ACTA ENVIRONMENTALICA UNIVERSITATIS COMENIANAE (BRATISLAVA)
Vol. 17, 2(2009): 58–65 ISSN 1335-0285

XYLEM GROWTH BY USING SPECIFIC TOOL FOR COLLECTING MICROCORES FROM TREES

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Abstract: ***Xylem growth by using specific tool for collecting microcores from trees****. Xylem growth and differentiation in Norway spruce (Picea abies) was studied (May 2008 – September 2009). Microcores were extracted weekly between May and mid-September in 2008 and 2009. Samples were embedded in paraffin and cut with a rotary microtome. The number of cells in enlarging, secondary cell wall formation and mature stages were determined microscopically. Gompertz function was fitted to the data. The onset of xylem formation occurred earlier in 2008. Xylem growth was completed faster in 2008 (61 days between 10 and 90% of growth in 2008 against 51 days in 2009).*

Key words: xylem, microcores, tree, Norway spruce

INTRODUCTION

The quality of wood is a big issue for the wood product market (Label et al. 2000, 2009). The properties of paper are dependent on the tracheid characteristics. Variation in tracheid properties between and within the trees is caused by the cambial growth rate and thus by the environment, cambial maturation and heredity (Wodzicki 2001; Mäkinen et al. 2002; Jakkola et al. 2005). The quality and the mechanical properties of wood are directly related to wood anatomy and structure of xylem (Aloni 1989; Label et al. 2000). Therefore, the economic importance of wood production has always emphasized understanding xylogenesis process and the mechanisms engaged in this process (Downes et al. 2002). Xylem formation begins with cambial division, the newly formed tracheid being involved in enlargement and then secondary wall formation before getting mature (Wilson et al. 1966). This process in boreal and temperate ecosystem is restricted to a certain period of year and is regulated by several factors including genotype, site, silviculture and climatic variation (Mäkinen et al. 2008). Rossi (2006) suggested that for conifers in cold environment the maximum growth rate would actually occur at the time of maximum day length, the achievement of photoperiod acting as a limiting factor to allow plants to safely finish secondary wall lignification before winter. However, the consecutive stages of formation of xylem cells are

independent process and their response to environmental factors could therefore be different (Antonova, Shebeko 1981; Antonova et al. 1983). Therefore, it is relevant to study more closely the timing of xylem growth and of each differentiation stage of cell formation along with their response to environmental factors in Norway spruce. The objective of this paper was to get more basic information concerning xylem growth and consecutive stages of differentiation of xylem cells in Norway spruce during two growing seasons and to link this information with environmental data. I determined the onset and pattern of xylem growth during the two growing seasons. I also defined the timing and the pattern of trees estimated by Gompertz – function.

MATERIAL AND METHODS

Site

The study was performed in Flakaliden (64°07' N; 19°27' E), in northern Sweden. The experiment site was established in 1987 in a Norway spruce stand planted in 1963 with 4 year old seedlings of a local provenance. The soil is a thin, podzolic, sandy glacial till with an average depth of 120 cm (Bergh et al. 1998). The thickness of the humus layer varies between 2 and 6 cm, with mean of 4,3 cm. The monthly mean temperature at the site varies from – 8,7 ºC in February to 14,4 ºC in July and the mean annual precipitation is approximately 600 mm, of which more than one-third falls as snow (Bergh et al. 1999).

Micro-sampling

The studies of radial growth of trees during two growth seasons are mostly based on repeated taking of small samples (so-called micro-sampling) of cambium and xylem tissue around the circumference of an individual tree by Trephor tool. First sampling date was 22nd of May in 2008 and 14th of May in 2009. The samples were taken twice on May, 4 times on June, 5 times on July, 4 times on August and 2 times on September. Micro-sampling is most vital in the beginning of the vegetation period, when the cambium area is wide, and cells of early wood with thin cell walls and large radial dimensions are created. The quality of the sample depends on the sharpness of the cutting edge of the tool (Fig. 1).

