WOOD RESEARCH
52 (2): 2007
1-10

WOOD FORMATION IN NORWAY SPRUCE *(PICEA ABIES)* STUDIED BY PINNING AND INTACT TISSUE SAMPLING METHOD

Jožica Gričar
Slovenian Forestry Institute, Ljubljana, Slovenia

Martin Zupančič, Katarina Čufar, Primož Oven
University of Ljubljana, Biotechnical Faculty, Department of Wood Science and Technology, Ljubljana, Slovenia

ABSTRACT

Seasonal dynamics of xylem formation were studied at a cellular level in Norway spruce *(Picea abies)* from the lowland forests Sorsko polje (350 m a.s.l.) and the sub-alpine site Pokljuka (1200 m a.s.l.) in Slovenia during the growing season 2003. The pinning technique and sampling of intact tissue were performed at weekly intervals to assess the usefulness of both methods for examining wood formation. Pinning induced desiccation of differentiated xylem, necrosis of cambial cells and undifferentiated xylem derivatives, and formation of callus, traumatic resin canals and wound-wood. The border between cambial cells and radially expanding cells located inside the callus was a reliable mark for defining the xylem increment reached from the time of wounding. Intact tissue sampling was superior for precise investigations of cambial activity, various developmental stages of tracheids, and for secondary phloem formation.

KEY WORDS: Norway spruce *(Picea abies)*, wood formation, cambial activity, intact tissue sampling method, pinning method

INTRODUCTION

Wood formation is an episodic process in trees of temperate climatic regions. Development of xylem begins with cell divisions in the cambium followed by successive phases of cell differentiation, which include expansion, secondary wall formation, lignification, and cell death with autolysis of protoplasts in xylem elements destined for water transport and mechanical support (Wardrop 1965, Wodzicki 1971, 2001, Larson 1994, Savidge 1996, Kozlowsky and Pallardy 1997, Dengler 2001, Plomion et al. 2001, Gričar et al. 2005).

The seasonal dynamics of cambial activity and wood formation in trees can be followed by a variety of different methods, such as pinning, micro-coring, using dendrometers, sampling of

intact blocks of tissues, tilting, and radiological methods (Whitmore and Zahner 1966, Wolter 1968, Wodzicki 1971, Skene 1972, Antonova and Stasova 1993, 1997, Bäucker et al. 1998, Gindl and Grabner 2000, Schmitt et al. 2000, Forster et al. 2000, Deslauriers et al. 2003a, b, Mäkinen et al. 2003, Schmitt et al. 2004, Deslauriers and Morin 2005).

The pinning method uses the ability of the cambium and its youngest derivatives to respond to a minute mechanical injury without affecting the physiological integrity of a tree (Wolter 1968). Pin insertion into the cambium causes minute wound reactions, which define the increment reached from the time of pinning. Therefore, precise knowledge of the species-related anatomical response to wounding is necessary when applying this technique (Wolter 1968, Kuroda and Shimaji 1983, 1984a, b, Yoshimura et al. 1981, Kuroda and Kuyono 1997, Mäkinen et al. 2003, Schmitt et al. 2000, 2004). A second method, which involves sampling of small blocks containing intact wood, cambium, and bark, seems to be a useful technique for directly examining the xylem increment at every moment of the growing season (Uggla and Sundberg 2002, Gindl et al. 2001, Schmitt et al. 2003, Gričar et al. 2005).

The objective of the current study was to assess the feasibility of the pinning method (PM) and the intact tissue sampling method (ITSM) for these kinds of studies. As a part of this, the anatomical response of Norway spruce to pin insertion was examined in order to define relevant anatomical markers for assessment of xylem growth in this species.

