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Cost Action E20
Wood Fibre Cell Wall Structure

FIBRE WALL

Program and Proceedings of the final Workshop

BUILDING A CELL WALL

September 4—6, 2003, Helsinki, Finland

Organised by
Metla
The Finnish Forest Research Institute
and
University of Helsinki
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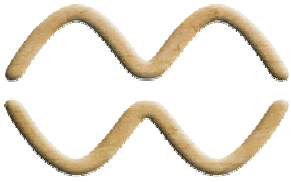

wood wisdom

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Scientific Program

Final Workshop "Building a Cell Wall" Helsinki, September 4−6, 2003

Thursday 4th September

12.00 : Registration

Session 1 - All WG Fibre Wall - research and applications *Lecture hall 1*
Chairman: Pekka Saranpää and Kurt Fagerstedt

14.00 : 14.15 Introductory Remarks, P. Ander, P. Saranpää

14.15 : 15.00 Leena Paavilainen, *WOODWISDOM - Wood Material Science Research Programme, Helsinki* - KEYNOTE:
Wood fibre research: importance for pulp and paper industry

15.00 : 15.20 L. Eriksson, *Stockholm*:
The COST system in a changing world - some personal views

15.20 : 15:50 Coffee break

15.50 : 16.10 R. Wimmer, G. Downes and R. Evans, *Vienna*, *Hobart and Clayton*:
Interpreting sub-annual wood-property variation in terms of stem growth

16.10 : 16.30 G. Daniel, *Uppsala*:
Cellulose aggregates an integral part of pulp fibre structure

16.30 : 16.50 K. Ruel and J.-P. Joseleau, *Grenoble*:
Contributions of lignins to the building of wood secondary walls

16.50 : 17.10 M. Lehtonen, K. Hildén, K. Marjamaa, K. Fagerstedt, P. Saranpää, T. Lundell, *Helsinki*:
Localization of peroxidases in developing xylem of Norway spruce (*Picea abies*)

17.10 : 20.00 Refreshments and Poster Session I (All WG) *Rooms 3 and 4*

18.00 : 19.00 Management Committee I *Lecture hall 1*

Friday 5th September

8.00 : Registration

Session 2 - WG1 Biosynthesis and Modelling *Lecture hall 1*
Chairman: Anne Mie Emons

8.45 : 9.30 Candace Haigler, *North Carolina State University, Raleigh, USA* - KEYNOTE: **Cellulose and lignin biosynthesis: xylem vessel formation in vitro**

9.30 : 9.50 B.M. Mulder, M.A.W. Franssen-Verheijen, J.H.N. Schel, A.M.C. Emons, *Wageningen*:
The geometrical model for cellulose microfibril deposition, extended to random wall texture

9.50 : 10.10 M. Ebskamp, M. Akkerman, A.M. Emons, *Wageningen*:
Using cellulose synthase - GFP fusions as a tool to investigate cellulose biosynthesis

10.10 : 10.30 Teemu Teeri, *Helsinki*:
Linkage specificity and (per)oxidases in lignin polymerization

10.30 : 11.00 Coffee Break

Session 3 - WG2 Characterisation and Ultrastructure
Chairman: Uwe Schmitt

11.00 : 11.45	Andrew Staehelin, *University of Colorado at Boulder, USA* - KEYNOTE: **Cell plate assembly: Insights from electron tomography**
11.45 : 12.05	J. Fahlén and L. Salmén, *Stockholm*: **Pore size distribution in transverse direction of the wood fibre wall**
12.05 : 12.25	M.-P. Sarén, M. Peura, S. Andersson, P. Saranpää, M. Müller, R. Serimaa, *Helsinki and Kiel*: **Study of microfibril angle by X-ray diffraction - present state, future possibilities**
12.25 : 12.45	S. Bardage, L. Donaldson, C. Tokoh, G. Daniel, *Uppsala, Rotorua and Kyoto*: **Application of high resolution electron microscopy and image analysis for characterising pulp fibre surfaces**
12.45 : 14.00	Lunch and poster viewing *Rooms 3 and 4*

Session 4 - WG3 Cell Wall Structure and Properties *Lecture hall 1*
Chairman: George Jeronimidis

14.00 : 14.45	Holger Militz, *Georg-August University Göttingen, Germany* - KEYNOTE: **Changes of biological features in softwood and hardwood species due to wood modification treatments**
14.45 : 15.05	I. Burgert, K. Frühmann, J. Keckes, M. Eder, P. Fratzl, S.E. Stanzl-Tschegg, *Vienna and Leoben*: **New insights into structure-property-relationships on the cell wall level by micro-mechanical examinations of single wood fibres**
15.05 : 15.25	K. Kölln, I. Grotkopp, C. Behrend, M. Peura, R. Serimaa, M. Dommach, S.S. Funari, S.V. Roth, M. Burghammer, M. Müller, *Kiel, Helsinki and Grenoble*: **Tensile properties of cellulose fibres investigated *in situ* using synchrotron radiation**
15.25 : 16.30	Coffee Break and poster session II (All WG) *Rooms 3 and 4*

Session 5 - WG3 Cell Wall Structure and Properties *Lecture hall 1*
Chairman: Ingo Burgert

16.30 : 17.15	Tuula Teeri, *KTH, Stockholm, Sweden* - KEYNOTE: **Chemo-enzymatic modification of cellulosic materials**
17.15 : 17.35	M.S. Gilani and P. Navi, *Lausanne*: **Influences of microfibril angles and natural defects on the force-extension behaviour of single wood fibre modelling**
17.35 : 17.55	A. Limare, P. Dole, C. Joly, Y. Liu, B. Kurek, *Reims*: **Destructuring of hemp fibres by solvents and lignin oxidants: characterization of the thermomechanical properties of the polymers within the cell wall**
17.55 : 18:30	Poster viewing
18.30	Buses to hotels
20.00	Workshop Dinner at *Restaurant Töölönranta, address: Helsinginkatu 56*

Saturday 6th September

9.00 : 10.30	Working Group Parallel Meetings (All WG-leaders and members) WG 1 *Lecture hall 1,* WG 2 *Room 3,* WG3 *Room 4*
10.30 : 11.00	Coffee Break
11.00 : 11.30	Working Group Reports *Lecture hall 1*
11.30 : 12.30	Management Committee II *Lecture hall 1*
13:00 : 18:00	Excursion and lunch *Tuusula lake road*

Posters

Working Group 1

1. Aalto, M.K., P. Heino, J. Laine, C. Li, K. Ojala, L. Paulin, T. Puhakainen, J. Ulvila, A. Welling and T. Palva, *Helsinki*:
   Genomics approach for studying the development of dormancy and winter hardiness in birch (*Betula pendula*).
2. Akkerman, M., A.M.C. Emons and J.H.N. Schel, *Wageningen*:
   Insertion of cellulose synthase into the plasma membrane.
3. Kauppinen L., S. Tähtiharju, M. Laxell, K. Nieminen and Y. Helariutta, *Helsinki*:
   The role of cytokinin signalling during wood development.
4. Koutaniemi, S., A. Kärkönen, J. Immanen, T. Warinowski, I. Kilpeläinen, L.K. Simola, L. Paulin and T.H. Teeri, *Helsinki*:
   Comparison of gene expression in a lignin forming tissue culture and a developing wood of spruce.
5. Kukkola, E.M., S. Koutaniemi, E. Pöllänen, M. Gustafsson, P. Karhunen, T.K. Lundell, P. Saranpää, I. Kilpeläinen, T.H. Teeri and K.V. Fagerstedt, *Helsinki and Oulu*:
   Dibenzodioxocin lignin substructure is abundant in inner part of secondary wall in Norway spruce and Silver birch xylem.
6. Piispanen, R, T. Aronen, X. Chen, P. Saranpää, and H. Häggman, *Helsinki*:
   The effect of *aux* and *rol* genes on cell structure and chemistry in silver birch wood.
7. Pöllänen, E., A. Kattan, P. Elomaa, T. Teeri and M. Kotilainen, *Helsinki*:
   Functioning within cell wall: geg1 and possibly other family members participate in the regulation of cell dimensions
8. Warinowski, T., S. Koutaniemi, M. Toikka, A. Kärkönen, M. Mustonen, I. Kilpeläinen, L.K. Simola and T.H. Teeri, *Helsinki*:
   Lignin-bound peroxidases and a laccase from *Picea abies* tissue culture.

Working Group 2

9. Andersson, S., M. Peura, P. Saranpää and R. Serimaa, *Helsinki*:
   Crystallinity of wood and the size of cellulose crystallites.
10. Anttonen, S., K. Kostiainen, F. Ek, P. Saranpää, M. E. Kubiske, E. P. McDonald, J. Sober, D. F. Karnosky and E.Vapaavuori, *Suonenjoki, Vantaa and Rhinelander*:
    Are changes in wood chemical properties maintained over five years of exposure to elevated CO_2 and O_3 in aspen clones?
11. Bikova T., and A. Treimanis, *Riga*:
    Characterisation of the cell wall hemicelluloses by multi-wave UV-detection during SEC analysis.
12. Brändström, J., *Uppsala*:
    Ultrastructure of compression wood fibres in fractions of a thermomechanical pulp.
13. Fernando, D. and G. Daniel, *Uppsala*:
    Micro-morphological observations on spruce thermo-mechanical pulp fibre fractions with emphasis on fibre cell wall fibrillation and splitting.
14. Fioravanti, M., S. Federici, S. Ciattini, M. Peura, M.-P. Sarén and R. Serimaa, *Florence and Helsinki*:
    Determination of microfibril angles using x-ray diffraction in symmetrical and perpendicular transmission mode.
15. Frankenstein, C., C. Grünwald and U. Schmitt, *Hamburg*:
    On the regeneration of woody tissue in poplar after wounding.
16. Hafrén, J., *Uppsala*:
    Pectin on mechanical pulp fibre surfaces.
17. Koch, G., *Hamburg*:
    Topochemical characterization of lignins and phenolic extractives in wood cell walls.
18. Kostiainen, K., S. Anttonen, P. Saranpää, B. Sigurdsson, S. Linder and E. Vapaavuori, *Suonenjoki, Vantaa, Uppsala*:
    Effects of elevated [CO_2] and nutrients on wood structure and chemistry of Norway spruce.
19. Kostiainen, K., H. Jalkanen, S. Anttonen and E. Vapaavuori, *Suonenjoki*:
    Does climate change affect wood chemistry in silver birch?
20. Rangsri, W., J. Gril and G. Jeronimidis, *Montpellier, Reading*:
    Large deformations in transverse compression of a tropical hardwood: observation and finite-element analysis.
21. Touzel, J.-P., G. Jeronimidis, B. Chabbert, B. Monties and B. Cathala, *Reims and Reading*:
    New model systems of secondary plant cell walls based on bacterial cellulose/pectins composite: synthesis, chemical characterisation and mechanical properties.

Working group 3

22. Ander, P., *Uppsala*:
    Dislocations in wood fibres.
23. Ander P, I. Burgert and K. Frühmann, *Uppsala and Vienna*:
    The possible relationship between dislocations and mechanical properties of different spruce fibres: A single fibre study.
24. Belkova, L. and R. Kalnina, *Riga*:
    Properties of print paper produced in the 1st half of the 20th Century.
25. Frühmann, K., I. Burgert and S.E. Stanzl-Tschegg, *Vienna*:
    Radial trends of mechanical and fracture mechanical behaviour on the growth ring level of Norway spruce (*Picea abies* [L.] Karst).
26. de Jong, E. and J.C. Dekker, *Wageningen*:
    New measurement techniques leading to more insight in beating of chemical pulps.
27. Gindl, W. and U. Müller, *Vienna*:
    Effects of variability in cell-wall microstructure on the axial compression strength of Norway spruce.
28. Koukios E. G. and E. Avgerinos, *Athens*:
    Developing molecular strategies for delignification and characterisation of annual plant fibres.
29. Madsen, F.T., M. Peura, T. Koponen, S. Andersson, I. Grotkopp, K. Kölln, M. Müller and R. Serimaa, *Helsinki, Taastrup and Kiel*:
    Simultaneous determination of structure and tensile properties of industrial hemp
30. Peltonen, J., Å. Korsman, A. Arranto, P. Haapanen and L. Suomi-Lindberg, *Turku, Tampere and Helsinki*:
    Polymer composites reinforced by natural fibres - the role of fibre surfaces.
31. Raiskila, S.,T. Laakso, M. Pulkkinen, P. Saranpää, R. Mahlberg, L. Paajanen, A. C. Ritschkoff and K. Fagerstedt, *Helsinki*:
    IR analysis of lignin in Norway spruce (*Picea abies* (L.) Karst.).
32. Thygesen, A., A. B. Thomsen, H. Lilholt and G. Daniel, *Roskilde, Frederiksberg and Uppsala*:
    Microscopical and cytochemical observation on hemp stems with emphasis on fibers.
33. Thygesen, L. and P. Hoffmeyer, *Copenhagen*:
    Quantification of dislocations in hemp fibres.
34. Warensjö M., C. Lundgren and G. Daniel, *Uppsala*:
    X-ray micro densitometry and microscopical analysis of compression wood in relation to an image analysis method.
35. Vainio U., N. Maximova, J. Laine, P. Stenius, J. Gravitis and R. Serimaa, *Helsinki and Riga*:
    A small-angle x-ray scattering study on the morphology of kraft lignin.

Lectures

Wood material research: importance for pulp and paper industry

Leena Paavilainen

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The cost-competitiveness of Nordic forest cluster companies is excellent at present. There are several Nordic pulp and paper companies and wood products producers among the top ten companies in this sector in the world. The technology leadership achieved trough cluster co-operation has played a key role in boosting the companies' competitiveness. However, as technology development is in the hands of equipment suppliers, and as new production capacity has been increasingly built in regions with low production costs, the benefits achieved through improved process efficiency can be easily lost. At the same time, the trends in the marketplace are generating increasing demand for innovative, eco-efficient and cost-competitive products, processes and services. Although the industry has responded to the impacts of the public's increased environmental awareness, the potential of wood as a renewable raw material is not fully utilised, and the industry is only now starting to make use of the opportunities offered by ICT.

Human knowledge and competence are fundamental for the forest cluster's future success

The main challenge of the forest cluster is how to stay competitive in the global competition. Today, R&D in the forest cluster focuses on improving, optimising and increasing the efficiency of products and processes. The industry has also successfully utilised technology developed in other sectors. To be able to develop innovative, eco-efficient and cost-competitive products, processes and services, the forest industry should transform itself from a technology leader into an innovator. Human knowledge and competence are fundamental for the forest cluster's future success. The focus should be on innovations and new business, which means investing in people and know-how and in building co-operation with other sectors.

Multidisciplinary research in wood material science promotes the forest cluster's competitiveness

Wood material science research plays a key role in developing innovative, eco-efficient and cost-competitive products and processes. It can for example offer a solution to the challenge posed by sustainable development: to reduce radically the use of raw material in wood and fibre-based products. Combining wood with other materials creates new functional properties for wood-based products. Modern biotechnology and molecular genetics open up new possibilities for improving and tailoring wood properties. Multidisciplinary research is essential in the forest cluster, and besides conventional technologies and material science also biotechnology and ICT knowledge are vital for innovations. To improve its capacity for generating innovations and to transform R&D investment into a sustained competitive edge, the forest cluster must combine its resources and actively promote transfer of knowledge and technology between its members and other sectors

The long-term work to create innovative forest-based products started under the Wood Wisdom Research Programme in the period 1998–2001. The members of the forest cluster then agreed that there is a need to continue and deepen the research in the material science of wood. At the same time, the strong globalisation of the forest industry has led to a need for more active international networking in research. An international approach is essential when aiming to generate new strategic knowledge in the area of wood material science.

Wood Material Science Research Programme (2003-2006)

The Wood Material Science Programme is a bilateral continuation of the Finnish Forest Cluster Research Programme, Wood Wisdom. The objective of the programme is to build a knowledge base and to strengthen international research co-operation in the area of wood material science as a means to promote development of innovative, eco-efficient and cost-competitive products, processes and services, and thus to improve the competitiveness of the forest cluster and to add value to the forest products industry.

The programme consists of two sub-programmes, one for basic research and the other for innovation-targeted research and development. The themes of the programme are:

- Raw material properties of wood
- Means to improve the material properties of wood and fibres
- Modification and processing of wood raw material into innovative, eco-efficient products
- Socio-economic aspects related to material-scientific innovations

The volume of the programme for 2003 – 2006 is around EUR 20 million. The public funding organisations of the programme are the Academy of Finland, the National Technology Agency (Tekes), the Ministry of Agriculture and Forestry in Finland, the Swedish Research Council for Environment, Agricultural Sciences and Spatial Planning (Formas), and the Swedish Agency for Innovation Systems (VINNOVA). The aim is to expand the co-operation further, so the programme is also open to new partners.

