td>210 ± 11</td>
<td>217 ± 6</td>
<td>NS</td>
</tr>
<tr>
<td>End of maturation process (DOY)</td>
<td>288 ± 12</td>
<td>289 ± 7</td>
<td>NS</td>
</tr>
<tr>
<td>Total duration of cambial activity (in DOYs)</td>
<td>90 ± 12</td>
<td>90 ± 10</td>
<td>NS</td>
</tr>
<tr>
<td>Total duration of radial enlargement (in DOYs)</td>
<td>91 ± 14</td>
<td>85 ± 8</td>
<td>NS</td>
</tr>
<tr>
<td>Total duration of maturation (in DOYs)</td>
<td>78 ± 2</td>
<td>71 ± 8</td>
<td>NS</td>
</tr>
<tr>
<td>Total duration of growing period (in DOYs)</td>
<td>168 ± 10</td>
<td>161 ± 12</td>
<td>NS</td>
</tr>
<tr>
<td>Tree-ring width (μm)</td>
<td>1571 ± 419</td>
<td>1655 ± 437</td>
<td>NS</td>
</tr>
</tbody>
</table>

Tab. 1: Wood phenological phases (Day of the Year: DOY) and tree-ring width (μm). The value following ± symbol depicts the standard deviation. (NS: Not significant).
were found to be approximately 5.0, 8.0 and 11.0°C for minimum, mean and maximum air temperature, respectively. The minimum, mean and maximum stem temperature thresholds were found quite higher for the 45-year-old European beech stand (5.5, 8.5 and 11.8°C, respectively), but the difference was not significant between the two examined stands. The thresholds temperatures values calculated for the two age-different stands were in line with previous studies conducted on conifers (Rossi et al. 2007, Deslauriers et al. 2008).

The period of CCP corresponds to the time window during which the produced wood cells are open to directly receive environmental signals (Frankenstein et al. 2005). Since the weather conditions were classified as normal during the examined year (2013), the environmental signal registered in the xylem growth was not clear. In both stands, the temperature was affecting the cambial activity more strongly than precipitation, but still not comprehensively enough, probably due to general normal weather conditions. It was found that CCP duration in the 130-year-old European beech stand was more sensitive to the respective temperature and precipitation. In the 45-year-old European beech stand, the environmental influence was not elucidated.

**Tab. 2**: Number of cambial cells (CC) and daily increment obtained from Gompertz function (weekly intervals) correlated (Pearson’s correlation) with the respective 7-days and 14-days temperature (°C) and precipitation (mm). **. Correlation is significant at the 0.01 level (2-tailed), *. Correlation is significant at the 0.05 level (2-tailed).

<table>
<thead>
<tr>
<th>Phenology</th>
<th>7-days Temperature (°C)</th>
<th>7-days Precipitation (mm)</th>
<th>14-days Temperature (°C)</th>
<th>14-days Precipitation (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>130-years-old stand</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of CC</td>
<td>.371</td>
<td>.214</td>
<td>.412*</td>
<td>.217</td>
</tr>
<tr>
<td>Daily increment</td>
<td>.357</td>
<td>.038</td>
<td>.347</td>
<td>.092</td>
</tr>
<tr>
<td><strong>45-years-old stand</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of CC</td>
<td>.387*</td>
<td>.202</td>
<td>.439*</td>
<td>.174</td>
</tr>
<tr>
<td>Daily increment</td>
<td>.367</td>
<td>.024</td>
<td>.358</td>
<td>.072</td>
</tr>
</tbody>
</table>

**Tab. 3**: Duration of cambial cell production (CCP), (Pearson’s correlation) with temperature (°C) and precipitation (mm) of the corresponding period. **. Correlation is significant at the 0.01 level (2-tailed), *. Correlation is significant at the 0.05 level (2-tailed).

<table>
<thead>
<tr>
<th>Duration of CCP</th>
<th>130-years-old stand</th>
<th>45-years-old stand</th>
<th>Both stands</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature (°C)</td>
<td>.953**</td>
<td>.395</td>
<td>.275</td>
</tr>
<tr>
<td>Precipitation (mm)</td>
<td>.882*</td>
<td>.448</td>
<td>.671*</td>
</tr>
</tbody>
</table>

**Tab. 4**: Minimum, mean and maximum threshold temperatures corresponding with the 0.5-probability of active wood formation forage-different European beech trees during 2013. Error stands for standard deviation.

<table>
<thead>
<tr>
<th>Air temperature (°C)</th>
<th>130-year-old stand</th>
<th>45-year-old stand</th>
<th>F value (p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minimum</td>
<td>4.98 ± .69</td>
<td>5.44 ± .94</td>
<td>.96 (.35)</td>
</tr>
<tr>
<td>Mean</td>
<td>7.98 ± .80</td>
<td>8.52 ± 1.07</td>
<td>.97 (.35)</td>
</tr>
<tr>
<td>Maximum</td>
<td>11.21 ± .79</td>
<td>11.76 ± 1.09</td>
<td>.99 (.34)</td>
</tr>
</tbody>
</table>
CONCLUSIONS

In this study, no differences were found in the seasonal dynamics of two age-different European beech stands growing under the same environmental conditions. Considering the fact that 1) the weather conditions were not found to be extreme during the examined year (2013) and 2) the age-difference between the two examined European beech stands was high enough to elucidate the effect of the age on the tree growth, further investigation under this perspective is suggested in the future. Conclusions on the age-dependent weather-xylem growth relationships under normal weather conditions were not clear, since trees are known to respond less strongly to climatic variation than those from extreme sites.

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