MATERIAL AND METHODS

Sampling was performed on dominant and co-dominant Norway spruce (*Picea abies* (L.) Karst.) trees growing at Sorsko polje (350 m a.s.l.) and Pokljuka (1200 m a.s.l). Sorsko polje is a typical lowland plantation of Norway spruce, growing on shallow soils of detritus of the Sava River. The site is subjected to high seasonal oscillations in the underground water table resulting in late summer water stress (Fig. 1). Regular bark beetle attacks seem to be one of the secondary drought effects in this forest. Moreover, many of the trees are affected by root rot disease. Pokljuka (1260-1290 m a.s.l.) represents a typical, natural, sustainable managed sub-alpine spruce forest *(Piceetum subalpinum)* characterized by low winter temperatures and high amounts of snow (Fig. 2).

On each site, seven trees with comparable biometric characteristics and age (about 70 years) were selected, including five for pinning and two for sampling of intact tissues. The mean diameter of trees at the breast height was around 30 cm at Sorsko polje and 37 cm at Pokljuka. Both sites were visited weekly during the year 2003 from April 25th until November 14th and experiments were made according to the procedure described below. Six pinning holes were set in a semi-helical pattern along the stem of each tree at the same experimental date using a needle that was 1.75 mm at its thickest part. The holes were carefully marked and numbered. After the 2003 growing season, the pinned trees were felled, and samples containing wounded tissue were removed and processed for light microscopy. For the intact tissue sampling, a hammer and chisel were used to remove samples at breast height on the entire stem circumference to avoid influence of wounds on tissues of next sampling locations. The sample blocks (30 x 10 x 10 mm) contained the inner part of the living bark, the cambium, the current xylem increment, and at least one previous fully formed xylem growth ring. Sampling was done with care to avoid compression of cambial tissue or separation of bark from the wood. The pinned and intact samples were further treated according to traditional anatomical procedures. Immediately after removal, the tissues were fixed in FEA (formalin-ethanol-acetic acid solution) and dehydrated in a graded

series of ethanol (30%, 50% and 70%). Transverse sections of approximately 25 μm in thickness were prepared using a Leica SM2000R microtome. They were stained with safranine and astra blue and mounted in Euparal. Microscopy was carried out with a Nikon Eclipse E800 light microscope under bright field or polarized light with the aid of a Lucia G 4.8 image analysis system.

RESULTS

Pinning induced desiccation of differentiated xylem, necrosis of undifferentiated xylem derivatives and cambial cells, as well as formation of callus, traumatic resin canals and typical wound-wood (Fig. 1a). The callus, comprised of cells of irregular shape with thick lignified walls, developed from cambial cells as well as from axial and radial xylem derivatives in the stage of postcambial growth. The cells of solitary files were not involved in the formation of callus. These cells died, but the anatomy of the tissue extant at the time of pinning remained almost unchanged. The cells adjacent to the pinning canal were often crushed and collapsed. The beginning of wood formation was defined by distinguishing between necrotic residuals of cambial cells and radially expanding xylem cells located in the callus adjacent to the 2002 xylem growth ring. During the growing season, the radial files of xylem cells were comprised of mature tracheids, necrotic cells in the stage of secondary wall deposition, and dead cells in the stage of radial postcambial growth, which were usually followed by the necrotic cambial cells. The tracheids in postcambial growth and cambial cells were found inside the callus in the solitary radial files only (Fig. 1b). The cessation of regular cambial activity was determined when the following conditions were fulfilled: the number of the cells of the current xylem increment coincided with the number of the cells in the intact part of the same growth ring aside of the callus, radially expanding cells were absent from the callus, and the latest formed late wood tracheids below the callus were in the final stages of differentiation. The termination of the cambial activity was often masked with reactivation of the cambium due to pinning, particularly in the trees from the low elevation Sorsko polje site (Fig. 1d). The occurrence of the traumatic resin canals exhibited high spatial and temporal variability. When present, solitary traumatic resin canals were distributed diffusely and formed later than the callus in the early wood. The temporal coincidence of formation of callus and traumatic resin canals, arranged in tangential series, predominated in the late wood. We had to prepare new sections from the parallel pinning samples when tissue below the callus was crushed and torn or when cambial cells and cells in postcambial growth were not preserved in the callus, as well as in the cases when traumatic resin canals developed in the callus (Fig. 1c, e). With the PM, it was not possible to investigate the dynamics of phloem growth ring formation or the variability in the number of cambial cells throughout the vegetation period, since these tissues were not usually preserved in the callus.