The COST system in a changing world – some personal views

Lennart Eriksson

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Box 5604, SE-114 86 Stockholm, Sweden

The author has been active within the COST Technical Committee Forest and Forestry Products (TC FFP) all since its start in 1994, and has acted as the first chairman of its Sector Group Pulp and Paper for many years. He was also one of the persons launching the very first TC FFP COST action E1 "Paper Recyclability". In the capacity as chairman of the board of the Wood Ultrastructure Research Center (WURC) at SLU/Uppsala, the author proposed to the TC FFP at its meeting in Sopron, Hungary in 1997 the cross-sectoral COST action E20 "Wood Fibre Cell Wall Structure". So, the author has a long time experience with the COST system. He has also since the early 1980ies been engaged in almost all aspects of EU Framework Program research – except actually working in the Commission itself.

Based on these and other experiences from the research and research management field, the author will reflect on the COST system – its pros and cons. Like everything else in society, the COST system works in a dynamic environment and has to adapt to change. What does and what may that mean? What could be the implications of the move of the COST secretariat to the European Science Foundation? How shall we perceive the development of the COST system in relation to the changes that are implemented in the EU Framework Programs (the ERA-concept and the new instruments Integrated Projects and Network of Excellence now launched in EU FP 6). What are the implications of the EU enlargement – to COST and to the EU FP.

These are examples of topics that will be dealt with in the presentation, together with some factual information on the COST TC FFP activities.

Interpreting sub-annual wood-property variation in terms of stem growth

Rupert Wimmer[1], Geoff Downes[2], and Rob Evans[3]

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[2]CSIRO Forestry and Forest Products, Hobart, Tasmania, Australia
[3]CSIRO Forestry and Forest Products, Clayton, Victoria, Australia

Radially measured stem-fluctuations were successfully combined with wood data from two contrasting species, i.e *Eucalyptus nitens (Shining gum)* and *Picea abies* (Norway spruce). On the one hand, the high-resolution scanning device *SilviScan*™ was capable of estimating a wide range of wood and fibre properties from increment cores. On the other hand, point dendrometers were used to monitor the radial movements of tree stems providing a linear frame of stem movements, which was related to stem growth. The cambial region undergoes water stress phases almost daily during the growing season because of high tensile forces that develop in the adjacent mature xylem. Under these conditions, the size of the meristematic cells and the duration of the cell division cycle in these cells determine the rate of cell production.

The distance based wood property measurements were converted to the time axis, which allowed synchronous comparisons of growth processes at almost daily resolution. The combination of these two techniques made it possible to monitor wood formation with time, at a particular point on the tree stem. It was found that interactions between climate and cambial growth are complex and variable. As an example, correlation coefficients between temperature and stem growth varied from positive in spring to zero or negative during summer. Irrigated-droughted eucalypts have shown obvious relationships between microfibril angles and soil water deficits with increasing angles responding to water stress releases (Figure 1).

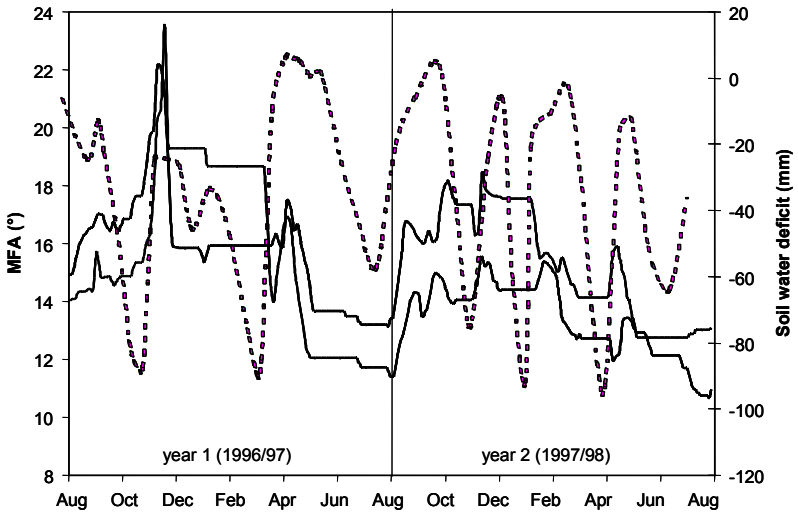

Figure 1: Time-trends for microfibril angles in two Shining gum trees exposed to (solid lines) periodic drought over two years, compared with soil water deficits (dashed line) measured each fortnight.

Preliminary results with Norway spruce have also proved utility of the applied method. Radial tracheid diameter trends seemed to show obvious associations with temperature and a stronger link to increment phase temperature, compared to average daily temperature (Figure 2). This provides another evidence that the presented methodology is able to record the relevant biological assays at high resolution; here, temperature during the increment phase being directly linked to tracheid formation.

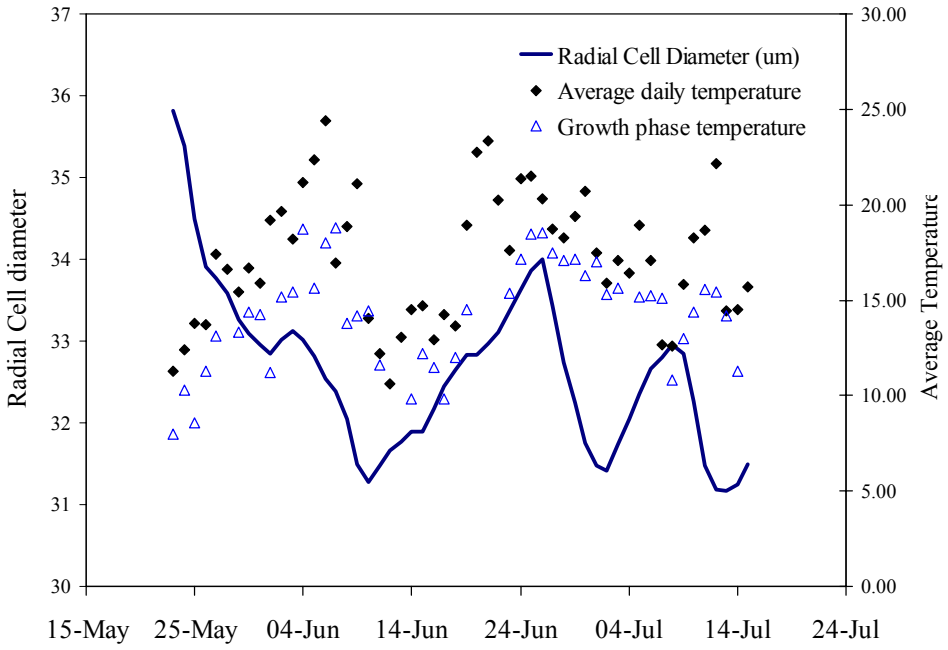

Figure 2: Radial cell diameter trend and temperature during increment and growth phase.

The employed methods help to understand the sources of variation in wood and fibre properties, which could be seen as a function of genotype, site or silviculture. These in turn greatly assist in understanding the physiology of wood formation when linked to studies on tree growth towards improved utilisation of timber resources.

Cellulose aggregates an integral part of pulp fibre structure

Geoffrey Daniel

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The improvement and development of new fibre products highlights the need for a better understanding of the fundamental structure and behaviour of wood fibres at the nano-level. While it is well known that the architecture of wood fibre cell walls play a major role for final properties (e.g. strength) of fibre-based products, the detailed contribution of the different morphological cell wall layers and in particular their nano-structure (ultrastructure) is still poorly understood. In this presentation a short overview of wood fibre micro- and nano-structure will be given, outlining our current understanding of fibre cell walls based on electron microscope observations. In particular, the use of various microscope and ancillary techniques (e.g. FE-SEM, Cryo-FE-SEM, TEM, TEM replicas) and their advantages and disadvantages for providing new information will be given.

Wood fibres are recognized as composed of primary and secondary walls, the latter composed of a tri-ply wall structure supporting 3 layers (S1, S2, S3) in which the cellulose components are arranged in a concentric and/or radial fashion across the cell wall. The S2 layer is the dominating layer and most important regarding properties. Using advanced electron microscope methods, the individual layers of pulp fibres are observed composed of microfibrillar aggregates (cellulose/hemicellulose) that lie above the order of individual cellulose microfibrils (i.e. 3-4 nm). Such aggregates vary in size (thickness and shape) and chemical composition and are orientated to reflect the local microfibrillar angle of the main fibre axis or accompanying pores. The aggregates are recognizable using both FE-SEM and TEM particularly in rapidly freezed and cryo-prepared pulp fibres, but are more difficult to recognize in conventionally prepared samples (i.e. air-dried and resin embedded fibres). The nano-aggregates are evident in both bleached- and unbleached chemical pulps, in mechanical pulps, in the gelatinous layers of tension wood and in native wood cells selectively degraded by fungi. In lignified tissues the aggregates are more difficult to discern because of the lignin matrix but may be revealed in mechanical pulp fibres during refining. The aggregates comprise the basic nano-structure of fibre surfaces (primary wall, S1) and intracellular structure (S2, S3) and thus the manner in which they are organized will greatly affect porosity and the penetration of polymers into fibres. Recent studies have concentrated on characterizing aggregates in whole pulp fibres (e.g. spatial distribution, surface chemical structure) and after their isolation from fibres. Changes in the nano-structure and the importance of the aggregates are noted in “beaten” pulp fibres during fibrillation and at sites of fibre dislocations.

While various electron microscope methods can be used to characterize the surface structure of individual aggregates, to date it has not been possible to document their internal organisation using electron microscopy due to problems of specimen preparation and electron beam damage.

Contribution of lignins to the building of wood secondary walls

Katia Ruel and Jean-Paul Joseleau

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BP 53, 38041 Grenoble, cedex 9, France

In the first stages of secondary wall formation, cellulose microfibrils show a large degree of loosening and disorder. With the progressive deposition of lignin, loosening and disorder decrease and a coherent wall builds up. Intermediary stages could be observed by transmission electron microscopy of thin sections from developing poplar plants. Similar observations were obtained on young stems of *Arabidopsis thaliana* and *Tobacco* plants. Immuno-labelling of lignin epitopes suggests a particular role of non-condensed lignin types in the early phase of cellulose microfibrils aggregation. Images of cell walls of plants genetically transformed on lignin biosynthesis and lacking the capacity to build a coherent secondary wall support this view. Another role of lignin in the building of the secondary wall is suggested by the lamellar structures released after delignification. The same type of lamellar arrangements could be observed in the incompletely lignified walls of young poplar and *A.thaliana* fibres, thus pointing out to a role of lignin in the aggregation of the lamellae.

Localization of peroxidases expressed in developing xylem of Norway spruce (*Picea abies*)

Mikko Lehtonen[1], Kristiina Hildén[2], Kaisa Marjamaa[1], Kurt Fagerstedt[1], Pekka Saranpää[3] and Taina Lundell[2]

[1] Department of Biosciences
[2]Department of Applied Chemistry and Microbiology, University of Helsinki, PO Box 56, FIN-00014 University of Helsinki, Finland
[3]Finnish Forest Research Institute, PO Box 18, FIN-01301 Vantaa, Finland

Background

Class III secretory plant peroxidases (POX, EC 1.11.1.7) are heme-containing oxidoreductases. POXs have several functions in plant cells including oxidative polymerisation of monolignols during lignin biosynthesis. Stems of trees contain large amounts of xylem tissue, where the cell walls of conductive cells are highly lignified. This, and the economical importance of trees for pulp and paper and timber industry, makes them interesting material for studying lignin biosynthesis.

Objectives

We have studied the participation of peroxidases in lignin biosynthesis in Norway spruce (*Picea abies*), a common gymnosperm tree species in Finland. Lignin polymerising peroxidases in Norway spruce have to fullfill at least two criteria: 1) They have to be able to oxidase coniferyl alcohol, the main lignin monomer in the softwood of gymnosperm trees. According to our *in vitro* studies, the ability to oxidise coniferyl alcohol is a general property of POX isoforms in spruce xylem. 2) They have to be localised to the lignifying cell walls.

Complete purification of peroxidases from spruce xylem has proven to be difficult due to low amounts of protein and high amount of disturbing extractives in wood, and therefore, a molecular biological approach was chosen.

Results

We extracted RNA from developing xylem of Norway spruce stems and expressed peroxidase genes (mRNA) were amplified using RT-PCR (reverse transcriptase polymerase chain reaction) techniques with degenerate primers designed for homologous regions of known genes of class III peroxidases. Three full-length cDNA clones that showed gene and predicted protein sequence similarity to other plant heme peroxidases, were obtained. Calculated molecular weigths of the translated proteins were over 33 kDa and their p*I* values showed that two of the cloned peroxidases are alkaline and one is acidic. All the three cloned spruce peroxidases start with predicted plant secretion signal leader peptides, and hence the hypothesis is that they are transported to ER. Fusion constructs of the spruce peroxidase signal sequences, EGFP (green fluorescence protein) and ER retention sequence were transferred to tobacco protoplasts to verify function of the predicted signal sequences. The peroxidase signal peptide directed localization of EGFP in tobacco protoplasts was studied with confocal microscope. ER typical network structures were seen in confocal images. In addition, two of the peroxidases contain a putative C-terminal propeptide, which may indicate further transport of the protein to vacuole. In future the localization of the three peroxidases will be studied further with peptide antibodies.

Cellulose and lignin biosynthesis: xylem vessel formation *in vitro*

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Over two decades ago, a system for *in vitro* tracheary element (TE) differentiation from isolated mesophyll cells of the first leaves of *Zinnia elegans* was perfected (Fukuda and Komamine 1980). Since that time, numerous papers have been published using this system to explore several areas of research including factors that promote and inhibit differentiation, differential gene expression related to TE differentiation, cell biology of TE differentiation, and mechanisms of cellulose synthesis and lignification. General advantages of this differentiation system are enumerated below, with citations to example publications. (1) Differentiation of TEs *via* patterned secondary wall deposition is inducible by an appropriate ratio of auxin and cytokinin, beginning about 48 h after cell culture (Fukuda and Komamine 1980). (2) To generate controls and experimental contrasts, alternative media are available that either induce cell expansion via primary wall deposition or support cell viability with or without cell division in the presence of auxin, cytokinin, or both (Roberts and Haigler 1992; 1994). (3) Differentiation in the whole culture is semi-synchronous, with visible differentiation in any one TE completing within about 12 hours and a "first wave" of differentiation in the culture completing within about 18 hours. (4) In some media, a "second wave" of differentiation occurs after expansion of cells that did not differentiate in the first wave. Other media cause initial cell expansion followed by differentiation; these large TEs more closely resemble metaxylem (Roberts and Haigler 1994). (5) Researchers are able to correlate differential gene expression and enzyme activity tightly with stages and percentage of TE differentiation (Demura et al. 2002; Milloni et al. 2002; Babb and Haigler 2001). (6) Because of differentiation in suspension culture, differentiating TEs are optimally accessible to drugs (Taylor et al. 1992; Nakashima et al. 1997a) and are able to be fixed by superior cryogenic methods for electron microscopy, including freeze fracture and immunolocalization (Haigler and Brown 1986; Salnikov et al. 2001). (7) It is possible to compare TE differentiation mechanisms *in vitro* with those occurring in intact *Zinnia* seedlings (Ye 1997). (8) Although a stable transformation system of *Zinnia elegans* is not available, a transient gene expression/knock-out system based on electroporation of nucleic acids into freshly isolated cells has been developed (T. Demura, unpublished). This also allows tracking of fluorescently tagged proteins during the differentiation process. A persistent disadvantage of the system is that large amounts of cellular material are not available for protein purification. Also, this system represents primary xylem rather than the secondary xylem that is important for wood formation.

Cellulose synthesis *in vitro* is apparently identical to the process *in vivo*. Particular results about mechanisms of cellulose synthesis derived from the *Zinnia* system will be discussed, including those enumerated below. (1) The plasma membrane rosettes associated with cellulose synthesis are assembled in the endomembrane system (Haigler and Brown 1986), but they fall apart in the membrane in the presence of some cellulose-synthesis-inhibiting herbicides (Kiedaisch et al. 2003). (2) Sucrose synthase is specifically localized at patterned sites of secondary wall deposition in TEs, thereby generalizing a model about sucrose synthase channeling of UDP-glucose to cellulose synthase that was derived from cotton fibers (Salnikov et al. 2001). (3) Higher activity of sucrose phosphate synthase, which can recycle the fructose released by sucrose synthase to additional sucrose to support cellulose synthesis, is correlated with TE differentiation (Babb and Haigler 2002). (4) Timed addition of

microtubule inhibitors and addition of direct dyes to alter cellulose crystallization showed that microtubules and crystalline cellulose work in an interdependent, biphasic manner to control patterned secondary wall deposition (Roberts and Haigler, in preparation).

