RADIAL GROWTH DYNAMICS OF SPRUCE (PICEA ABIES) MEASURED BY MICRO-CORES*

by

Ernst Bäucker¹, Claus-Thomas Bues¹ & Michael Vogel²

¹Institute of Forest Utilization and Forest Technology, ²Institute of Forest Growth and Forest Computer Science, Dresden Technical University, 01735 Tharandt, PF 10, Germany

SUMMARY

Using medical cannulas of 0.5 mm inside diameter, micro-cores were taken at three circumferential heights from five spruce trees [Picea abies (L.) Karst.] in the Ore Mountains (Germany). The micro-cores were taken every second week during the growing seasons of 1996 and 1997 and light-microscopy studies were made on the micro-cores. The number of xylem and phloem cells, and total cell count in the cambial zone, were tabulated for each sample. Determination of the intra-annual cellular growth rate of conifers is simplified and will be possible also in long-term experiments. Preliminary evaluations of the wound response indicate that this micro-coring technique causes minimal injury.

Key words: Increment micro-cores, micro-coring, cambial activity, wood formation, phloem, Picea abies (L.) Karst.

INTRODUCTION

Wood formation is influenced by genetic factors that differ from tree to tree, as well as by environmental factors. In addition to short-term weather changes the rate of new cell formation is modified by long-term impacts (Denne & Dodd 1981; Dünnisch & Bauch 1994; Larson 1994). Extreme weather conditions, periodic climatic fluctuations, and anthropogenic air pollution are additional factors that affect the rate of cell formation (Fritts 1976; Antonova & Stasova 1993).

Using band dendrometers, the diamic development of trees can be continuously monitored with a resolution of 1 μm (e.g., Lövdahl & Odin 1988; Vogel 1992). However band dendrometer movements are influenced not only by the formation of xylem, but also by phloem cell collapse, by growth processes taking place in the outer bark, and by reversible changes in the diameter. These reversible variations are mainly due to changes in water storage capacity of the stem during the growing season, and by daily fluctuations in the xylem water potential (Vogel 1994; Vogel et al. 1996; Kuroda & Kiyono 1997). Hence dendrometer measurements alone are of limited value for a precise measurement of cell formation.

The intra-annual formation of xylem cells can be monitored through cambial wounding (Wolter 1968; Denne 1977; Brix & Mitchell 1980; Kuroda 1986; Dünnisch & Bauch 1993).

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1994). Cambial markings supply results only with a timelag. However, this requires the sampling of square wood pieces, or the felling of the sample trees and thus the end of the experiment.

Precise analysis of the cambial activity can be made by direct sampling and investigation of the cambial samples (Dünisch et al. 1996). However, this generally causes significant injury which influences the long-term growth of the tree (Schöpfer 1962; Lenz & Oswald 1971), especially if repeated sampling is done at the same sampling height over several years.

Loris (1981) examined xylem formation using small samples which were removed with medical cannulas (1.6 mm) and were prepared for scanning electron microscopy.

This paper will present a modification of the method of Loris, applied to spruce trees for the investigation of radial increments and shows its applicability in ecological studies. Our experiment began in 1996 and will be completed by the autumn of 1998. This paper is aimed at giving early access to the methodology. The examples are chosen in such a way that wide variations in cell formation are included, thus providing a broad spectrum with regard to sampling, preparation and evaluation.

MATERIALS AND METHODS

The investigations were conducted on trees from a 43-year-old spruce stand of yield class M 32 [The stand will reach a height of 32 meters at age 100 (Yield table: Wenk et al. 1984)]. The forest is at 739 m at sea level in the eastern part of the Ore Mountains (Germany). The area has an average annual temperature of 5.5 °C (May through August 12.5 °C), an average annual precipitation of 900 mm (May through August 380 mm). The site is stressed by a high SO₂ concentration over many years, and by presently increasing O₃ concentrations.

In the current investigation, three spruce trees (A1, A2, and A3) [Picea abies (L.) Karst.] of a drought-stress experiment were selected. These trees are protected from rainfall by a roof structure, and isolated from surface groundwater flow by peripheral trenching. The experiment started in the spring of 1996. Two spruce trees in the same stand (not covered and not trenched around) serve as controls (VA1, VA2). A control tree (VA0) was felled August 13, 1997 for studies on wound reaction to the micro-coring. All the trees are co-dominated except suppressed A3 (Table 1).

Table 1. Data of the experimental trees (DBH = diameter at breast height; CB = crown base; CM = middle of the crown; N = North; S = South; E = East; W = West); * = dimensions in autumn 1995.