Fig. 1: One of newest specific tool for collecting microcores from trees is Trephor, developed by an Italian group of researchers (Rossi et al. 2006)

Sample preparation

The samples were dehydrated in an ascending series of ethanol, after which the samples were cleared with Tissue-clear, a xylene substitute that is miscible with both ethanol and paraffin. The samples were then immersed into liquid paraffin that penetrates into the wood and fills the cells lumens, thus supporting the fragile tissue. After that, the samples were fixed on supports by means of paraffin blocks, using a paraffin smelter and a hot plate. The samples were cut into thin sections (8 μm thick) with a rotary microtome (Fig. 2). The sections were then deposited on microscopic slides coated with egg albumin. After drying, the slides were immersed into baths of Tissue-clear in order to remove the paraffin from the sections. Then, they were stained with Safranin (stains lignin in red) and Alcian blue (stains cellulose in blue) and dehydrated in ascending series of ethanol (Fig. 3). The slides were mounted into Canada balsam.

Fig. 2: Microcore included in a paraffin block cut by a rotary microtome

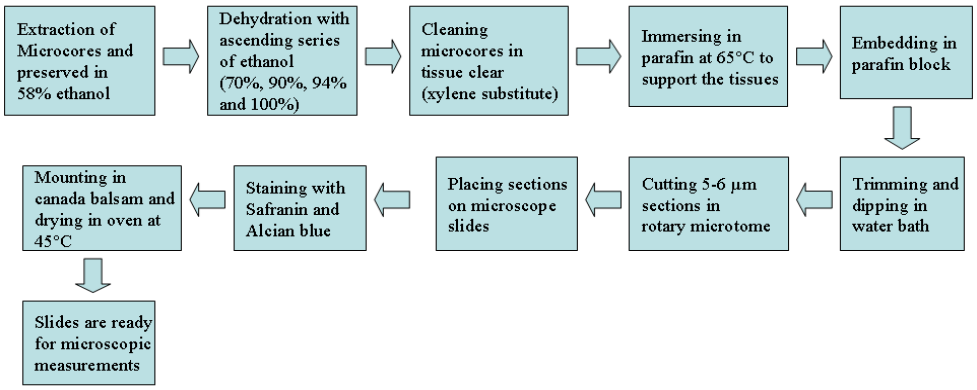

Fig. 3: Flow chart of sample preparation

Microscopic measurements

From the thin sections, the dynamics of xylem formation were measured with the help of both bright field and UV light under microscope. Due to birefringence, the lignin appeared very bright under UV on the microscope and permitted to determine the stages of xylogenesis of xylem cells. I measured from each sample:

1. The number of tracheids formed during the current growing season

2. The radial diameter of tracheids

3. The number of tracheids in different stages of xylogenesis with 3 classes:

- class A tracheid in enlargement
- class B tracheid in secondary wall formation
- class C mature tracheid.

The deposit of lignin was detected under UV light due to lignin birefringence. The lignification begins in the corner of the cells, then extends on the intercellular layer and finally, on the secondary wall. Cells in zone c are enlarging in radial, tangential and longitudinal direction. They contain no lignin, thus they do not show any birefringence. Cells in zone b show some brightness on their wall. Cells in zone a show birefringence throughout their wall, they are considered to be mature.

4. The width of the previous annual ring.

Digital images were taken by a video camera plugged on a microscope (Olympus U-TV1 X, Japan) and were analysed using Image Pro Plus Software (Media Cybernetics, L.P). The magnification used was 100x and picture resolution was 2,154 Pixels per µm. The images were converted to JPEG-format by Irfan view (Irfan Skiljan, Vienna). To cover the whole annual ring, the successive overlapping images were merged with PhotoStitch (Canon Inc.). The sections usually contained the previous four annual rings, and the annual layer of xylem in formation with the cambial zone and adjacent phloem. The newly divided cells were counted along three radial rows for each sample (Fig. 4). The growth was considered to have begun on the date when the first new cell was found and to have ceased when no more cells were found (compared to the previous measurements) and all the cells were mature.