Regarding mechanisms of lignification, *Zinnia* TEs differentiating *in vitro* form only G lignin (R. Hatfield, personal communication), which simplifies analysis of one biochemical pathway as contrasted with diverse pathways that may occur in woody tissues with several lignifying cell types. Examples where analysis of the *Zinnia* system has been particularly valuable in understanding mechanisms of lignification will be discussed, including those enumerated below. (1) Cellulose is the ultimate scaffold upon which the cell wall assembles, including lignin. When cellulose synthesis is inhibited, lignin becomes delocalized over the whole cell surface (Taylor et al. 1992). (2) The structure of the wall can be visualized over the time-course of lignification (Nakashima et al. 1997; Haigler and coworkers, unpublished). (3) Lignification is supported by synthesis of monomers by other cells in the culture that are not differentiating (Hosekawa et al. 2001; Goffner et al. personal communication). (4) Caffeoyl coenzyme A 3-O-methyltransferase (CCoAOMT) plays a key role in lignification in diverse species (Ye 1997).

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The geometrical model for cellulose microfibril deposition, extended to random wall texture

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We have formulated a theory for wall deposition consistent with present day experimental data on walls and cellular processes. It appeals to a very generic origin, geometrical constraints, as the underlying cause of the architecture of the cellulose microfibrils (CMF) in a wall. This mathematical model is fully explicit, allowing for specific predictions of qualitative and quantitative nature. The key point of the geometrical theory is the coupling of the CMF synthase (rosette) trajectories to the density of these synthases. This provides the cell with a route to manipulate wall structure by creating controlled local variations of the number of active rosettes. We have published how the model can describe known wall textures, underlining the flexibility of the proposed mechanism.

In the **helicoidal** case in which microfibrils in every lamella, of one microfibril thickness, make a constant angle with the previous and subsequent lamellae, the rosette life time matches to the size and velocity of the rosette insertion domain (RID). In the **crossed polylamellate** wall texture, successive lamellae have CMF orientations at right angles to each other, which is attained if the rosette creation rate is so high that the maximum density is achieved instantaneously, giving rise to the first CMF orientation. In a **helical** texture, all CMFs make an approximately constant angle with the cell axis. In solutions for helical textures the lifetime of the rosettes is taken to be much larger than unity, while the size and speed of the RID does not match with the build-up to the maximum rosette density, so that locally the creation of rosettes stops before the maximum density is reached. In the **axial** texture, all CMFs run parallel to the cell axis, which will occur when the diameter of the cell has shrunk considerably; so, in a sense it is a finite size effect. This case is similar to the one discussed for the helical texture, except that the rosette density is maximal for essentially the whole cycle.

Now we show by experimental data and theoretical modelling that the so-called random cell wall, which is seen in meristematic cells and at root hair tips, is in fact a helicoidal texture with microfibrils wide apart.

Using cellulose synthase – GFP fusions as a tool to investigate cellulose biosynthesis

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Since the identification of the catalytic subunit of the cellulose synthase complex in Gossypium hirsutum and Arabidopsis thaliana, these so-called CesA genes have been isolated from many species like Poplar, Eucalyptus, Maize and Rice. Despite the identification of this catalytic subunit in many species, little is known about the other components of the cellulose synthase complex. Furthermore little is known about the catalytic subunit itself. The trafficking route from the ER to the cell membrane, the assembly of the complex and interactions of the subunits are all examples of this gap in the current knowledge. To get more insight in several of these aspects we chose *Arabidopsis thaliana* as a model system and transformed these plants with different fusions of the N terminal parts of the CesA1 gene and a Green Fluorescent Protein (GFP). Here we present data on the phenotypes of these transformants and the routing of these fusion proteins.

Linkage specificity and (per)oxidases in lignin polymerization

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After cellulose, lignin is the most abundant polymer in wood. It provides compressive strength to timber but, on the other hand, its removal in paper and pulp production is expensive and often environmentally problematic. Genetic control over the amount and type of lignin in forest trees is thus of high interest. Lignin is a polymer of relatively few monomeric units (monolignols), but they combine in a very complex manner, and unlike other biopolymers, without repetitive units. Lignin monomers are derived from the amino acid phenylalanine, and the enzymatic steps leading to their biosynthesis are relatively well characterized. In contrast, the final steps of lignin biosynthesis, polymerization of the monolignols, are still under dispute.

It is known that polymerization starts by oxidation of the monolignols into reactive molecular radicals. However, the classical view of random polymerization of monolignols does not explain certain structures found in native lignin (e.g. the 8-ringed dibenzodioxocin structures). As a model for lignin biosynthesis in spruce, we are utilizing a special cell culture that deposits native-like lignin in its growth medium. By isolating and purifying secreted oxidizing enzymes of the cell culture (peroxidases and laccases) and by determining their product specificities, we wish to uncover their roles in lignin biosynthesis.

Research on lignin polymerization is part of the Academy of Finland Center of Excellence in Plant Molecular Biology and Forest Biology, and collaboration between Department of Applied Biology, Department of Biosciences (Prof. Liisa Simola) and Institute of Biotechnology (Prof. Ilkka Kilpeläinen) at the University of Helsinki.

Cell plate assembly: insights from electron tomography

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Until recently, the analysis of cellular structures at the electron microscopical level has been limited by both specimen preparation methods and by image analysis problems. For example, when cells are preserved by chemical fixatives, the preservation of different types of cellular structures in their native state is limited both by the slow rate of cross-linking reactions and by the selective nature of the cross-links that are formed. On the other hand, the deciphering of 3D cellular structures is limited by the thickness of the serial thin sections used for the reconstructions. We have now overcome both of these limitations by combining cryofixation / freeze-substitution methods in conjunction with dual-axis high voltage EM tomography of serial thick sections (0.25 to 0.4 μm thick). This new methodology produces tomographic slices that look like electron micrographs but are only ~2 nm thick versus 60-80 nm for normal thin sections. Overall, the 3D resolution in our reconstructed specimens is about 7 nm, which enables us to see and identify large molecules such as clathrin triskelions, dynamin spirals, vesicle-tethering molecules, and kinesin motor proteins within the thick sections. By tracing the outlines of cellular structures in the individual tomographic slices we can also produce high resolution, 3D models of membrane compartments and cytoskeletal systems. Furthermore, because all of the data are recorded in digital form, the data sets can be used to gain quantitative information about cellular components. This capability has enabled us to produce quantitative 3D information on the distribution of molecular complexes as well as of the diverse membrane compartments and cytoskeletal structures of cells.

My lecture will demonstrate how electron tomography has lead to the discovery of three types of cytokinesis in plants: somatic-type, endosperm and pollen syncytial-types of cell plate formation. However, the main focus will be on somatic-type cell plate formation in apical meristem cells of Arabidopsis. Cell plate assembly begins during anaphase B with the accumulation of vesicles and the assembly of the ribosome-excluding "cell plate assembly matrix" (CPAM) at the equatorial plane of residual polar spindle microtubules (MTs). The vesicles and the CPAM appear to travel together along the MTs to the cell plate-forming region with the help of kinesin motors (mostly two per vesicle). All cell plate growth occurs within the CPAM. The first signs of growth are the appearance of vesicles tethered by exocyst-type tethering molecules and of dumbbell-shaped vesicles, which have twice the surface area of the Golgi-derived cell plate-forming vesicles. Lengthening of the constricted neck of the dumbbells involves dynamin-like spiral complexes that expand possibly with energy provided by the hydrolysis of bound GTP. During this lengthening, the surface area of the dumbbells remains constant, while their volume is reduced by up to 50%, presumably due to the loss of water. A corresponding increase in concentration of cell wall-forming molecules is evidenced by an increase in staining of the dumbbell contents. Assembly of the solid phragmoplast with a continuous CPAM across its entire width signals the beginning of the next phase of cell plate formation. During this phase, dumbbell-shaped vesicles are formed across the entire width of the cocoon-like CPAM and then expand by the fusion of vesicles to their bulbous ends. This creates tubulo-vesicular membrane structures that fuse together to form a "tubulo-vesicular network" (TVN). This network extends across the width of the solid phragmoplast. Upon completion of the TVN, both the CPAM and the MTs of the solid phragmoplast break down and reform in a ring-like configuration around the periphery of the TVN. This creates a "peripheral growth zone", which mediates centrifugal cell plate growth and eventually leads to the fusion of the cell plate with the cell wall. Simultaneously, the TVN

undergoes a series of maturation steps (formation of a "tubular network" and then a "fenestrated sheet"), which are accompanied by very little net cell plate membrane surface area growth. However, where large fenestrae develop in the fenestrated sheet, a CPAM and attached MTs reform over the fenestrae to focus local growth to these specific cell plate regions. An unexpected finding of the quantitative analysis of these membrane events is that formation of the TVN during somatic-type cell plate formation requires less half the number of vesicles than the formation of the corresponding wide tubular membrane network during syncytial-type cell plate formation in the endosperm.

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Pore size distribution in the transverse direction of the wood fiber wall

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In the paper making process it is important that the individual wood fibers, separated in the pulping process, are flexible for achieving a good contact in between them. The interactions between individual fibers are crucial for several paper properties including tensile and tearing strength. The fiber flexibility is closely related to degree of fiber swelling where a higher swelling increases the fiber flexibility. The water uptake by the fiber cell wall can be increased in numerous ways including mechanical treatments (beating, refining) that open up the cell wall structure and chemical treatments that remove the lignin. All these treatments have an impact on the pore size distribution. The water adsorption is thus important for the fiber swelling and hence flexibility, which in turn are important for the quality of the paper produced. In order for the fibers to adsorb water the water molecules must gain access to the inner of the cell wall. Water is held by the amorphous parts of the fiber wall and in open areas such as pores, rays, pits and lumen.

Several techniques are available for examining the pore structure and the pore size distribution in fibers such as NMR, water retention value, size exclusion, solute exclusion, inverse size exclusion chromatography and DSC, still none of them are able to explore the pore size distribution across the fiber wall in the transverse direction. AFM (atomic force microscopy) has earlier been shown to be a useful tool for fiber wall characterizations on the nano-scale[1,2]. Based on such findings a new method using AFM and image analysis for investigating the pore size distribution across the fiber wall was developed. In this new method freeze-dried pulp fibers are impregnated with a hydrophilic polymer (PEO- poly (ethylene oxide)) with a molar mass of 5000-7000, a radius of gyration between 3-4 nm and a melting point between 52-57ºC. This PEO polymer was chosen since it is suitable for penetrating into the fiber wall and can fill up all pores larger than 4 nm. Transverse cross-sections of the pulp fibers are investigated with the AFM at room temperature and at 50ºC were the PEO polymer is softened. From the phase contrast images, were high and low surface hardness can by recorded, obtained at 25 and 50ºC, it was possible to detect differences in the PEO stiffness due to softening and hence the pores filled with PEO. AFM images were examined with an image processing software for calculation of pore size and the pore size distribution across the fiber wall. With this method the pore size distribution across the fiber wall has been investigated for pulp fibers before and after refining.

Study of microfibril angle by x-ray diffraction – present state, future possibilities

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Microfibril angle or MFA, the orientation of cellulose microfibrils with respect to the cell axis, is of importance when describing the structure and mechanical properties of wood. It has been shown, that the average MFA has a notable effect on the mechanical properties of wood, thus setting a frame on the end-use capabilities of the material. From a biological point-of-view there exists a consensus that average MFA is highest near the pith and decreases as the stem matures. When varying growth conditions are taken into consideration, diverse opinions exist whether the increase of growth rate has an effect on the average MFA.

New developments in methods for measuring the orientation of cellulose microfibrils in wood samples by means of x-ray diffraction are described. Macroscopic pieces of wood containing numerous cells and microscopic samples containing one or few cells have been examined and the results compared. The results suggested that while macroscopic samples can be used to obtain information on growth-related effects and average response in terms of cellulose microfibril orientation, microscopic samples are needed to analyse the structure of individual cell walls [1]. In addition to MFA determination, with x-ray diffraction one is also able to determine the average shape of cell cross-section from the same measurements. It has been shown by comparison to image analysis of thin-sections, that the shape can be determined reliably by x-ray diffraction analysis [2].

The sample material is Norway spruce (*Picea abies* [L.] Karst.), obtained from a nutrient optimisation experiment in Flakaliden, northern Sweden. Along the x-ray diffraction analysis of the effect of enhanced growth rate on the average MFA and the average shape of cell cross-section, the effect on the chemical composition was studied by x-ray absorption analysis. Fertilisation, when begun in the mature phase of wood, had no effect on average MFA, whereas density and chemical composition were affected (Peura et al., manuscript in preparation).

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Application of high resolution electron microscopy and image analysis for characterising pulp fibre surfaces

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In recent years, several studies (Daniel and Duchesne, 1998, Duchesne and Daniel 2000, Duchesne et al., 2001, Hult et al., 2001a, b) have shown the fibre cell walls of spruce after kraft pulp processing to consist of cellulose fibril aggregates (i.e. macrofibrils). Both the primary and secondary cell wall layers (i.e. S1, S2, S3) have been shown to consist of cellulose macrofibrils and evidence obtained showing a tendency for aggregating during kraft processing as lignin and part of the hemicelluloses are removed. In previous studies, changes in fibril aggregate size have been determined using NMR techniques (Hult et al., 2001a, b), by measuring the sizes (widths) of fibril aggregates by AFM in sample sections after embedding in resin, or by manual measurements on SEM images of the S1 layer of freeze dried samples from selected pulps (Duchesne et al., 2001; Fahlén and Salmén, 2002).

In the present work, pulp samples for electron microscopy were prepared by a rapid-freeze-deep-etching technique (RFDE) and thereafter the surface ultrastructure of fibres characterized by TEM and the size of cellulose fibril aggregates and intra-fibrillar spaces in the secondary cell walls layers (S1, S2) measured using an automatic computer technique based on image analysis. Measurements of cellulose fibrils and fibril aggregate widths were performed using the digital image analysis system V++ from Digital Optics. Measurements were performed on micrographs of the replica casts of the longitudinal surface structure of pulp fibre cell walls. More than 300 measurements per micrograph were performed. Intra-fibrillar spaces were also measured using V++ scripts.

Laboratory kraft pulps were produced according to three industrially relevant pulping processes, isothermal continuous cooking (ITC), rapid displacement heating technique (RDH) and 2-step polysulphide pulping (PS). The pulps were further oxygen delignified and bleached according to three different sequences or chlorite bleached (CLD). A prehydrolyzed kraft pulp (PH) was also included in the series. A total of 21 pulps were produced in the WURC (Wood Ultrastructure Research Centre) “Pulp 2000” project.

Results have shown the S1 and S2 layers in all pulps to have a wide range of fibril sizes - from ca 4-5 nm up to 40-55 nm despite having a mean aggregate size that are not significantly different. This is confirmed by observations on electron micrographs from the various pulp types and individual layers. All pulp S1 and S2 layers show a distinct aggregation at *ca* 18-20 nm consistent with the aggregation of *ca* 4-5 fibrils in width. Measurement of intra-fibrillar spaces showed pulps to have different degrees of fibrillar arrangement. Some correlations of the results could be made with chemical and physical properties of the pulps studied.

A complete paper on this work will soon be published in an international scientific journal.

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Changes of biological features in softwood and hardwood species due to wood modification treatments

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Abstract

In the recent years, in Europe several attempts where made to improve wood properties by non-biocidal wood modification treatments. Heat treatment processes, resin modification systems, furfuryl alcohol and acetylation processes are examples and are introduced on industrial level or are at least under process development on pilot plant level. These techniques use high temperatures and often aggressive process conditions, possibly causing cell wall changes. In this presentation, the results of some anatomical studies on anatomical changes of modified wood are presented. Furthermore, the mode of attack of fungi during the degradation process of modified wood is presented.