<table>
<thead>
<tr>
<th>Tree</th>
<th>Height* (m)</th>
<th>DBH* (cm)</th>
<th>Sample 1996</th>
<th>Sample 1997</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>14.6</td>
<td>17.3</td>
<td>1.3 N</td>
<td>1.3 N, S; CB N, S; CM N, S</td>
</tr>
<tr>
<td>A2</td>
<td>14.7</td>
<td>18.6</td>
<td>1.3 N</td>
<td>1.3 N, S; CB N, S; CM N, S</td>
</tr>
<tr>
<td>A3</td>
<td>9.6</td>
<td>15.3</td>
<td>1.3 N</td>
<td>1.3 N, S</td>
</tr>
<tr>
<td>VA1</td>
<td>14.5</td>
<td>17.7</td>
<td>1.3 N, S</td>
<td>1.3 N, S, E, W</td>
</tr>
<tr>
<td>VA2</td>
<td>11.9</td>
<td>17.3</td>
<td>1.3 N, S</td>
<td>1.3 N, S, E, W</td>
</tr>
<tr>
<td>VA0</td>
<td>15.6</td>
<td>20.9</td>
<td>—</td>
<td>1.3 S</td>
</tr>
</tbody>
</table>
Fig. 1–5. Sampling, preparation and wound response by using micro-cores. – 1: Sampling of a micro-core using a medical cannula (near older marked puncture points). – 2: Microsection produced from a micro-core, diameter 0.5 mm, tree A3, October 1 1997. – 3: Micro-section made from a core, xylem (X), extended cambial region (C), phloem (Ph), tree A2, July 15. – 4: Wound response at the puncture canal (WP), following sampling of May 13 and felling of the tree in August. – 5: Wound response 2 mm above (at stem axis) the puncture with a medical cannula presented in Fig. 4. — Scale bars: 2 = 500 μm, 3 = 100 μm, 4 & 5 = 500 μm.

As noted earlier, the sampling for the radial cell increment investigation was based on the method presented by Loris (1981) and further developed by the authors. Our medical cannulas (Sterican 0.80 × 40 mm, Braun) have an outside diameter of 0.8 mm and an inside diameter of 0.5 mm. They were specially sharpened prior to each sampling. After the outer bark scales had been removed, the cannulas were inserted 10 to 12
mm (Fig. 1). After sampling, the hole was closed with wound paste [Tervanol (Stähler)], and marked. With twice-weekly sampling (plus weekly sampling in July 1997), the trees have become well-punctured by sampling points; however, we have kept a 2 cm horizontal distance between adjacent coring points. Band-dendrometers were placed close to all core-sampling circumferences.

In 1996 samples were taken from the north side of the drought-stressed trees (A1, A2, and A3) at breast height; in 1997 we took samples from both the north and the south side at breast height and also from the crown base and the middle of the crown (trees A1 and A2 only). In 1996 core samples were taken from the north and south sides of control trees VA1 and VA2; in 1997 we sampled these control trees at quarter points around the trees (at breast height). When a circumference becomes completely encircled, a second sampling circumference is set slightly below the initial ringing.

After sampling, the micro-cores were pushed out of the cannulas with a thin wire from the inside, and stored in a phosphate-buffer solution. In the laboratory the cores were lined up for thin-sectioning with a Leica 1206 Freezing-Microtome. The cores were embedded in Jung embedding medium (Leica), frozen to −30 °C, and sectioned at 12 µm thickness. The sections were stained with astra-blue and safranin (Gerlach 1977) and embedded in a glycerol gelatine fixer (Merck).

The xylem and phloem of the current years, and generally the extended cambial region, could be successfully prepared (Fig. 3). The microsections from the drought-stressed trees (lower increment) were easier to prepare and of higher quality than those of the faster grown control trees. The microscopic analysis of the 1996 sampling was done by counting the xylem cells of the annual ring which had formed up to the date of sampling. The xylem cells of the older, complete annual rings present within the microsection were also counted. Additionally, the number of phloem cells (sieve cells and phloem parenchyma cells) of the youngest four annual rings of the phloem, and the number of cells in the extended cambial zone, were counted in 1997 (Fig. 3). The border between xylem and the extended cambial zone (the beginning of lignification) was made visible by red staining (safranin).