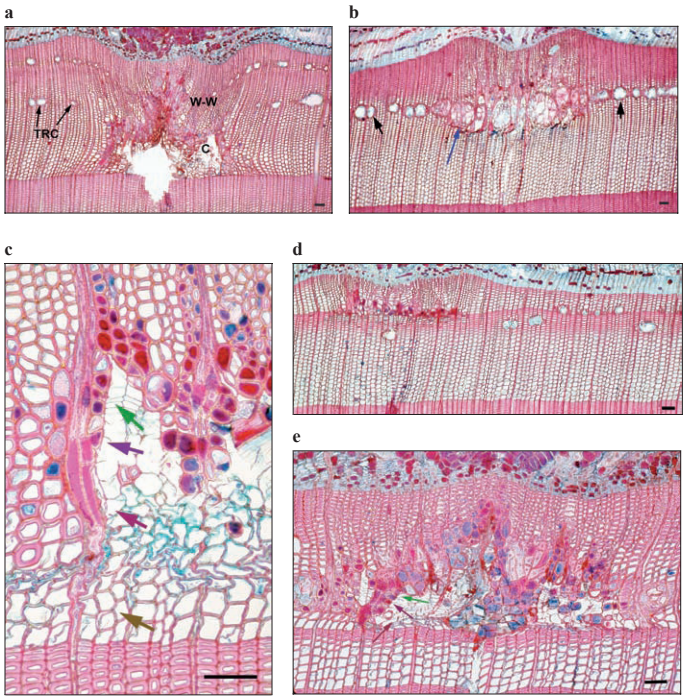

Fig. 1: Light micrographs of transverse sections of pinning samples: a – Callus (C) traumatic resin canals (TRC) and wound-wood (W-W) formed as a response to wounding; b – Tangentially arranged TRC (black arrows) and formation of TRC in the callus (blue arrow). Wavy appearance of annual ring boundaries at the wound as observed in all examined samples; c, e – Residuals of cambial cells (green arrows) and radially expanding tracheids (violet arrow) in the callus. Well visible cells in the phase of secondary wall formation and lignification (red arrow) and mature tracheids (brown arrow) below thecallus; d – reactivation of the cambium as a response to wounding of the meristem after the cessation of its regular activity. Scale bars = 100 µm

The ITSM enabled relatively easy and unambiguous definition of the onset and cessation of cambial activity. It was also possible to study the seasonal dynamics of the individual phases of xylogenesis (Fig. 2a, c). The initiation of the secondary wall thickening was determined by birefringence in the cell walls under the polarized light (Fig. 2b). The blue-stained cell walls and the protoplasmic content in the cell lumina indicated incompletely developed tracheids (Fig. 2c). The fully matured tracheids had red-stained cell walls and empty lumina (Fig. 2d). In contrast to the PM, the ITSM enabled us to follow the dynamics of phloem growth ring formation during the vegetation period.

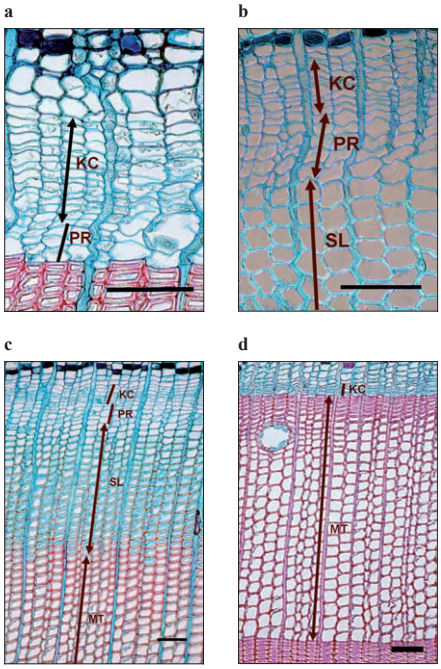

Fig. 2: Transverse sections of the intact tissue samples: a – Beginning of the cambial activity; b – Cells undergoing secondary wall thickening as observed under polarized light; c – Developing tracheids with blue stained cell walls and protoplasmic content in the lumina; d – Entire 2003 xylem growth ring where all cells are fully matured. KC – cambial cells, PR – xylem cells in postcambial growth, SL – xylem cells undergoing secondary cell wall deposition and lignification, MT – mature tracheids. Scale bars = 100 μm