New insights into structure- property-relationships on the cell wall level by micro-mechanical examinations of single wood fibres

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Wood has excellent mechanical properties with regard to its low density. From the point of view of material science wood can be looked at as a fibre composite on several hierarchical levels. According to the tissue level the fibre is the most important parameter, since tissue properties are determined by the structure and shape of every single cell and the fibre/fibre interactions. With respect to the cell wall level cellulose microfibrils embedded in a matrix of hemicelluloses and lignin result in an optimised fibre composite.

Microtensile tests on single wood fibres can provide new insights into structure- property-relationships on the cell wall level. Even though cell wall components were already characterized in detail, we are still lacking information on the polymer interaction. By examining single fibre properties with respect to specific structural features a better understanding of the basic interrelations behind the cell wall properties can be obtained. In this way we like to present recent progress in the understanding of polymer interactions on the cell wall level of wood.

Tensile properties of cellulose fibres investigated *in situ* using synchrotron radiation

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The crystalline structure of cellulose itself is well known. On the contrary, there are still open questions on the morphology and the mechanical properties of cellulose as a composite material.

We investigated the tensile properties of flax cellulose fibres *in situ* using microfocus wide-angle X-ray diffraction (WAXD) at the European Synchrotron Radiation Facility ESRF (Microfocus Beamline ID13) and standard WAXD at HASYLAB (Beamline A2). Single flax fibres and small bundles of flax fibres, respectively, were mounted in a stretching device. Tensile load was applied along the fibre direction. The measurement of the displacement of the jaws and the longitudinal force yields stress-strain curves of the whole fibres. Simultaneously, the stress-strainK. Kölln1, I. Grotkopp1, C. Behrend1, M. Peura[2], R. Serimaa[2], M. Do change in lattice spacings using the recorded WAXD pattern. Assuming an isotropic distribution of the stress within the composite material, Young's modulus and Poisson's number were calculated for the crystalline parts (microfibrils). Changes of the orientational distribution of the microfibrils were obtained as well. Modelling of the mechanical properties, treating cellulose as a composite material (microfibrils in a disordered matrix) based on our new data is currently under way.

Since water can only penetrate disordered, but not crystalline cellulose, moisture drastically changes the mechanical properties of cellulose. We are planning to systematically investigate the influence of water and present preliminary results.

The elastic properties of wood fibres are based on those of cellulose, their main constituent. However, the helical arrangement of microfibrils, characterised by the microfibril angle MFA (measured with respect to the longitudinal axis), plays an important role as well. First experiments on wood are reported.

Chemo-enzymatic modification of cellulosic materials

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Wood and pulp fibres constitute a renewable raw material, which can be processed using enzymes during post-harvest processing. A number of different enzyme systems contribute to the cell wall formation and the resulting fibre structure and properties in trees. An improved understanding of the biochemistry of the cell wall biosynthesis thus provides new biomimetric means for engineering the fibre structure and chemistry. Functional genomics provides powerful tools to identify new enzymes involved in wood-formation. An EST database of 100.000 sequences has been assembled from hybrid aspen, Populus tremula x tremuloides Mich. followed by expression profiling of genes activated during different stages of xylogenesis [1-3]. One of the enzymes identified among the first 3000 ESTs investigated was the xyloglucan endotransglycosylase (XET) [4]. Xyloglucan is practically irreversibly bound to cellulose thus providing a dynamic linkage between the cellulose microfibrils. During cell expansion, the bound xyloglucan is processed by XET, which can cleave and rejoin xyloglucan polymers. This principle is now being developed into a tool to chemically reactivate cellulose. The XET reaction is exploited to couple chemically modified xylogluco-oligosaccharides to isolated xyloglucan. The modified xyloglucan is then bound to pulp fibres or cellulosic surfaces thus enhancing their chemical reactivity. The natural cell walls are likely to provide several more examples on effective ways to join ands cross-link different natural polymers with one another thus providing the basis for their efficient exploitation for example in biocomposites.

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Influences of microfibril angles and natural defects on the force-extension behaviour of single wood fiber modelling

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Abstract: Experimental results show that the average microfibril angle (MFA) of the wood fibres highly influences their force-extension behaviour. The stress-strain diagram of the fibres having small MFAs is usually a one segmented curve and the fibre behaves as a brittle material under tension while the fibers with larger MFAs demonstrate few segmented curves and a ductile behaviour before failure. To understand the mechanism underlying the wood fibers extension a micromechanical based model was developed. In this model the existing natural defects known as fiber dislocation, microcompressions, slip planes and misaligned microfibrils around the pits were represented as a change in microfibril orientation. An isotropic damage model for cell wall degradation under tensile loading was used and different mechanisms involved in force-extension behavior of single wood fibers were discussed.

Introduction and methods: Experimental results given by Page and El-Hosseiny [3] in 1983 show that the load-extension behavior of single wood fibers is non-linear. The stress-strain curves for the fibers with small MFAs are almost one segmented curve and for the fibers with large MFAs consist of several segments and show important strain potential before failure.

To understand the physical mechanisms underlying such behavior, a micromechanical approach is used. In this approach the wood fibre is considered as a long hollow cylinder of length L, only consisting of S_2 layer. This layer is composed of lignin and hemicellulose matrix reinforced by helically- wound microfibrils (cellulose). The distribution of MFA in S_2 layer is non-uniform.

To calculate the effective elastic constants of S_2 layer the volume fractions and the stiffness tensor of each constituents given by Persson[4] were used and the formulation given by Chou and Carleone [1] was implemented. Fig 1 shows the calculated longitudinal Young's modulus of the fibre for given MFAs, assuming the fiber porosity is Φ = 30%. Also the Young's modulus of the fibers after damaging at different damage states is presented in the same figure. It is assumed that the damage of the fiber starts by the degradation of matrix and in the micro-structural level the matrix stiffness reduces corresponding to the equation $\sigma = (1 - \omega) E \varepsilon$, where ω is the damage parameter and is equal 0 in the intact state. It is shown in Fig 1 by lines AA" and BB".

Experimental results show that the wood fibers after a certain tensile force level and yield point undergo mainly irreversible deformation and the Young's modulus of the fibers remains almost unchanged during the loading-unloading tensile test (Navi et al [2] and Wild et al [5]).

To compensate the reduction of stiffness, which is caused by the damage of the matrix (justifying the macroscopic elasto-plastic behavior) the MFA should be reduced (lines AA' and BB'). Although Fig 1 is calculated using the volume fractions of the constituents of unmodified wood fibers, the presented phenomenon remains the same when the matrix volume fractions reduce during chemical maceration (for isolating the wood fibers).

Results and Conclusion: To verify the above mentioned assumption, we have tried to build up the Page and El-Hosseiny experimental results[3] for three different wood fibers. The geometrical details of these fibers with non-uniform MFAs distribution are presented in Table 1. This table also gives β, the final changed MFAs measured in the weak parts when ω=90 %.

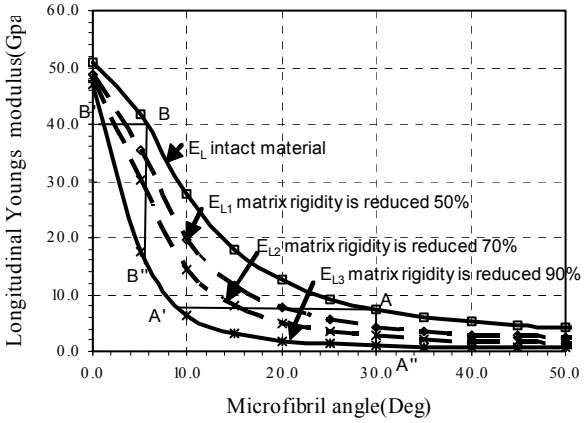

Fig.1. Longitudinal Young's modulus of a single fiber in terms of MFA α, for virgin fiber (E_L) and for the same fiber with reduced matrix constants (E_{L1}, E_{L2}, E_{L3})

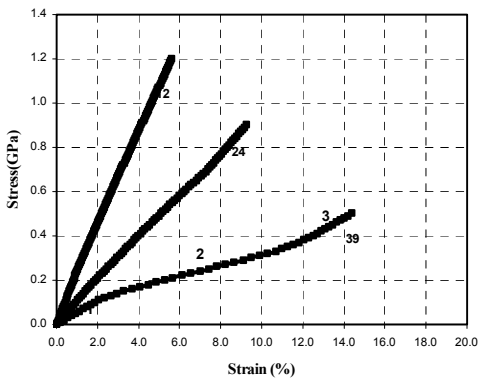

Fig.2. Simulated force-extension behavior of single fibers with different mean MFAs

During the degradation of the matrix under the incremental applied force, the MFA reduces accompanied by an irreversible strain, ε_α (corresponding to the damaged zone), which should be added to the elastic strain, ε_e (corresponding to the whole fiber), $\varepsilon_t = \varepsilon_e + \varepsilon_\alpha$. Calling the final reduced MFA β, the strain ε_α can be calculated using the following relation:

$$\varepsilon_\alpha = \left(\frac{\cos\beta}{\cos\alpha} - 1\right) \qquad (1)$$

Table 1. Characteristic and geometry of three single fibres and reduced MFA after damage

Fiber No.	L_1 *(μm)*	L_2 *(μm)*	L_{total} *(μm)*	α_1 *(Deg)*	α_2 *(Deg)*	α_m *(Deg)*	E_1 *(Gpa)*	E_2 *(Gpa)*	E_{eff} *(Gpa)*	β_1 *(Deg)*
1	240	1760	2000	50	37.5	39	4.312	5.6	5.4	11
2	100	1900	2000	35	22.5	24	6	11.2	10.73	10.5
3	100	1900	2000	30	10	11	27.67	7.25	24.25	10

In Fig 2, the simulated force elongation curves of three mentioned fibres are presented. As it was shown in Fig 1, in the tensile test of fibers with small MFAs, the MFA does not reduce a lot to compensate the local stiffness reduction of the fiber, therefore contrary to the fibres with larger MFAs, these fibers demonstrate small deformation before failure. So the slope of the second segment of their stress-strain curve is higher and they show a brittle mode of failure.

Acknowledgement: The financial support of the Swiss federal office of education and science in the framework of COST E20 is gratefully acknowledged.

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Destructuring of hemp fibers by solvents and lignin oxidants: characterization of the thermomechanical properties of the polymers within the cell wall

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Looking at the mobility of the polymers constituting the lignocelluloses is an interesting observation level between the biochemistry analysis and the mechanical or other use properties. The determination of the viscoelastic properties of the plant cell wall polymers is particularly adapted to this objective: the positions of the relaxations of different components on the temperature scale can be considered as a mobility classification; the viscoelastic response of minor constituents like pectins and hemicelluloses can be exacerbated by a careful choice of the environmental conditions (cellulose is maintained in glassy state while the other components are passing from glassy to rubbery state). Moreover, the viscoelastic response is generally expressed in terms of damping peaks. This variable can be considered to be independent of the sample geometry and of the deformation mode, a positive point when manipulating natural substrates. We present here the viscoelastic properties of annual plant fibres (hemp) obtained in controlled environment.

Experimental

Plant material: The hemp samples were prepared as follows: Well retted hemp bast fibres were first washed by cold water and manually cleaned to remove the attached wood part and then dried in oven at 30-40°C.

Treatments of fibers and preparation for analysis: The fibres bundles were treated by the ligninolytic binuclear manganese complex (Mn-TPA; 85µmol/g dry fibres) in the presence of hydrogen peroxide (2.3 mmol/g dry fibres) for 6h at 90°C. The fibre bundles were then selected under microscope and only undamaged samples with a constant section were kept for the analysis.

Biochemical characterisation of the hemp fibre bundles: The lignin content and structure, as well as the determination of neutral and acidic polysaccharides contents were carried out .

Dynamic mechanical testing: In our work, a TA Instruments Dynamic Mechanical Analyser (DMA) 2980 was used in two configurations. The first one allowed the study of viscoelastic properties as a function of temperature at different values of the relative humidity. The existing DMA instrument was coupled with a climatic chamber (SECASI Technologies, France) especially designed to this purpose. In the second configuration, the clamped sample was accommodated in a container filled with either ethylene glycol or water and heated by two heating films glued on two of exterior walls. A constant rate (1°C/min) temperature increase was applied using a PID controller. Temperature decrease with respect to ambient was also possible by using two Peltier elements glued on the other two exterior walls. Only results obtained in the second configuration and in ethylene glycol immersion are shown in this summary.

Results and discussion

The most important biochemical modification observed after Mn-TPA treatment was a 50% decrease of pectin content. A slight decrease of lignin content was also observed, accompanied of a significant structural change. Hemicelluloses seem not to be affected by the Mn-TPA treatment. An apparent cellulose content increase (10%) after Mn-TPA oxidation cannot be explained only by the pectin and lignin removal. Such phenomenon indicate instead that ~ 5 to 10% in weight of low molecular polar components are leaching from the cell wall during the reaction (oligomers, phenols, proteins, etc.).

The evolution in time of the DMA spectra obtained from hemp fibre bundles immersed in ethylene glycol was followed. Twelve successive multifrequency scans were recorded in the temperature range from 0°C to 90°C at a temperature slope of 1°C/min, at a rate of three scans per day. From spectrum number 1 to 4, only one relaxation was visible, shifting slightly to lower temperatures. Spectrum number 5 shows a shoulder, revealed as a second peak at the seventh scan. From the ninth scan on, all spectra are superposed. DMA spectra remain qualitatively unchanged after Mn-TPA treatment. In the Figure, only the spectra at equilibrium and obtained at 1Hz are shown. The existence of a second peak on both untreated and treated sample indicated that this relaxation is likely to belong to molecules slightly or not affected by the lignin oxidizing treatments and also not sensitive to the succinic acid extraction of polar compounds from the cell wall: the hemicelluloses.

A surprising result was the difference in the kinetics observed between the reference and Mn-TPA destructured sample. Indeed, the Mn treated sample required a longer equilibrium time: the first initial peak splits into two peaks at the tenth scan, again the first peak being very sensitive to ethylene glycol plasticization. This unexpected result could be explained by the following points:

- The treatment indeed destructured the sample, but the lignin which remains in the sample is more condensed, as evidenced by the decrease of the release yields of lignin products
- The treatment corresponded mainly to a structuring treatment, probably through in situ repolymerisation of lignin by Mn-TPA and H2O2, again in accordance to the lignin structure analysis.
- The extraction of plasticizing species by the catalyst (10% w/w of yet unidentified products) led to the decrease of the accessibility of solvent to lignin and left the other components and/or lignin less plasticized.

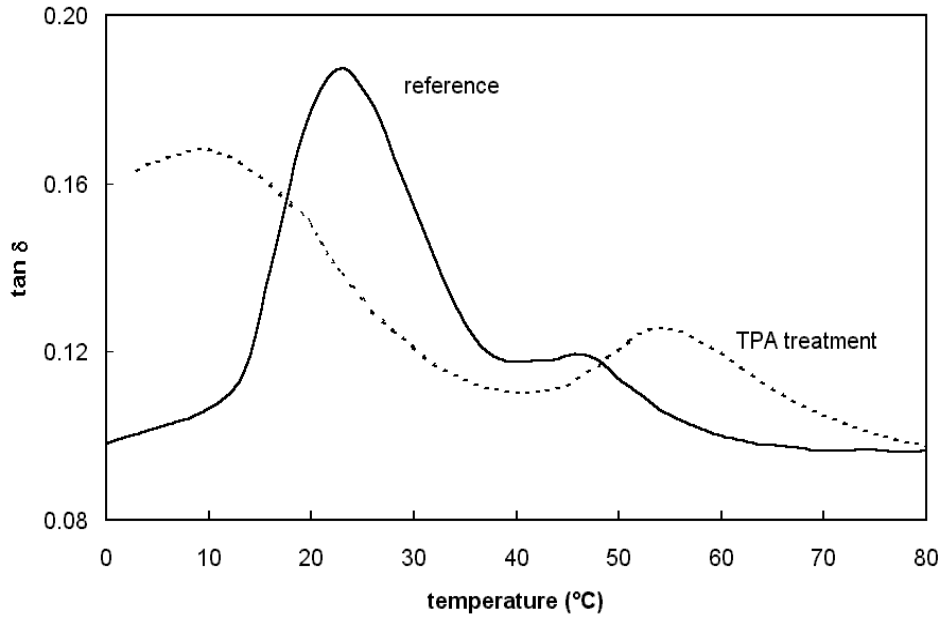

Figure 1. Loss tangent (tan δ) as a function of temperature for untreated hemp fibre and TPA treated sample, for a frequency of 1Hz, in ethylene glycol immersion, at equilibrium.

Posters

Genomics approach for studying the development of dormancy and winter hardiness in birch (*Betula pendula*).