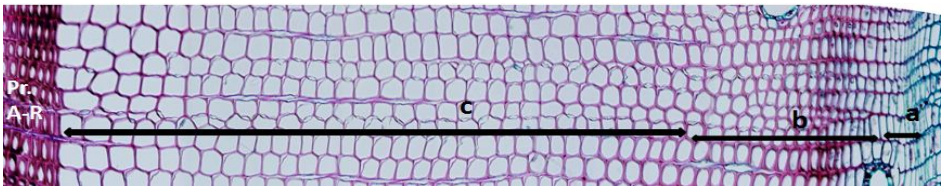

Fig. 4: Three different stages of xylogenesis: a) enlarging cells b) wall thickening cells and c) mature cells; Pr. A-R: part of previous year annual ring

Statistical analysis and testing of data

To describe the progress of xylem growth during two growing seasons, both linear and non-linear models were tested and non-linear one was chosen because it had the best fit to the data. The Gompertz function was fitted to the data to establish the xylem growth profile for Norway spruce. The SAS ANOVA procedure (SAS Institute Inc. 2002, USA) was used to test the effect of Norway spruce on xylem growth.

The Gompertz function was fitted to the measured successive number of tracheids using NLIN (Non Linear regression) of the SAS statistical package as follows:

$$y = A \exp[- e^{(\beta - kt)}]$$

Where: **y** is the number of tracheids at date t

t is date in Julian days

A is the upper asymptote, i.e. the maximum number of cells

β is a parameter defining the placement on the x-axis

k is a parameter defining the rate of change.

RESULTS

Dynamics of xylem growth

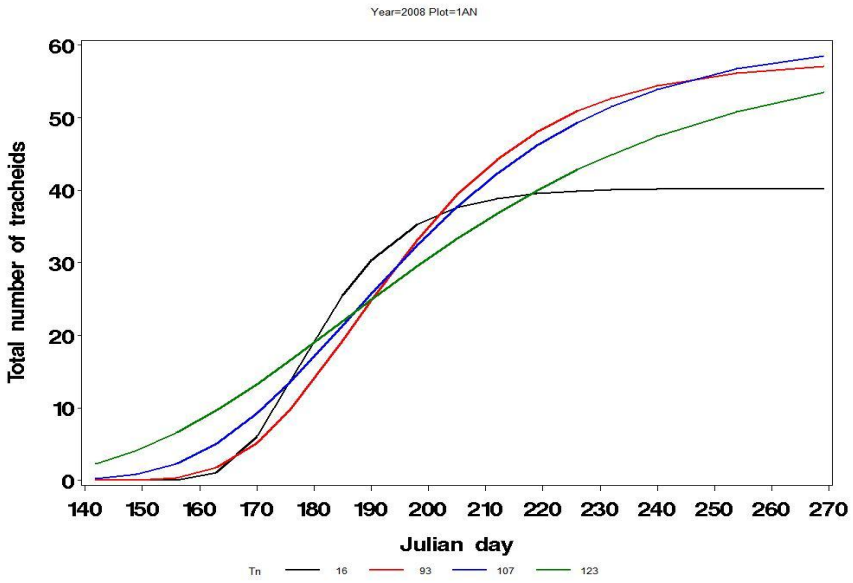

Fig 5: Number of cells given by Gompertz prediction in the annual ring in function of time in 2008