DISCUSSION

Both the pinning and the ITS method proved to be suitable for investigations of seasonal dynamics of wood formation in Norway spruce. However, a thorough understanding of the cambial wound response is needed when applying the pinning technique in research of cambial activity. The process of wound healing in trees has been described as a formation of dedifferentiated callus tissue, followed by a vascular tissue formation within it (Kuroda and Shimaji 1984a). New cambium developed in a continuation of the old one (Oven 1997). Polar basipetal flux of auxin is necessary for spatial organization of vascular tissues, as well as for maintenance of cell divisions in the cambium. Wounding of the cambium interrupted flux of auxin causing disorganized cambial growth (Uggla et al. 1996, Lachaud et al. 1999, Sundberg et al. 2000). Cambial cells isolated from regular supply of auxin dedifferentiated into parenchyma cells (Savidge 1996, 2000). Cells of wound-wood formed after callus were disorientated and irregular shapes. Oven (1997) reported that first formed cells were tracheoids, then short disorientated tracheids gradually followed by normal tracheids arranged in radial files.

An examination of pinned tissue in Norway spruce revealed that the border between the

cambial cells and the first cells in postcambial growth located inside of the callus represented a reliable marker for defining the xylem increment realized by the time of wounding. These cells contributed to callus formation in the vicinity of the pinning canal, but remained preserved in the developmental stage from the time of pin insertion in sporadic radial files. It was previously reported that the cambial cells were not involved in differentiation of traumatic tissue after pin insertion in conifers (Kuroda and Shimaji 1984a, b). The site of the cambium at the date of the wounding in the investigated Norway spruce trees was located in the callus and not above it, as in the case of *Chamaecyparis obtusa, Cedrus deodara* (Kuroda and Kuyono 1997) and *Pinus taeda* (Yoshimura et al. 1981). Contrary to previous observations of Kuroda and Shimaji (1983), we could not confirm the traumatic resin canals as a reliable marker for pinpointing cambial cells in Norway spruce. Using the PM, the cessation of regular activity of the cambium could be masked with wound-induced reactivation of the meristem, resulting in formation of false growth rings. This response was observed in samples pinned in the last part of the growing season. Such abnormal reactivation is very likely a consequence of hormonal unbalance caused by wounding (Kuroda and Kiyono 1997). Our experience with the pinning method confirms previous reports that information on seasonal dynamics of xylem formation remained preserved in the xylem growth rings for many years after the experiment.

CONCLUSIONS

Contrary to the PM, the critical part of sampling intact tissue blocks was at the height of the growing season when a wide, thin-walled cambial region and a layer of cells in postcambial growth were very sensitive to mechanical rupture. In addition, difficulties with sampling may appear in trees with a very thick rhytidome because great part of bark needs to be removed. Also, sampling over several years at the same sampling height of a stem would induce numerous wounds that could influence cambial growth in the vicinity of subsequent samplings. However, this method was found to be far more suitable then the PM for distinguishing various developmental stages of xylem cells, cambial cells, as well as for analysis of secondary phloem formation.

ACKNOWLEDGEMENTS

We are grateful to Peter Cunder for invaluable assistance in the field. We are indebted to the Slovenian Forest Service, GGO Bled, and GE Pokljuka for providing access to the experimental sites. The work was funded by the EU project PINE (EVK2-CT-2002-00136) and the Ministry of Education, Science and Sport of the Republic of Slovenia.