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Controlling tree growth is central to forestry. The main limitation in tree growth in boreal forests is due to the specific combination of light and temperature. Survival of the temperate-zone tree species, such as birch, during the normal summer-winter cycle depends on proper timing of growth cessation and development of winter hardiness. Northern populations set bud and develop frost hardiness earlier than the southern ones even when grown under identical environments. The presence of such climatic ecotypes has been clearly demonstrated in a number of tree species including birch. However, the genes involved in photoperiod responses in woody species are only poorly characterized and the functions required for growth cessation, bud set and frost hardiness have not been elucidated. Our aim is to explain the molecular mechanisms for controlling the growth of birch in boreal climates. The focus is on elucidating the molecular mechanisms of photoperiod perception and the development of dormancy and winter hardiness.

A collection of 75 000 ESTs is being used to make a specific stress array which includes birch cDNAs showing homology to known stress inducible genes from other plants. Example of expression analysis of the target and regulatory genes will be presented. Expression of selected genes is being correlated with physiological data.

Insertion of cellulose synthases into the plasma membrane

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The organization of cellulose microfibrils determines important mechanical properties of plant cell walls. Especially the orientation of cellulose microfibrils appears to be crucial. Emons and Mulder proposed a geometrical model that predicts this orientation (Emons, A.M.C. and Mulder, B.M., 1998, Proc. Natl. Acad. Sci. USA 95, 7215-7219. Emons, A.M.C. and Mulder, B.M., 2000, Trends Plant Sci. 5, 35-40. Emons et al. 2002, Plant Biology 4, 22-26). In the model, wall texture is explained as a space-time process, mainly dependent on the number of cellulose synthases at the location of cellulose microfibril deposition. The model quantitatively relates the deposition angle of cellulose microfibrils to (1) the density of active synthases in the plasma membrane, (2) the distance between individual microfibrils within a wall lamella, and (3) the geometry of a cell.

The density of active cellulose synthases in the plasma membrane depends on the insertion, the activation and the lifetime of the cellulose synthases. The research presented here focuses on the insertion into the plasma membrane.

Because it is supposed that cellulose synthases are delivered to the plasma membrane by Golgi bodies, the dynamics of Golgi bodies were studied in transgenic *Arabidopsis thaliana*, in which a Green Fluorescent Protein is targeted to the Golgi bodies (Boevink et al, 1998, The Plant J. 15, 441-447). For this study root epidermal cells were used.

The Golgi bodies appear to arrest at certain sites close to the plasma membrane unlike in trans-vacuolar strands. CLSM movies provide spatial-temporal patterns of the stop sites. In root hairs, the number of Golgi bodies that arrest at a site, as well as the duration of each arrest, was measured. It appeared that most of the arresting Golgi bodies stop for 5-25 seconds. They can either stop alone, or, as in most cases, join other arresting Golgi bodies.

Because the geometrical model supposes that insertion/activation sites move, also the movement of the stop sites was examined.

To study whether the stop behavior of Golgi bodies is dependent on cytoskeleton elements, cytoskeleton depolymerizing drugs were used in atrichoblasts (epidermal cells without a root hair). The density of stop sites at the plasma membrane was determined before and after drug treatment.

The location and movement of the cellulose synthase complexes themselves will be imaged in transgenic *Arabidopsis thaliana* plants. In these plants, the catalytic subunit of the cellulose synthase complex, AtCESA1, was fused to a GFP. These GFP-AtCESA1 constructs were made in collaboration with Michel Ebskamp from Gene Twister (see abstract Ebskamp et al.).

The role of cytokinin signaling during wood development

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Vascular tissue is first established during embryogenesis as an undifferentiated procambial tissue in the innermost domain of the plant embryo. Later in development, a lateral meristem (the cambium) is formed, as the undifferentiated cells begin to divide in the procambial tissue between the phloem and xylem strands. Therefore, the pattern of cell divisions is an important determinant of the cellular organization of vascular tissue in plants. We have recently shown that a recessive mutation, *wooden leg (wol)*, in the CRE1/WOL/AHK4 gene coding for a cytokinin receptor, results in reduced cell proliferation during procambial development in Arabidopsis root (Mähönen *et al.* 2000, Genes Dev. 14:2938-2943). This indicates the involvement of a specific cytokinin mediated morphogenetic pathway during primary phase of vascular development.

Consequently, we have started to investigate the role of CRE1/WOL/AHK4 like receptors and cytokinin signaling during the secondary phase of wood development. We have identified a putative cytokinin receptor gene family (Birch Histidine Kinase 2, 3 and 4) from a perennial tree species silver birch. Each of the three BHK genes is active in the cambial zone of the birch trunk. Furthermore, several other genes homologous to the members of various elements of the recently identified cytokinin core signaling pathway in Arabidopsis have been identified as RNA based PCR fragments or as ESTs in our collection of approximately 20 000 sequences derived from the cambial zone of birch trunk. We are currently studying the expression of the identified genes and performing functional analyses of the BHK genes in Arabidopsis and in the tree system.

Comparison of gene expression in a lignin forming tissue culture and developing wood of spruce

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A lignin forming tissue culture of *Picea abies* (Norway spruce) has been used as a model system to study the final polymerisation steps of lignin biosynthesis. In this system, lignin formation is spontaneous, no elicitors are needed and the structure of the formed polymer resembles native lignin according to NMR analysis. To compare this system to developing woody tissue of spruce, an EST sequencing project was carried out. Three cDNA libraries were constructed, two from the suspension culture at the 1st and 3rd day after inoculation and one from the developing xylem of spruce using the λ-ZAP II cDNA library kit (Stratagene).

Together, about 8000 ESTs have been sequenced form the three libraries. From the 1st and 3rd day tissue culture libraries, 2067 and 1875 sequences, respectively, have been obtained. In addition, 4098 ESTs have been sequenced from developing wood. Using homology searches, 79% of the sequences could be preliminary identified, and 74% were singletons. Among the 8000 sequences we have found e.g. 23 putative peroxidases (18 clusters), 34 laccases (19 clusters; all from the wood library), as well as most of the monolignol biosyntesis pathway genes.

Dibenzodioxocin lignin substructure is abundant in inner part of secondary wall in norway spruce and silver birch xylem

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A specific lignin substructure, dibenzodioxocin produced by oxidizing 5-5 biphenyl together with coniferyl alcohol was immunolocalized in Norway spruce (*Picea abies* (L.) H. Karsten) and silver birch (*Betula pendula* Roth) differentiating xylem cell walls. For antibody production dibenzodioxocin was conjugated to keyhole limpet hemocyanin (KLH) and antiserum against the dibenzodioxocin-KLH conjugate was raised in rabbits. The specificity of the antiserum was determined with indirect competitive enzyme-linked immunosorbent assay (ELISA), dibenzodioxocin and lignin model compounds 5-5, β-5, β-β and β-O-4 were used as competitors. Dibenzodioxocin was the only lignin model compound able to inhibit the binding of dibenzodioxocin antibodies to dibenzodioxocin-BSA conjugate coated microtiter plate wells (Kukkola et al. 2003).

Alexa Fluor 488 goat anti-rabbit IgG A fluorescence probe was used as a secondary antibody. Cryo-sectioned 25 μm xylem cross sections of both Norway spruce and silver birch were viewed with a confocal microscope with the krypton/argon laser. A X63 oil-immersion objective and FITC fluorescence filters were used. In very young tracheids of Norway spruce and vessels and fibers of silver birch where secondary cell wall layers were not yet formed, the presence of the dibenzodioxocin structure could not be shown. With the fluorescent label used here in combination with confocal laser scanning microscopy, we were able to show that dibenzodioxocin structure in mature tracheids was concentrated mainly in the inner part (lumen side) of the secondary wall (S3 layer), a results which could not be pinpointed in our earlier study (Kukkola et al. 2003). In this study no signal was detected in outer S2 layer suggesting that the number of biphenyls is lower there than in the S3 layer where the signal was intense. In the last phases of cell wall lignification it is possible that there is a burst of free coniferyl alcohol in the cell wall when the protoplast dies, and it is used to produce dibenzodioxocin substructures, which may explain its abundance in the S3 layer of the secondary wall.

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The effect of *aux* and *rol* genes on cell structure and chemistry in silver birch wood

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The effects of *Agrobacterium* pRiA4 *aux* and *rol* genes on wood cell structure and chemistry were studied in 5- and 7-year-year-old silver birch (*Betula pendula* Roth) trees. The transferred genes were controlled by their endogenous promoters. Southern hybridization confirmed the following *rol* and *aux* gene combinations: control plants (no genes transferred); plants with *rolC* and *rolD* genes; plants with *rolA*, *rolB*, *rolC*, and *rolD* genes; and plants with *rolA*, *rolB*, *rolC*, *rolD*, *aux1* and *aux2* genes. Transgene mRNA was detected most abundantly in phloem/cambium samples and in the developing xylem and not in the leaves. The properties of xylem cells were measured from transverse and tangential sections and macerated fibres and vessel members with the aid of image analysis. The total concentration of acid-soluble carbohydrates was measured after Klason hydrolysis with GC-MS. Plants with *rol* genes produced less xylem than control plants or plants with both *rol* and *aux* genes. Tension wood was detected in both control and transgenic plants irrespective of their gene combination, probably as a result of greenhouse cultivation. The transgenic plants had smaller cell wall index (cell wall area in % of total transverse area), vessel area, vessel diameter and fibre length than the controls. The total concentration of acid-soluble carbohydrates was lower and the vessels were shorter in plants with all the *rol* genes than in the controls or plants with both *rol* and *aux* genes.

Reference

Piispanen et al. 2003. Silver birch (*Betula pendula* Roth) plants with aux and rol genes show consistent changes in morphology, xylem structure and chemistry. Tree Physiology 23: 72—733.

Functioning within cell wall: GEG1 and possibly other family members participate in the regulation of cell dimensions

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GEG1 gene product has a role in the regulation of cell expansion during the maturation of *Gerbera hybrida* petals. *GEG1* expression is detected in petals and carpels and it coincides spatiotemporally with flower opening and cessation of cell elongation. At this same stage of development the inner structure and organization of petal cells changes rapidly allowing petals to bend and flowers to open. In transgenic plants constitutively expressing *GEG1*, reduced corolla lengths with concomitant reduction of longitudinal cell expansion were found (Kotilainen *et. al*. 1999, Plant Cell 11: 1093-1104).

In addition to *GEG1*, five other GEG-like genes have been found from gerbera EST collection. The deduced amino-acid sequences of these proteins share high similarity with previously characterized putative small cell wall proteins encoded by GA-inducible genes, for example, *GAST1* (for GA-stimulated from tomato), *GASA1-4* (for GA-stimulated from arabidopsis) and *GIP1-5* (for GA-induced gene of petunia). Taken together our studies suggest that GEG1 plays a role in phytohormone-mediated cell expansion in late stages of petal development while others might be important in earlier stages and /or in other organs.

In this poster we present the localization of GEG1 protein in cell walls of gerbera petals. We also introduce five other GEG-like gene products from gerbera. At this point our main goal is to gain knowledge of the actual mechanism of function of GEG-like proteins as well as their possible interacting companions in the surrounding cell wall.

Lignin-bound peroxidases and a laccase from *Picea abies* tissue culture

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Lignin is a significant constituent of plant cell walls. It forms from monolignol precursors by a reaction catalysed by peroxidases and putatively also laccases. A *Picea abies* tissue culture, which spontaneously forms lignin in the culture medium, was utilised in this research. Several peroxidase isoenzymes and one laccase have been characterised from the liquid phase of the medium. Some peroxidase and laccase activity can be extracted from the culture medium lignin by a high-salt buffer. Since these enzymes are bound to the lignin, they probably play a role in lignin synthesis.

The lignin-bound enzymes were partially purified, digested with trypsin and some of the resulting peptide fragments were sequenced. The sequence fragments were compared with sequences obtained from the liquid medium peroxidases. The results indicated that the lignin-bound enzymes are distinct from those found in the liquid medium. The laccase has not yet been compared. Sequencing the relevant genes is in progress.

A dehydrogenation polymer (DHP) is an artificial “lignin” made *in vitro* from monolignols using enzyme catalysts. A DHP was produced from coniferyl alcohol monolignol using the total protein eluted from culture medium lignin as a catalyst. The resulting polymer was characterised with NMR. The proportion of 8-ring structures was greater than with any characterised liquid medium enzyme. This is in accordance with the theory that the formation of the 8-ring structure requires close association of nascent lignin and the catalysing enzyme.

Crystallinity of wood and the size of cellulose crystallites in Norway spruce and Scots pine

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The crystallinity of wood and the average thickness of cellulose crystallites in Norway spruce (P*icea abies* (l.) Karst.) And Scots pine (P*inus sylvestris* l.) Were determined using x-ray diffraction [1]. The samples were from stems that were grown in Finland in medium fertile [1] (average growth ring width 2 mm) or exceptionally fertile site [2] (average growth ring width 6 mm). Also samples from a nutrient optimisation experiment in Sweden [3, 4] were investigated. For samples grown in average conditions, the crystallinity of wood increased in juvenile wood as a function of the year ring and was constant in mature wood, about (30 $\pm$ 4) %. For fast-grown samples slightly lower crystallinity values were obtained. The Average thickness of the crystallites (3.2 $\pm$ 0.1 nm) remained at the same level from the pith to the bark and it did not depend on the growth rate.

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Are changes in wood chemical properties maintained over five years of exposure to elevated CO_2 and O_3 in aspen clones?

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The aim of this study was to test the effects of elevated [CO_2] and [O_3] on wood chemistry and anatomy of five clones of *Populus tremuloides* Michx. Wood chemistry was studied also on *Betula papyrifera* Marsh.and *Acer saccharum* Marsh. Material for the study was collected from the Aspen FACE experiment in Rhinelander, Wisconsin, USA, where the trees had been exposed to four treatments: control (C; ambient CO_2, ambient O_3), elevated CO_2 (EC; 560 ppm during daylight hours), elevated O_3 (EO; 1.5x ambient during daylight hours) and their combination (EC+EO) for three growing seasons (1998-2000).

In aspen, genetic variation was significant on concentrations of total, gravimetric and acid-soluble lignins, acetone-soluble extractives, soluble sugars, starch and nitrogen in wood. Increases in soluble sugars and starch were found under the EC exposure, but due to the interaction between clone and the EC treatment, the effect was significant only in clone 8L for soluble sugars or clones 259 and 271 for starch. Significant interactive effects of the EC and EO treatments were detected on α-cellulose, total lignin (= gravimetric + acid-soluble lignin) and nitrogen. The interaction for α-cellulose was due to the fact that in the EC treatment its concentration decreased while in the EC + EO treatment the concentration increased. For total lignin, the EO exposure increased the concentration but the EC treatment nullified the EO effect. Also an interaction between the EO exposure and clone for gravimetric lignin was found: all other clones except clone 42E had increased concentrations under the EO treatment. The interaction of EC and EO for nitrogen was because the EC treatment alone did not have an influence while the EC + EO treatment decreased the nitrogen concentration. In paper birch wood, the EO exposure increased total lignin and hemicellulose, while the EC treatment decreased the concentrations. In sugar maple wood, statistically significant interaction between the EC and EO treatments were found for starch concentration. This was due to decreased starch concentrations in the EC and EO treatments alone while the combination treatment had no effect on starch concentration. The above results will be compared to the results of five-year fumigation (sampling in 2002) that are under processing at the moment.

In aspen, genetic variation was significant on all studied structural parameters. Diameter of the stem, i.e. distance from pith, increased in the EC treatment. The EC exposure had no effect on the structure of secondary xylem while the EO treatment had a significant impact. In the EO treatment vessel and fibre diameter decreased while the percentage of cell walls and the thickness of radial fibre wall increased. Treatments did not have significant effect on vessel percentage. The implications of these findings, in the context of climate change and wood properties, will be discussed.

Characterisation of hemicelluloses by multi-wawe UV-detection during SEC

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Any treatment of wood and pulp leads to oxidation of hemicelluloses, i.e. accumulation of carboxyl and carbonyl groups. These chromophore groups absorb at 200-220nm and 270-290nm, respectively. The heteroaromatics of furan- and pyron-type, which are formed during wood delignification and pulp fibre bleaching, absorb additionally at 230-250 and 290-320nm. The charge transfer complexes originated from conjugated heteroaromatics causes the absorbance above 300 nm with maximums around 350, 390 and 430nm. Based on this chromophore composition, the SEC techniques of simultaneous characterisation of hemicelluloses by molecular mass (MM) and chemical composition is proposed. It includes multi-wave UV-detector operating at the set of the wavelengths λ and connected in line with an RI-detector.