RESULTS

Wound response

The wound response was studied by microscopic analysis on serial sections of tree VA0 at the micro-core of May 13, 1997; the tree was felled August 1997. An embolism in the sapwood occurred only very close to the puncture canal, but was as deep as the maximum puncture depth. This embolism extended approximately 3 mm out tangentially from the coring point. The puncture canal was filled with wound parenchyma which extended several millimetres into the annual ring of the previous year (Fig. 4). From the middle of the 1997 annual ring, the series of tracheids indicated that the cambium layer was again completely closed at the site of the puncture and was able to function normally. At the time of felling of VA0 the periphery of the annual ring had a normal surface without visible wound response (e.g. no bumps). At a distance of 2 mm above the puncture (Fig. 5) wound parenchyma formed within the
Fig. 6. Number of xylem cells in the 1996 annual ring at breast height. Trees A1, A2 and A3 sampling direction (beginning) north, trees VA1 and VA2 average values of sampling directions north and south.

Fig. 7. Number of xylem cells in the 1997 annual ring, at breast height, trees A1, A2 and A3 average values from the two sampling directions, trees VA1 and VA2, average values from 4 sampling directions.
Fig. 8. Tree A1, breast height, number of cells in the xylem, cambium and phloem in the 1997 annual ring, average values as of two sampling directions each.

Fig. 9. Tree A1, 1997, number of xylem cells in the three tree heights investigated (breast height, crown base, middle of the crown).
zone of earlywood, and in latewood the number of resin ducts tended to increase. However, there were hardly any irregularities visible in latewood or at the boundary of the annual ring.

Cell formation

In 1996, following a cool spring, the xylem formation in all trees did not start until the beginning of June (Fig. 6). The end of cell formation was recorded as the first week of September. On the suppressed tree A3, the differentiation of the xylem cells started about 4 weeks later and came to an end 4 weeks earlier compared with the other trees.

In 1997 xylem formation in all trees began in mid-May (Fig. 7). The two control trees (VA1, VA2) produced 40% more cells (in cell count) than in 1996. The drought-stressed trees stopped growing at the beginning of July (A1) and August (A2, A3). Later, only a change in the numbers of cells could be determined at the various points of puncture, probably due to a variation of the annual ring width.

The date of the end of cell division was indicated by the beginning of a clear reduction in the number of cells in the extended cambial zone (Fig. 8, of July 8). During the development of cambial dormancy only a differentiation of the xylem and phloem cells took place; the total number of xylem and phloem and cambial zone cells did not increase further. A comparison of the samples from the different tree heights showed differences in the formation of the xylem cells. In tree A1 the rate of cell formation of xylem clearly increased relative to tree height (Fig. 9). In contrast,
in tree A2, no difference was detected in the number of xylem cells in the 1997 annual ring at the three different heights. The influence of the drought stress was visible by the small number of xylem cells formed, as well as by the time between beginning and end of cell division.

**Phloem**

The difference in the number of cells formed in the annual phloem rings at breast height (sieve cells and phloem parenchyma cells) between the drought-stress trees and the control trees was less significant than differences between the samplings at the different tree heights (Fig. 10). The number of cells is higher in the crown-base and middle-of-the-crown locations.

The 1996 annual phloem ring had a lower number of cells than the annual phloem rings of 1994 and 1995; in 1997 the non-stressed trees appeared to again reach phloem cell numbers of the earlier years.

In trees A1 and A2 a reduction in cell numbers at breast height appeared to occur. This can be interpreted as a reaction of these trees to increasing drought stress, analogous to the reduced number of xylem cells. In both these trees, the greatest reduction in cell numbers was present at the crown base, and in the middle of the crown. The suppressed tree A3 formed the least number of phloem cells, without any clear indication of drought stress influence in 1997.

**DISCUSSION AND CONCLUSIONS**

Preliminary results on wound response to micro-coring indicate that such sampling can take place frequently and at a high density without causing great physiological impact on the tree. Thus comparisons to dendrometer measurements or physiological measurements (e.g., sap velocity measurements) can be made without requiring large distances between points of puncture. Subsequently the wound parenchyma formation also allows assessments similar to the methods established for cambium marking (pinning method). In addition, a control of the results of xylem cell formation is possible at the end of the experiment.

The big advantage of this micro-coring method is the possibility of frequent counting of the actual number of xylem cells simultaneously with the number of cells in the cambial zone, as well as the number of phloem cells in the actual annual ring at every date of sampling. With the evaluation of the cells (cell border thickness and cell lumen width) it becomes possible to determine more of the radial growth process which controls the changes in bole diameter. Only the growth processes taking place in the outer bark are not covered by this technique. If the cannulas are inserted deeply enough, it is possible to evaluate the radial cell formation of several annual rings, and of variation between cell numbers and dimensions of these previous annual rings. Using this relatively time-saving method, it is possible to do frequent, near-simultaneous sampling of a number of trees, or to do multiple-height sampling of trees. More secure comparative data than hitherto available can be assembled, on rates of cell division, radial cell dimensions, and the relationship to band-dendrometer measurements.
REFERENCES