REFERENCES

1. Antonova, G. F., Stasova, V.V., 1993: Effects of environmental factors on wood formation in Scots pine stems. Trees, 7: 214-219
2. Antonova, G.F., Stasova, V.V., 1997: Effects of environmental factors on wood formation in larch (*Larix decidua* Ldb.) stems. Trees, 11: 462-468
3. Bäucker, E., Bues, C., Vogel, M., 1998: Radial growth dynamics of spruce *(Picea abies)* measured by micro-cores. IAWA Journal, 3: 301-309
4. Dengler, N.G., 2001: Regulation of vascular development. Journal of Plant Growth regulation, 20: 1-13
5. Deslauriers, A., Morin, H., Urbinati, C., Carrer, M., 2003a: Daily weather response of balsam fir (*Abies balsamea* (L.) MIll.) stem radius increment from dendrometer analysis in the boreal forest of Quebec (Canada). Trees, 17: 477-484
6. Deslauriers, A., Morin, H., Begin, Y., 2003b: Cellular phenology of annual ring formation of *Abies balsamea* in the Quebec boreal forest (Canada). Canadian Journal of Forest Research, 33: 190-200
7. Deslauriers, A., Morin, H., 2005: Intra-annual tracheid production in balsam fir stems and the effect of meteorological variables. Trees, 19: 402-408
8. Gindl, W., Grabner, M., 2000: Characteristics of spruce (*Picea abies* (L.) Karst) latewood formed under abnormally low temperatures. Holzforschung, 54: 9-11
9. Gindl, W., Grabner, M., Wimmer, R., 2001: Effects of altitude on tracheid differentiation and lignification of Norway spruce. Canadian Journal of Botany, 79: 815-821
10. Forster, T., Schweingruber, F.H., Denneler B., 2000: Increment puncher – a tool for extracting small cores of wood and bark from living trees. IAWA Journal, 21: 169-180
11. Gričar, J., Čufar, K., Oven, P., Schmitt, U., 2005: Differentiation of terminal latewood tracheids in silver during autumn. Annals of Botany, 95: 959-965
12. Kozlowsky, T.T., Pallardy, S.G., 1997: Growth control in woody plants. Academic Press Inc. San Diego, 641 pp.
13. Kuroda, K., Shimaji, K., 1983: Traumatic resin canal formation as a marker of xylem growth. Forest Science, 29: 653-659
14. Kuroda, K., Shimaji, K., 1984a: Wound effects on xylem cell differentiation in a conifer. IAWA Bulletin n.s., 5: 295-305
15. Kuroda, K., Shimaji, Y., 1984b: The pinning method for marking xylem growth in hardwood species. Forest Science, 2: 548-554
16. Kuroda, K., Kiyono, Y., 1997: Seasonal rhythms of xylem growth measured by the wounding method and with a band-dendrometer: an instance of *Chamaecyparis obtusa*. IAWA Journal, 18: 291-299
17. Lachaud, S., Catesson, A.M., Bonnemain, J.L., 1999: Structure and functions of the vascular cambium. Life Sciences, 322: 633-650
18. Larson, P.R., 1994: The vascular cambium: development and structure. Springer. Berlin Heidelberg New York, 725 pp.
19. Mäkinen, H., Nöjd, P., Saranpää, P., 2003: Seasonal changes in stem radius and production of new tracheids in Norway spruce. Tree Physiology, 23: 959-968
20. Oven, P., 1997: Odziv sekundarnega floema in ksilema ter kambija na mehanske poškodbe bele jelke (*Abies alba* Mill.), navadne smreke (*Picea abies* Karst.), rdečega bora (*Pinus sylvestris* L.) in evropskega macesna (*Larix decidua* Mill.). Doctoral thesis. University of Ljubljana, Biotechnical faculty, Department of Wood Science and Technology. Ljubljana, 167 pp.