While both organic solvents (DMSO, DMAA) and alkaline solutions (KOH, NaOH, Cadoxen) are useful, the UV-transparent (starting from 190nm) NaOH or KOH solutions are preferable aiming at chemical composition analysis of hemicelluloses. In our case, 5-10% KOH or 10-18% NaOH are used as solvent and 50 mM aq. NaOH is used as eluent. SEC analysis is carried out with prepacked titanium or cartridge glass Separon HEMA BIO 1000 column (TESSEK). The MM values for hemicelluloses are calculated via universal calibration with corresponding Mark-Houwink constants using dextran or carboxymethyl cellulose as calibrants. The chemical composition is described by the chemical composition distribution (CCD) curves based on absorptivity of the chromophore groups and structures.

The hemicelluloses isolated from wood and/or pulp fibres contain hydroxyl-, carboxyl-, aldehyde- and keto-groups, conjugated and non-conjugated heteroaromatics. Therefore the direct analysis of the chemical composition from UV-chromatograms is hindered due to the interference of different chromophore groups. We propose the CCD curves approach based on the ratio between the UV chromatograms obtained at different λ and that between UV- and RI-chromatograms. The ratio between the UV- and RI-chromatogram heights is equal to the absorbance per mass unit, i.e. it is the absorptivity value at the given wavelength. So, the UVλ/ RI ratio determined in each elution volume (Ve), representing the CCD curve, reflects the concentration profile of absorbing at the corresponding λ functional groups and/or extended chromophore structures in terms of the absorptivity value through the MMD of the sample. On the other hand, as the UV-response at the given λ (absorbance value Aλ) is proportional to the concentration of the corresponding chromophore groups/ structure, the ratio between Aλ1 and Aλ2 reflects the concentration of λ2-absorbing group/structures per λ1-absorbing ones. The Aλ1/Aλ2 (Ve) curves also represent certain kind of CCD curves but they are based on the absorbance values. In our studies, the absorbance of carboxyl groups at 215 m was used as the basis.

Xylans isolated from wood, holocellulose, kraft unbleached and TCF bleached pulp fibres were investigated by the method described. Although the CCD curves showed a non-uniform distribution of functional groups and heteroaromatics through MMD not depending on the xylan origin, the degree of oxidation as well as the degree of conjugation of heteroaromatics significantly depended on the pulping and bleaching process. Significant contribution of conjugated heretoaromatics absorbing above 360nm into absorbance of pulp's xylans was

found. The change in the composition and distribution of MM of the products of thermo-oxidative destruction in hardwood xylan after the pulp bleaching process was discovered.

These results on the attribution of the lignin- and heteroaromatic- substances originated UV/VIS absorbance above 270nm require further careful examination as they seem to be important in pulp fibres bleaching technology.

Ultrastructure of compression wood fibres in fractions of a thermomechanical pulp

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The microstructure and ultrastructure of compression wood fibres in mechanical pulp have been insufficiently characterised previously. This kind of information is required in order to understand how compression wood fibres are treated in the mechanical pulping process and how these fibres affect products produced from such pulp. In the present study, the ultrastructure of compression wood was characterized in four Bauer McNett fractions of a commercial thermomechanical pulp (TMP) produced from Norway spruce roundwood. Comparisons were made with TMP fibres of normal wood and unrefined native compression wood. Compression wood fibres in the TMP ware identified using the Wiesner reaction (Schneider et al. 1999) and studied using scanning electron microscopy. Compression wood fibres were found in all fractions, and thus compression wood fibres are not broken into fines, as suggested previously. The cell wall layers observed on fibre surfaces were the S1 and/or the S2 secondary wall layers (Fig 1.). Compression wood fibres were almost never collapsed. It was suggested that compression wood fibres might contribute to the surface roughness of printing paper grades based on TMP.

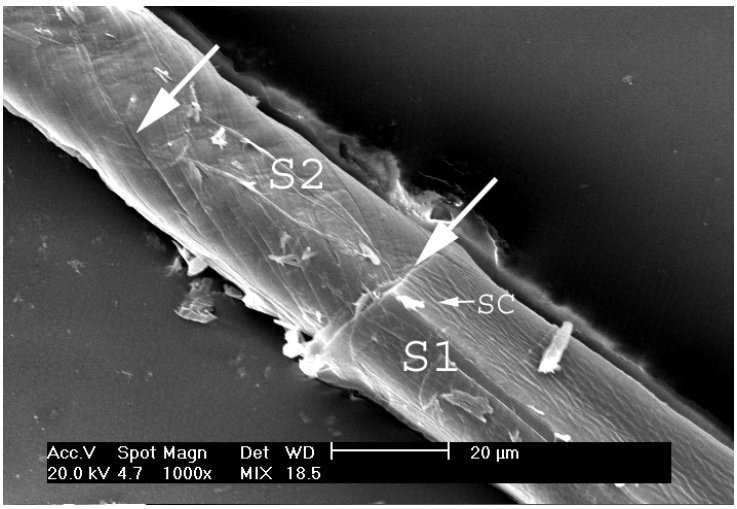

Fig. 1. SEM of a compression wood tracheid selected from the BMN 30 fraction. The fracture between S1 and S2 is oriented perpendicular to the tracheid axis (lower right large arrow). Surface corrugations (SC) approximately parallel to the tracheid axis are present on the S1 layer. Cracks originating from the helical striations/cavities are present in the S2 layer (upper left arrow).

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Micro-morphological observations on spruce thermo-mechanical pulp fibre fractions with emphasis on fibre cell wall fibrillation and splitting

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Important properties sought for papers from mechanical pulps are fibre strength and light scattering. In addition, fibre wall thickness and stiffness have been shown to be important criteria for creating smooth paper surfaces. All these properties are enhanced through the longitudinal splitting of pulp fibres. Split fibres tend to collapse irreversibly during processing combining a smooth paper surface with improved strength and light scattering. Thus, the manner by which pulp fibres split and ways to enhance this process are of considerable economic interest.

A detailed electron microscope (SEM) study was performed on thermochemical (TMP) spruce fibre BauerMcNett mechanical pulp fractions (BMN, 15, 30, 50, 200) in order to obtain a greater insight into the origin and development of splits and fibrillation within the fibre wall. Observations showed two major types of fibre fibrillation to occur: “flake-like” derived from the S1 layer and “sheet and ribbon” fibrillation from the S2 cell layer for both early- and latewood fibres. In both cases fibrillation developed from the initial cracking and subsequent splitting of the individual fibre cell wall layers along the orientation of the native cellulose microfibrils (i.e. along the microfibril angle, MFA) for both the S1 and S2 layers. Flake-like fibrillation was derived from the fragmentation of shorter pieces from the fibre S1 layer. S2 fibrillation was derived from helical cracking of the cell wall layer following the MFA. The outer fibre S2 wall split into regular sized sheets (frequently concentric orientated) and/or ribbons and sheets; each ribbon composed of a set number of cellulose aggregates. Observations suggested that fibre cell wall splitting was “clean” lying between the cellulose aggregates rather than across them. Additional sites for the initiation of splits included the bordered- and cross-field simple pits and rays. It is concluded that fibre fibrillation results from the cracking of the fibre wall along sites of weakness already present in the cell wall. These weak sites lie between the cellulose aggregates and are possibly rich in hemicellulose and lignin. Severe fracturing of the fibre wall derived via splitting from the pits and in earlywood fibres resulted in the fibre “opening up” to expose the fibre cell lumen. In addition to providing more details on the effects of refining on fibre wall structure, novel information is being obtained on fibre cell wall ultrastructure.

Determination of microfibril angles using x-ray diffraction in symmetrical and perpendicular transmission mode

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Two x-ray diffraction (XRD) modes, symmetrical (Cave 1966, Paakkari and Serimaa 1984) and perpendicular transmission (Lichtenegger et al. 1999), for determination of the microfibril angle (MFA) have been compared. For comparison, MFA was also determined with a drying checks method. The wood samples were taken from stems of European larch (*Larix decidua* Mill.), Oak (*Quercus sp*.), Beech (*Fagus sylvatica* L.), Poplar (*Populus sp*.), Norway spruce (*Picea abies* Karst.) grown in Italy or in Finland. Both early wood and late wood were studied for each annual ring analysed. Furthermore, samples of reaction (compression and tension) wood were also measured.

In data analysis the MFA distribution in one cell wall was presented as a sum of Gaussian functions whose positions, intensities and widths were obtained by fitting the model symmetrically in the intensity curve. The effects of the shape of the cell and the orientation of cell walls on the measured intensity curve were considered (Sarén et al. 2001, 2003).

In the case of perpendicular transmission mode all cell walls affect the intensity curve regardless of the shape of the cells, which complicates the data analysis. Most unambiguous results are obtained using the symmetrical transmission mode and the reflection 004 (Cave 1966). However, in this study experiments in the perpendicular transmission mode were much faster than those in the symmetrical transmission mode, since a rotating anode based setup with an area detector was used. The measuring times were between 60-90 seconds. However, additional microscopy studies were needed for determining the shape of the cell. Measurements in the symmetrical transmission mode were carried out using a conventional sealed x-ray tube and a scintillation counter and measuring times from 60 to 120 minutes were needed for both reflections 200 and 004, but the shape information was obtained from the same data (Cave 1966, Sarén et al. 2001).

When the data-analysis is performed carefully, the same mean MFA for the same sample is obtained using the two XRD measuring modes. The mean MFA agreed with that obtained with the drying checks method. The agreement was good for all samples, both softwood and hardwood. We point out that biological aspects in the sample preparation need to be considered as both the sample preparation and the measurement mode determine, which cell walls give a major contribution to the scattering intensity. It was observed that for Norway spruce the mean MFA in radial cell walls was larger than that in tangential walls (Sarén et al. 2003). This was attributed to the bordered pits in the radial wall, which affect the alignment of microfibrils.

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On the regeneration of woody tissue in poplar after wounding

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Mechanical wounding of trees into the xylem induces the formation of callus tissue along the wound edges. The present study deals with stem wounds set in poplar trees during the vegetation period. Light and electron microscopy revealed that young callus tissue develops by cell divisions in the phloem parenchyma, in the differentiation zone of xylem and phloem as well as in the cambium. With increasing duration of wound response, i.e., within two to six weeks, a wound cambium is regenerating pereferably by re-differentiation of phloem parenchyma cells. Later on wound xylem and phloem is laid down. Wall structures of cells in the callus tissue are characterised.

Pectin on mechanical pulp-fibre surfaces

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The quality of mechanical pulp is partly determined by the processing method used for defibration but also by inherent properties of the pulp fibres, such as physical characteristics like shape and size, as well as surface ultrastructure and chemical composition. The surface and composition of mechanical pulp fibres are heterogeneous and depends on wood species, and which part of the cell wall is exposed to the surface since the various fibre cell wall layers have different chemical composition and structure. The middle lamella region contains relatively higher amounts of lignin, whereas the secondary cell wall is rich in polysaccharides like cellulose and hemicelluloses. Pectin is restricted to middle lamellae, primary cell walls, ray cells and pit membranes. Pectin is a negatively charged polysaccharide composed of numerous different monosaccharides, but mainly galacturonic acids, whose carboxylic groups can affect the fibre-surface chemistry and may thereby influence pulp properties. After mechanical pulping, residues of compound middle lamellae remain on the pulp fibre surface, and immunolabelling of pectin in mechanical pulps has shown that both thermomechanical pulp (TMP) and chemi-TMP (CTMP) have patches of high concentrations of pectin on the fibre surface localised in these remaining compound middle lamella areas. [1]Using a newly developed bioassay based on colorimetric determination of the amount of pectin-specific antibodies that bind to the pulp fibre surfaces, it has been shown that TMP has more methyl esterified pectin exposed on the fiber surfaces than CTMP. [2]This may be due to the chemical treatment in the CTMP process altering the composition of pectin or the fibre surface structure.

This work was performed within the framework of WURC (Wood Ultrastrucutre Research Centre) at the Swedish University of Agricultural Sciences in Uppsala, Sweden (Project 15 at http://www-wurc.slu.se). The aim is to study the fibre surface structure and chemistry, fibre collapsibility, and their subsequent effects on final paper quality.

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Topochemical characterization of lignins and phenolic extractives in wood cell walls

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The topochemical distribution of lignin and phenolic extractives in woody tissue is determined on a cellular level by using scanning UV microspectrophotometry (UMSP). This improved cellular analytical technique enables direct imaging of the lignin distribution within individual cell wall layers with a resolution of 0.25 µm^2. The technique is based on the ultraviolet illumination of semi-thin transverse sections of the woody tissue which can be related semiquantitatively to the concentration of lignin within the cell wall. Furthermore, the UV absorption maximum is sensitive to structural differences of the lignin allowing discrimination between hard- and softwood lignins due to different ratios of their guaiacyl- and syringylpropane units. For detailed investigations of the extensive (three dimensional) distribution of lignin and aromatic phenolic compounds in the wooden tissue a specific UV-spectrometric-scanning-analyses has been developed. The UV-microscope is combined additionally with a scanning stage which allows to scan the transverse sections with a characteristic defined wavelength by using the scan program APAMOS® ZEISS (Automatic-Photometric-Analysis of Microscopic Objects by Scanning). The scan programme digitises rectangular fields with a local geometrical resolution of 0.25 µm² and a photometrical resolution of 4096 grey scale levels which are converted in 14 basic colours to visualise the absorbance intensities. The scans can be depicted as two- or three-dimensional image profiles including a statistical evaluation (histogram) of the semi-quantitative lignin distribution. The method was found to be ideally suited for the study of lignification during wood formation, the detection of phenolic extractives, the removal of lignin during pulp processes, and other applications in chemical technology of wood.

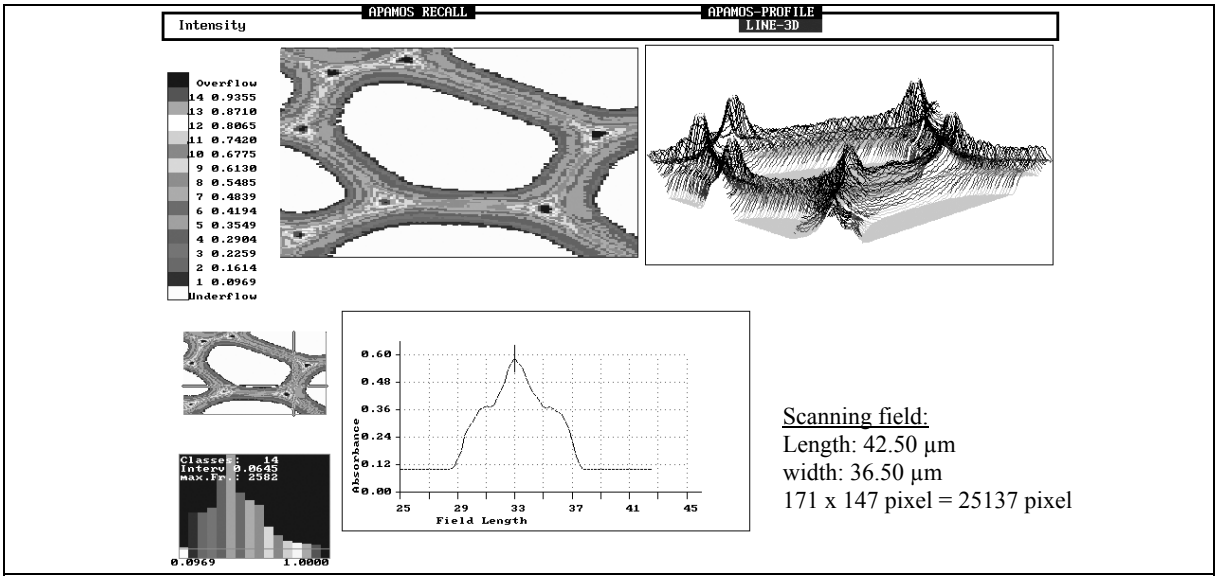

Fig. 1. UV microscopic image profiles of an individual tracheid of Picea abies scanned with a geometrical resolution of 0.25 µm². The grey scales indicate the different UV-absorbance values at a wavelength of 280 nm.