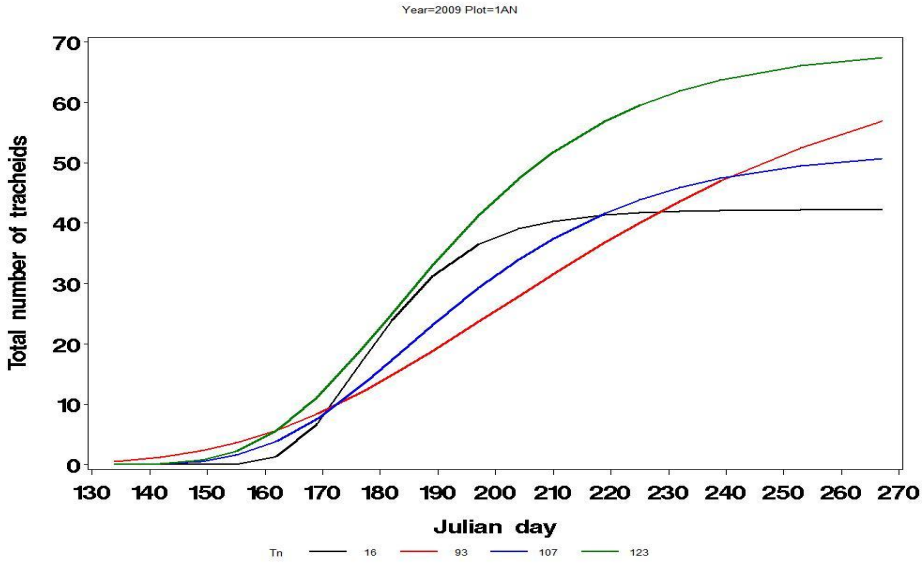

Fig. 6: Number of cells given by Gompertz prediction in the annual ring in function of time in 2009

The qualities of micro-cores were sufficient for defining the onset and cessation of cambial activity and examination of xylem cells in various stages of differentiation during two growing seasons. The pattern of xylem growth was similar in both years 2008, 2009. The S-shape was more accentuated in 2008. The variation between trees in 2008 and 2009 in days when they complete 5%, 10% and 25% of growth were significant. The growing season started earlier in 2008 and also the growing period in 2008 was longer (61 days), than in 2009 (51 days). The differences could be caused by different temperature during the growing season.

DISCUSSION

Tree growth affected by the environmental factors, influence onset, duration, dynamics and the conclusion of individual phases of xylogenesis (Wodzicki 2001). Rossi et al. (2006) focused on coniferous trees in cold areas of northern hemisphere. Period of maximum cell production was, regardless to tree species or growth site, always only before middle of June. Similar conclusions had Horaček et al. (1999) for spruce (*Picea abies*) in Czech Republic. Mäkinen et al. (2003) reported the period of maximum cell production later, in the middle of July. This agrees with Mäkinen et al. (2008) who compared three different methods for measuring radial increment for

Norway spruce. They found that the onset of xylem formation occurred around early June for Norway spruce. In such cases the cell divisions in cambium begin normally in spring, but with the lack of precipitation or long snow cover the trees are forced to stop radial growth sooner than usual, by which as a consequence the period of maximum cell production is shifted towards the beginning of vegetation period.

CONCLUSIONS

The results of the study showed that the frequency of cambial division, a reduction of duration of cambial growth, and a resultant reduction of the wood formation period and the width of annual xylem increment, are most of apparent growth responses of Norway spruce trees in the Flakaliden, Sweden. The research confirmed the micro-coring technique, which has been successfully applied to conifers (Rossi et al. 2006) as an appropriate method for research of wood formation.

SÚHRN

V príspevku sú prezentované výsledky štúdia xylémového rastu a diferenciácie v smreku obyčajnom (*Picea abies*). "Microcores" boli extrahované týždenne od mája do polovice septembra v rokoch 2008 a 2009. Vzorky boli vložené do parafínu a krájané za pomoci rotačného mikrotónu (obr. 2). Počet zväčšujúcich sa buniek formujúcej sa sekundárnej bunkovej steny a zrelej fázy boli stanovené mikroskopicky. Gombertzová funkcia slúžila na štatistické vyhodnotenie dát. Nástup xylémovej formácie nastal skôr, v roku 2008. Xylémový rast bol dokončený rýchlejšie v roku 2008 (61 dní medzi 10 % a 90 % rastu v roku 2008, oproti roku 2009, kde to trvalo 51 dní).

Výsledky štúdie ukázali (obr. 5, obr. 6), že frekvencia kambiálneho delenia, redukcie dĺžky trvania kambiálneho rastu, výsledná redukcia formácie dreva, zníženie ročného prírastku xylému v dreve, sú väčšinou zjavné v odpovedí rastu smreka obyčajného (Picea abies) vo Flakalidene, Švédsku. Výskum potvrdil, že metóda "micro-coring" bola úspešne aplikovaná na ihličnaté dreviny (Rossi et al. 2006) ako vhodná metóda pre výskum tvorby dreva.

ACKNOWLEDGEMENTS

I would like to thank Harri Mäkinen, Tuomo Kalliokoski for supervising and to all Metla´s staff. I would like to thank also Peter Hohti for helping me with the laboratory work. The study has been supported by APVV-0231-07, VEGA 1/0238/08 and UK grant 579/2010 projects.

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