21. Plomion, C., Leprovost, G., Stokes, A., (001: Wood formation in trees. Plant Physiology, 127: 1513-1523
22. Savidge, R.A., 1996: Xylogenesis, genetic and environmental regulation – a review. IAWA Journal, 17: 269-310
23. Savidge, R.A., 2000: Biochemistry of seasonal cambial growth and wood formation – an overview of the challenges. In: Cell and Molecular Biology of Wood Formation (eds. R. A. Savidge, J. R. Barnett, R. Napier). Pp. 1-30, BIOS Scientific Publishers Limited. Oxford, UK.
24. Schmitt, U., Möller, R., Eckstein, D., 2000: Seasonal wood formation dynamics of beech (*Fagus sylvatica* L.) and black locust (*Robinia pseudoacacia* L.) as determined by the "pinning" technique. Journal of Applied Botany 74: 10-16
25. Schmitt, U., Grünwald, C., Gričar, J., Koch, G., Čufar, K., 2003: Wall structure of terminal latewood tracheids of healthy and declining silver fir trees in the Dinaric region, Slovenia. IAWA Journal 24: 41-51
26. Schmitt, U., Jalkanen, R., Eckstein, D., 2004: Cambium dynamics of *Pinus sylvestris* and *Betula* spp. in the northern boreal forest in Finland. Silva Fennica 38: 167-178
27. Skene, D.S., 1972: The kinetics of tracheid development in *Tsuga canadensis* Carr. and its relation to tree vigour. Annals of Botany 36: 179-187
28. Sundberg, B., Uggla, C., Tuominen H., 2000: Cambial growth and auxin gradients. In: Cell and Molecular Biology of Wood Formation (eds. R. A. Savidge, J. R. Barnett, R. Napier). Pp. 169-188, BIOS Scientific Publishers Limited. Oxford, UK.
29. Uggla, C., Moritz, T., Sandberg, G., Sundberg, B., 1996: Auxin as a positional signal in pattern formation in plants. Proceedings of the National Academy of Sciences USA 93: 9282-9286
30. Uggla, C., Sundberg, B., 2002: Sampling of cambial region tissues for high resolution analysis. In: Wood formation in trees. Cell and molecular biology techniques. (ed. N. Chaffey). Pp. 215-228, Taylor & Francis. London New York.
31. Wardrop, A.B., 1965: Cellular diferentiation in xylem. In: Cellular ultrastructure of woody plants. Proceedings of the Advanced Science Seminar Pinebrook Conference (ed. W. A. Cote.). Pp. 61-97, Syracuse University Press. New York.
32. Whitmore, F.W., Zahner, R., 1966: Development of the xylem ring in stems of young red pine trees. Forest Science, 12: 198-210
33. Wodzicki, T.J., 1971: Mechanism of xylem differentiation in *Pinus sylvestris* L. Journal of Experimental Botany, 22: 670-687
34. Wodzicki, T.J., 2001: Natural factors affecting wood structure. Wood Science and Technology, 35: 5-26
35. Wolter, K.E., 1968: A new method for marking xylem growth. Forest Science, 14: 102-104
36. Yoshimura, K., Itoh, T., Shimaji, K., 1981: Studies on the improvement of the pinning method for marking xylem growth. Mokuzai Gakkaishi, 27: 755-760

Jožica Gričar
Slovenian Forestry Institute
Večna pot 2
SI-1000 Ljubljana
Slovenia
Tel: 386 1 200 78 00
Fax: 386 1 257 35 89
E-mail: jozica.gricar@gozdis.si

Martin Zupančič
University of Ljubljana
Biotechnical Faculty
Department of Wood Science and Technology
Rožna dolina
Cesta VIII/34
SI-1000 Ljubljana
Slovenia

Katarina Čufar
University of Ljubljana
Biotechnical Faculty
Department of Wood Science and Technology
Rožna dolina
Cesta VIII/34
SI-1000 Ljubljana
Slovenia

Primož Oven
University of Ljubljana
Biotechnical Faculty
Department of Wood Science and Technology
Rožna dolina
Cesta VIII/34
SI-1000 Ljubljana
Slovenia