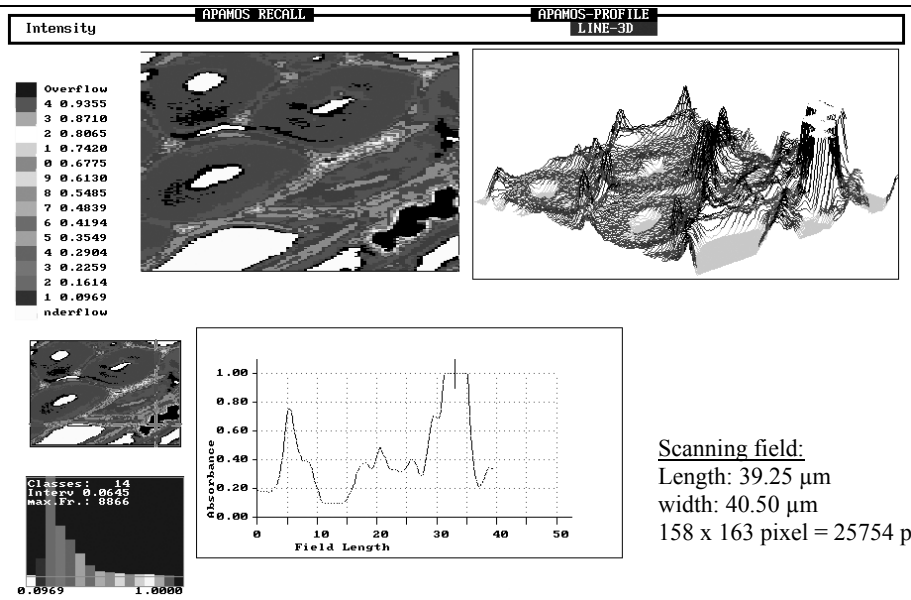

Fig. 2. UV microscopic image profiles of Fagus sylvatica tissue measured at λ_{278nm} showing the deposition of phenolic extractives (arrows) in lumina of ray parenchyma cells

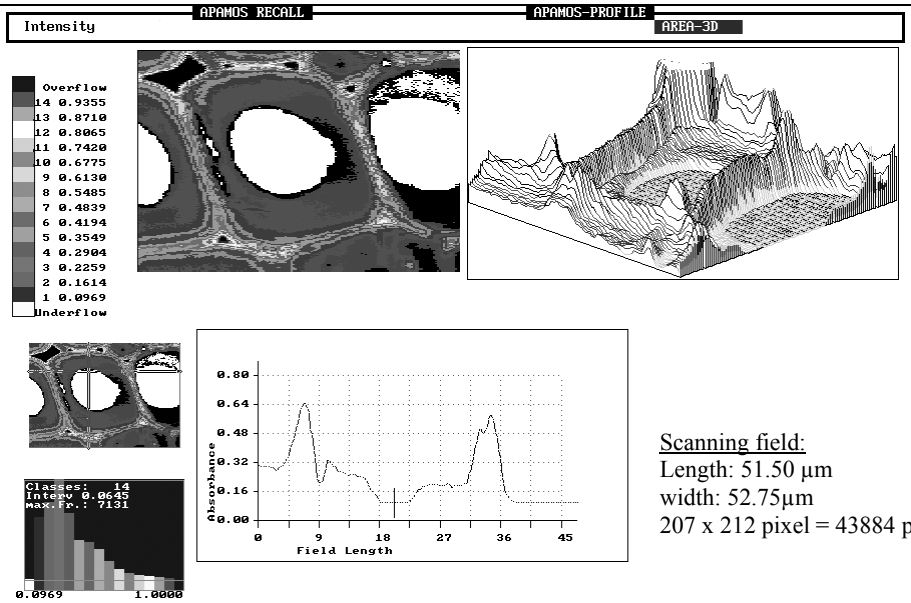

Fig. 3. UV-microscopic image profiles of bisulfite pulped Picea abies latewood tracheids (Cooking stage 60 min). The grey scales indicate the different UV-absorbance values at a wavelength of 280 nm.

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Effects of elevated [CO_2] and nutrients on wood properties of Norway spruce

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Many studies have reported that an increase in atmospheric [CO_2] enhances photosynthesis, growth, and productivity depending on plant species and growing conditions. Although wood quality is of great importance in forest industry, few studies have so far examined the effects of elevated [CO_2] on wood properties. The objective of the present study was to investigate the effects of elevated [CO_2] and nutrient availability on wood properties of 40-year-old Norway spruce trees (*Picea abies* (L.) Karst.). Material for the study was obtained from a CO_2-experiment in a long-term nutrient optimisation trial in Flakaliden, Sweden. The treatments were obtained by means of temperature-controlled whole-tree chambers with ambient [CO_2] and elevated [CO_2] (twice ambient) and outdoor controls. The trees, with or without nutrient optimisation, had been exposed to the CO_2 treatments for three years. We investigated the interactive effects of elevated [CO_2] and nutrient availability on stem wood structure (annual ring width, latewood percentage, tracheid length, tracheid lumen diameter, cell wall index and thickness) and chemistry (lignin, cellulose, hemicellulose, acetone-soluble extractives, soluble sugars, starch, and nitrogen). Elevated [CO_2] increased annual ring width and decreased radial tracheid lumen diameter in the second year of exposure. The increase of annual ring width was significant in non-fertilised trees. Decrease of concentrations of soluble sugars and nitrogen, and increase of acid-soluble lignin in elevated [CO_2] was dependent on sampling height and/or nutrient availability. Nutrient optimisation affected both stem wood structure and chemistry. Cell wall thickness of tracheids, cell wall index, and latewood percentage decreased as an effect to nutrient optimisation, while annual ring width and radial lumen diameter increased. Nutrient optimisation increased concentrations of gravimetric lignin and nitrogen.

Does climate change affect wood chemistry in silver birch?

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Effects of elevated CO_2 and O_3 on wood chemistry of two silver birch clones (clones 80 and 4) were studied after a fumigation of three growing seasons 1999-2001. Altogether 32 trees were exposed in open-top chambers the field to the following treatments: ambient [CO_2], 2*ambient [CO_2], 2*ambient [O_3], 2*ambient [CO_2] and 2*ambient [O_3] in combination, and 8 trees served as outside controls. After the 3-year study the trees were harvested and samples were taken at three different heights of the stem (1.3m, 40% of stem height and the 1998 stem section) for analyses of the chemical composition of secondary xylem. In the analyses the annual rings that developed during the exposure period were used. There were significant differences in the chemical composition between the two clones. In clone 4 the concentrations of extractives, starch, soluble sugars and nitrogen were greater than in clone 80, while in clone 80 the concentrations of α-cellulose and acid-soluble lignin were greater. Sampling height also had a significant effect on concentrations of extractives, α-cellulose, acid-soluble lignin, soluble sugars, starch and nitrogen, that were greater higher in the stem, while the C/N –ratio decreased towards stem top. The effects of the treatments on chemical composition of secondary xylem were small and the response did not differ between the two clones. The only significant effect was found in starch concentration that increased under elevated CO_2. Interestingly, the concentration of α-cellulose was greater under elevated [O_3] when compared with the combined treatment of elevated [CO_2] and [O_3]. This was due to a tendency of α-cellulose to decrease under elevated [CO_2] and to increase under elevated [O_3].

Large deformations in transverse compression of a tropical hardwood: observation and finite-element analysis

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The transverse compressive deformation of a tropical hardwood (*Triplochiton scleroxylon*) was observed and analysed at the microscopic level. Uniform compression and indentation tests allowed to study the local deformation mechanisms in the transverse plane in the case of large strains. Localization originating from vessels and the role of rays were evidenced through observations under microscope of the wood compressed in the radial or tangential direction. A finite element simulation of the deformation processes is attempted using MSC.Marc software. Due to the complexity of the multi-level structure, the numerical analysis has to be divided into successive steps. First, it is considered that fibres and axial parenchyma, made of small cells, should be replaced by an equivalent foam made of the superposition of an isotropic compressible Ogden model and reinforcing elements; a similar approach can be applied to the rays, to be replaced by the same type of model with different parameters. Then the behaviour of a typical wood portion containing all types of cellular tissues is simulated in order to compare the two calculations, that with the detailed description of the cellular arrangement and that with the reinforced foam. When the use of a foam model will have been validated in this way, it will become possible to produce a simplified mesh of the compressed zone in an indentation test and simulate the 3D compression process.

New model systems of secondary plant cell walls based on bacterial cellulose/pectins composite : synthesis, chemical characterisation and mechanical properties.

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Mats of bacterial cellulose and of a pectin/cellulose composite were used as a host matrix for *in vitro* polymerisation of coniferyl alcohol in order to build up model systems of secondary plants cell walls. A diffusion cell was used in order to allow the diffusion of both hydrogen peroxide and coniferyl alcohol into the peroxydase impregnated cellulose mats through dialysis membranes. The resulting binary and ternary blends were imaged by Scanning Electron microscopy (SEM) and characterized by chemical means. The presence of pectin induces a better dispersion of the synthetic lignin in the cellulose network and enhances the proportion of alkyl-aryl-ether in the polymer. Mechanical properties of lignified and unlignified systems were also evaluated.

Dislocations in wood fibres

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Deformations in pulp fibres have been called dislocations, nodes, slip planes, micro-compressions, minute compression failure, misaligned areas or zones. Although they make fibres more flexible they are also points for chemical, mechanical and enzymatic attack which can decrease paper strength (Nyholm and Ander 2000; Nyholm et al. 2001; Ander 2002).

Dislocations appear when there is a change in microfibril alignment as a result of fibre cell wall compression. Dislocations are most easily studied using polarised light microscopy, while scanning (SEM) and transmission electron microscopy (TEM) may be used for more detailed investigations on the changes in structure of different cell wall layers in dislocations. Dislocations may be rather small only involving the S1 layer, or larger involving both S1 and S2 layers.

Since dislocations are considered important for pulp and paper properties there is a considerable interest in the pulp and paper industry to analyse for the number of dislocations in pulp fibres after cooking and bleaching. Therefore a technique to measure HCl-induced fibre cleavage of dislocations and other weak points has been developed (Ander and Daniel 2003). It was earlier found (Ander 2002), that HCl at pH 1.8 at 80°C did not cleave spruce kraft pulp fibres. Later it was found however that stronger HCl, namely 0.1 to 2 M (pH 1 to – 0.35), resulted in dislocation cleavage. The newly developed technique involves swelling/ defibration of the fibres, HCl-treatment and washing. Afterwards the fibres are analysed in a Fibremaster to give mean fibre lengths, which are used for calculation of dislocation number.

Treatment scheme:

Fibre separation and swelling for 10 min with stirring bar
Treatment with 1 M HCl pH 0 for 4h at 80°C, shaking but no stirring bar
Transfer to room temperature and stirring for 30 min during cooling (to finalise cleavage)
Wash and adjust to pH 4-5
Fibremaster analyses

In addition to other parameters, the Fibremaster analyses give length weighted fibre length distribution.

Cleavage per fibre: $l_0 / l - 1$

where l0 and l = length weighted fibre length distribution for control and sample respectively. Cleavage per fibre is due to dislocations and other weak points. Typical values for industrial kraft pulp fibres are 2-3 cleavages per fibre, while laboratory pulp fibres give slightly lower cleavage per fibre. For 2M HCl, 6-10 cleavages per fibre has been obtained. The differences are statistically different, repeatable and the method can be used to evaluate wood and pulp fibres of different kinds. Using polarised light microscopy it was seen that the cleavages occurred in both earlywood and latewood fibres (Figure), although dislocations are more easily detected in latewood fibres. The above method will in the future be compared with cellulase-induced dislocation cleavage using light and electron microscopy.

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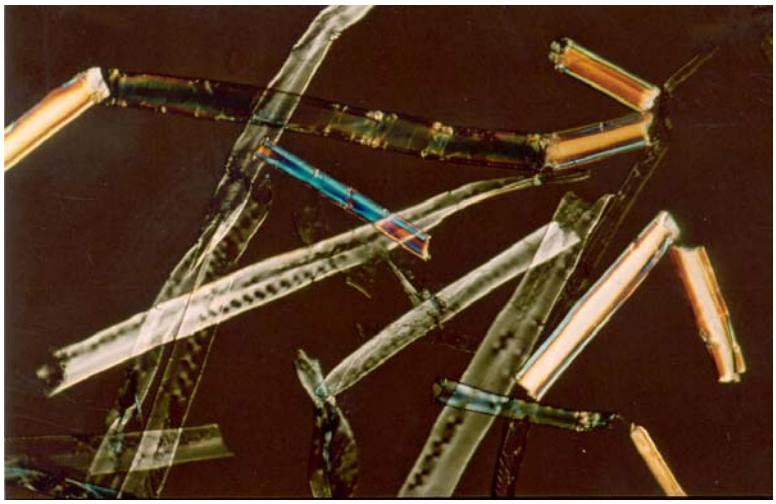

Figure 1. Cleavage of spruce earlywood and latewood pulp mill fibres by HCl after 4h at 80°C.

The possible relationship between dislocations and mechanical properties of different spruce fibres: A single fibre study

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Dislocations appear in wood fibres during chipping and further increase in number during pulping and bleaching [1,2]. Since dislocations can lead to decreased pulp and paper strength, it is important to study the development, properties and morphology of dislocations in softwood fibres. Single fibre studies are one way to obtain more information how dislocations may influence mechanical properties of a single fibre. Thus, some spruce kraft pulp fibres were tested for mechanical properties in a newly constructed apparatus. Fibre samples were latewood fibres, either partly-delignified latewood fibres with 6% lignin or H_2O_2/HAc-delignified fibres. In these fibres, dislocations were easily detected in delignified fibers, but did not exist or were covered by lignin in the lignin-containing fibres. Some earlywood fibres were also tested.

Fibres: Latewood LW5 annual ring 5, LW26 annual ring 26-30 (LW-0%), Earlywood EW5 annual ring 5, EW26 annual ring 26-30. These fibres had been delignified by H_2O_2/HAc [3]. Non-delignified LW26-30 (LW-6%) with 6% lignin were also used.

Single fibres were transferred to a frame and elongated in a tensile apparatus designed to measure force and elongation versus time of single fibres. Values were combined to load-displacement curves [4,5]. The fibre cell wall cross sections were measured in an ESEM and used for stress-strain calculation. Preliminary results indicate that the partly-delignified and wholly delignified fibres show similar moduli of elasticity, but differ significantly in their ultimate tensile stress. The reason may be that delignification together with appearance of dislocations decreases tensile strength. However, ESEM micrographs of the fibre fracture surfaces indicate that the relation of these surfaces with dislocations is uncertain.

Modulus of elasticity:	LW-6%	31606 MPa
	LW-0%	27425 MPa
Tensile stress:	LW-6%	1452 MPa
	LW-0%	899 MPa

Thus non-delignified fibres with 6% lignin were strongest (Figure) and juvenile fibres (LW 5) were less strong than mature fibres (LW 26-30). One reason is probably the microfibril angle (MFA), which is lower in matured fibres closer to the bark. The EW fibres were rather delicate with thin cell walls and hard to prepare and flattened, making fixing in the frame difficult. Only some results were obtained. These indicate that the tensile strength of earlywood fibres are low.

The type of breakages for the LW fibres were very different, with breakage angles ranging from 90 to 45 degrees ("brash-type") indicating dislocations and down to a very low angle perhaps due to macrofibril breakage. New fibres with high and low number of dislocations were picked out using polarizing microscopy and sent to Vienna for measurement. However, many of them were fractured during the picking procedure and the glycerine/water mixture (1:1) used for the flight transport of the fibres was not good for gluing of the fibres before testing. Further testing should be done. It may however be mentioned that measuring fibre cell wall cross sections, necessary for stress-strain calculation, is very time-consuming.

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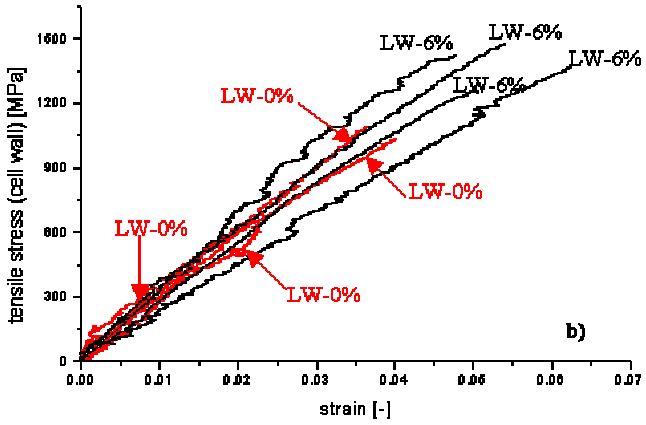

Figure 1. Stress-strain diagram of LW-6% and LW-0% spruce fibres, stress calculated on the basis of cell wall cross section (Ander et al. 2003).

Properties of print paper produced in the 1st half of the 20th century

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To elucidate the preservation degree of the print paper produced in the first half of the 20th century, samples of print paper from 53 books stored at the Latvian National Library (LNL), a major state book repository in Latvia, were taken.

The following characteristics were determined and analysed: brightness; paper surface pH; pH of aqueous extracts (20°C, 100°C); lignin content; acid-insoluble ash (fillers); tearing resistance; tensile properties, etc.

For almost all print paper samples, the pH indices are considerably below 7, even reaching pH 2.88; For 60 % of samples, lignin content is above 10 %. The most typical quantity of fillers is 14–20 %. Breaking length of the samples range from 265 m to 3665 m. Tear index ranges from 1.62 mN m^2/g to 7.19 mN m^2/g.

A comparative analysis has shown that:
for 80 % of paper, brightness is, on the average, by 43 % below the standard;
for 70 % of paper, the pH index is, on the average, by 70 % below the standard;
for 40 % of paper, tear index is, on the average, by 30 % below the standard;
for 50 % of paper, the tensile properties – breaking length (m) is below the standard – in this case, for a half of the samples, this index is below the limit value.

In order to increase the preservation degree of the paper excess acid should be eliminated first for print paper with the pH below 5.0.

Radial trends of mechanical and fracture mechanical behaviour on the growth ring level of Norway spruce (*Picea abies* [L.] Karst)

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The gradual change of material properties in a growth ring of softwoods is correlated by a gradual change of the mechanical and fracture mechanical behaviour of the layered wood structure. Local density and as well anisotropy and cell wall design are the most important parameters. In this study tensile experiments on thin specimens (0,2 mm) of Norway spruce (*Picea abies* [L.] *Karst*) have been performed in the longitudinal and tangential direction in order to determine MOE and strength depending on the radial position of the specimen in several growth rings.

The MOE profiles could be explained by the variations in density showing the same tendency in its profile. The strength parameter obtained from a notched tensile specimen is also depending on the position in the growth ring. The crack path of the tensile specimens was investigated utilising light- and scanning electron microscopy and showed remarkable differences depending on the radial position of the specimen. The results are discussed regarding the changes in local anisotropy, density and tissue structure.

New measurement techniques leading to more insight in beating of chemical pulps

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The project "Development of new mechanical upgrading technologies" is a part of the EET-program subsidised project "Fibre raw material technology for sustainable paper & board production". In this project the project partners: Sappi R&D, Kappa Roermond Papier, Voith Fibre Systems, Bouma Technical Services and ATO Fibre and Paper Technology work together to develop new techniques to improve the fibre and paper properties by mechanical action. The project is co-ordinated by the Dutch Competence Centre Paper and Board.

Papermaking is finding the technological and economic compromise between product properties and processing. With refining both product and processing properties increases like strength, formation and wet web strength. However, refining also decreases processing properties like drainage and drying efficiency. New beating techniques should improve the positive effects with a minimum decrease in processing properties.

To optimise the effect of mechanical treatment we must know the changes in fibre structure due to beating and know how these changes correlate with changes in product and processing properties. In general the most important effects of beating are summarised as:
- Fines formation
- External fibrillation
- Internal fibrillation

The effect of fines and external fibrillation on different fibre and pulp properties and the importance of fibre flexibility of a bleached softwood kraft pulp is determined in this study.

Removing the fines from a PFI-mill beaten bleached softwood kraft pulp resulted in a large increase in drainability but only a minor decrease in strength properties. The amount of created fines in beating can be addressed by subtracting the specific surface area of the washed pulp from the surface area of the whole pulp. Comparing the results of a PFI-mill beating curve with a refiner beating curve shows that the same strength levels can be reached with a PFI-mill at much lower fines contents compared to refiner beating.

The amount of external fibrillation can be addressed by measuring the specific surface area of the beaten and washed fibres and subtracting the surface area of the washed unbeaten fibres. The PFI-mill shows a much lower increase in external fibre surface at comparable strength development than refiner beating.

The fibre flexibility, measured with the Cyberflex, shows a fast increase when it is plotted against the amount of beating. This fast increase corresponds well with the fast increase in strength.

From these results we concluded that the increase in strength due to beating is mainly an effect of fibre flexibility increase. Fines and external fibrillation contribute only a little to the paper strength. However fines and fibrillation have a tremendous effect on paper processing like drainability and drying. By only increasing fibre flexibility the strength properties of pulp can be increased with a minimal decrease in de-watering properties.

At ATO a special device is used to control the amount of shear in a batch laboratory beating process. Applying mainly compression forces instead of shear forces made clear that it is possible to increase strength with a minimum increase in beating degree. At the moment the project is focused on the translation of the batch compression beating to a continuos beating process.

Effects of variability in cell-wall microstructure on the axial compression strength of norway spruce

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BOKU Vienna

Variability in the axial tensile strength and Young's modulus of wood is mostly due to changes in the main orientation of the cellulose microfibrils with respect to the cell axis. By contrast, the causes of variability in the axial compression strength of wood are less well understood. Therefore, the axial compression strength and density as well as microfibril angle and lignin content of Norway spruce specimens were examined. 84 % of the variability of compression strength could be explained by density. After normalisation of compression strength for density, the experimental results showed that variability in the microfibril angle in the secondary cell wall is not responsible for variability in the axial compression strength of the cell wall. This finding is supported by theoretical considerations using a composite failure criterion. Deviations of the microfibrils from a strictly axial alignment in the vicinity of rays are most probably the cause for the initiation of compression failure in Norway spruce. The lignin content of the secondary cell wall showed a positive relationship at low statistical significance with the compression strength of the cell wall. A positive effect of increasing lignin content on compression strength seems therefore possible, but very weak.

Developing molecular strategies for delignifiaction and characterisation of annual plant fibres

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Industrial utilisation of annual plant fibres has always been a major item on the BTU/NTUA research agenda, since the very beginning of the group's life. Historically, R&D by BTU in this field has gradually evolved around a small number of largely interrelated ideas:

(a) Physico-chemical fractionation of main macromolecular components (cellulose, hemicellulose and lignin), while preserving (or upgrading) fibre qualities;
(b) Development of sulphur- and chlorine-free delignification processes adapted to the characteristics of annual plants, mainly based on the oxygen and organosolv concepts; and
(c) Formulation of analytical protocols and, where necessary, novel "intelligent" ones with the aim to support industrial applications with relevant information at the molecular level.

In this context, a number of annual fibrous feedstocks have been investigated, including

- agricultural and agro-industrial residues, e.g., wheat, barley, and rice straws, corn stover, cotton, tobacco, grape and rape stalks; and
- potential dedicated industrial/fibrous plants, e.g., sorghum (fibre and "sweet" varieties), miscanthus, arundo donax, okra.

The most significant achievements of this research can be summarised in the following:

The development (at the pilot-plant level) of a prehydrolysis-chemical pulping process especially for wheat straw, making possible the co-production of printing-paper grade straw pulp along with fermentable monosaccharides appropriate as substrate for biotechnological applications.
The development (at the laboratory level) of a prehydrolysis-organosolv scheme for the optimal fractionation of the three annual fibre cell wall components.
The formulation of a non-empirical mathematical model adequately describing the kinetics of the fractionation process for process optimisation and design purposes.
A contribution to the establishment of the fibre production line of the first European agro-biorefinery (on the island of Bornholm, Denmark); establishing a "Mediterranean" biorefinery is among the strategic targets of the BTU in the years to come.
Putting together, and improving throughout the years, a comprehensive protocol for the chemical characterisation of annual fibre, permitting detailed mass balance calculations.
The development of a new analytical concept (AFFLUENCE) for fibre/fibrous products characterisation, combining fluorescence spectroscopy/microscopy with chemometrics.

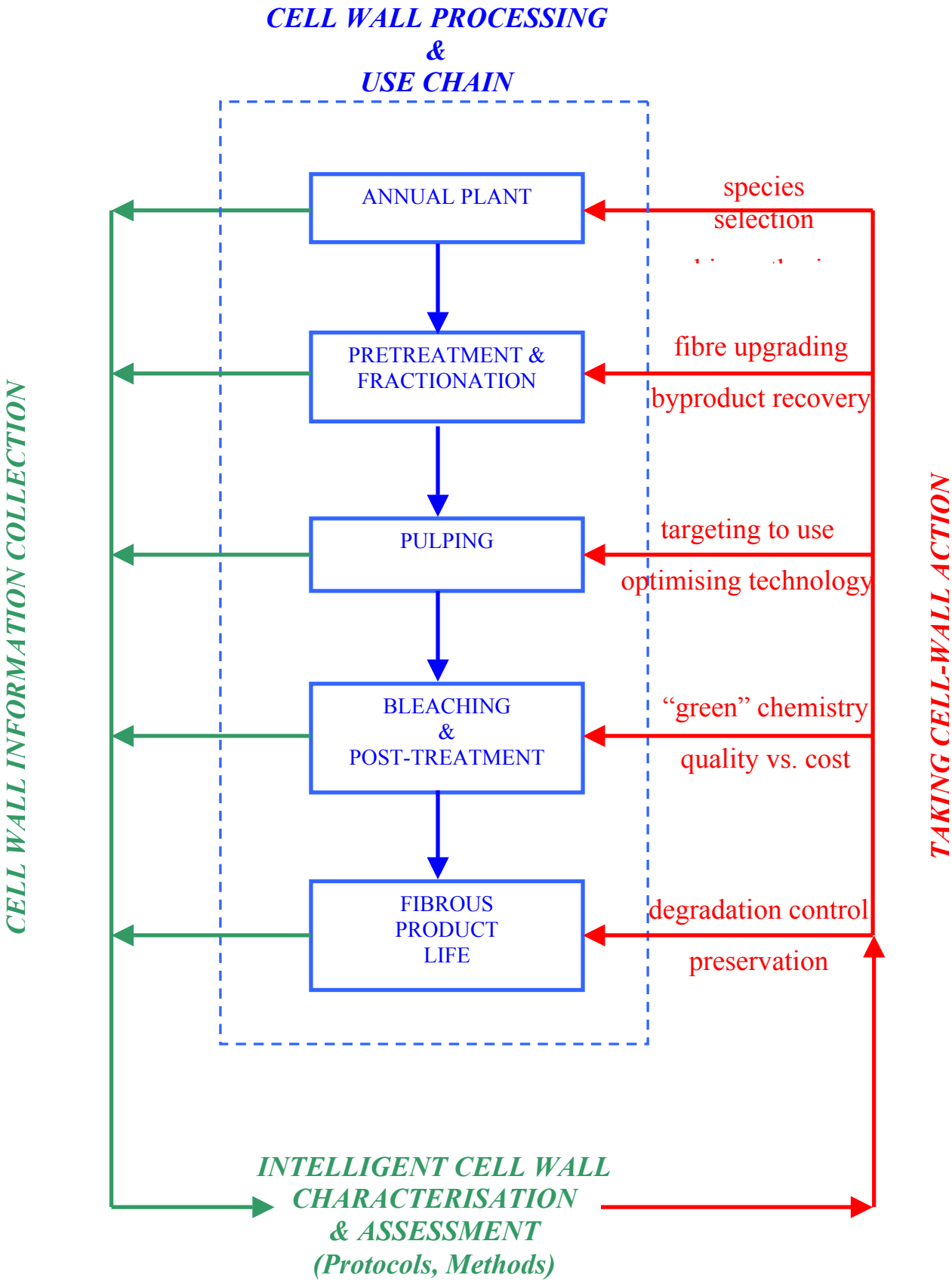

Figure 1. A strategic view at the annual plant fibre system, based on structural / functional information at molecular level.

Simultaneous determination of structure and tensile properties of industrial hemp

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Pieces of industrial hemp (*Cannabis sativa* [L.]) were ground into fine powder (bark and core areas separately) and from these the average width of cellulose crystallites and crystallinity in core and bark was determined by Wide Angle X-ray Scattering (WAXS). The orientation of cellulose microfibrils in hemp core and bark was determined from other, intact pieces of hemp by using x-ray diffraction. [1] In a separate experiment small samples from industrial hemp were stretched and *in situ* WAXS measurements were made. The aim was to study simultaneously both the structure and the tensile properties of this material. The tensile properties of the samples can be determined from the load/strain curves while x-ray diffraction gives information on cellulose crystallisation, crystal structure and fibre texture.

From the x-ray diffraction measurements of powdered hemp it can be concluded that hemp bark contains more crystalline cellulose than hemp core, the crystallinity of bark is 42 % while in the core the value is 28 %. Also the average thickness of cellulose crystallites was different, in hemp bark it was determined to be 35 Å and in hemp core 31 Å. Based on x-ray diffraction analysis of the intact pieces, the expectation value of microfibril angle distribution (average orientation of cellulose microfibrils with respect to the cell axis) is 11 ^{o} in the core and 5 ^{o} in the bark.

In order to achieve sufficient time resolution for the tensile test, a high intensity x-ray beam was needed. Thus synchrotron radiation was used. Measurements were carried out at beamline A2 of HASYLAB, Hamburg. Samples were stretched with a constant rate of $2*10^{-4}$ mm/s and diffraction patterns were measured simultaneously using a MAR CCD detector. The exposure time was either 24 or 34 seconds per sample, depending on the scattered intensity. When the dead time of the detector is taken into account, the time resolution of the tests was 30 or 40 seconds, respectively.

The results from four samples, two from hemp core and two from hemp bark are presented. All of these exhibited a clear fracture point (catastrophic failure) in the load/strain curves. Diffraction patterns recorded before stretching and right before fracture were compared and from these patterns the reflections *004* and *200* of cellulose were investigated. The reflection *004* gives information on the dimension of the cellulose unit cell parallel to the cellulose chains, whereas the reflection *200* gives dimensional information perpendicular to the chains. The intensities of the reflections became lower as a consequence of the applied strain, but the effect was not uniform in magnitude in the samples. Changes in the shapes of the reflections occurred as well, but the changes were not unambiguous among the samples. The reflection *004* shifted towards smaller and *200* towards larger scattering angles upon stretching, this indicates changes in the dimensions of the unit cell. In the direction of the cellulose chains the unit cell elongated, while perpendicular to chain direction the unit cell became thinner. A similar type of shift of the reflection *004* towards smaller scattering angles has been reported earlier on thin foils of wood [2].

There exists a clear difference in the tensile behaviour of hemp bark compared to the core, as can be seen from the table below. Hemp bark is much more rigid and can withstand twice as much elongation as the core before fracture, also the cellulose unit cell becomes more elongated and thinner in the bark than in the core. The differences in tensile behaviour most likely stem from the structural differences (crystallinity, MFA, crystallite size) between the two regions. Further analysis of the intensity profiles of both reflections and the load/strain curves is in progress.

Sample	Tensile strength (TS) [MPa]	Elongation of sample at TS [%]	Elongation of cellulose unit cell at TS [%]	Thinning of cellulose unit cell at TS [%]
Core 1	84	2.3	0.3	0.1
Core 2	82	2.5	0.1	0.2
Bark 1	426	5.1	1.0	0.8
Bark 2	313	4.5	0.7	0.4

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Polymer composites reinforced by natural fibres; the role of fibre surface

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The properties of composites consisting of natural fibres in a polymer matrix (e.g. polypropylene, PLLA) have been studied. Different types of fibres were studied: dew- and water-retted flax, cotton, and mechanical pulp fibres. A thorough analysis of especially the surface characteristics of the fibres was carried out. The surface chemical composition was determined by the survey and high-resolution elemental XPS-spectra. Further, molecular information and the distribution of the components on the fibre surface was provided by imaging ToF-SIMS. The morphology was studied by SEM and AFM.

The SEM results obtained for the composites revealed clear differences in compatibility between the fibres and the polymer matrix. A correlation was also observed between the mechanical tensile properties (strength, modulus, etc.) and the surface chemical composition (XPS data) of the fibres. It was concluded that the surface structure of the fibres does influence the mechanical strength of the composite.

IR analysis of lignin in Norway spruce (*Picea abies* [L.] Karst.)

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The purpose of our study is to investigate the variation of the amount and polymer structure of lignin in Norway spruce clones and in individual trees in natural stands. In addition, the effect of changes in lignin structure and amount in wood on durability, UV-tolerance and mechanical properties of the wood material will be studied. The structure of lignin can be altered and weaker lignin structures produced, which is important for the removal of lignin by environmentally hazardous chemicals in pulping. On the other hand, lignin structures could be strengthened, resulting in more durable timber and wood products, which may be more resistant to fungal attacks.

Lignin modifications include enzymatic and microwave treatments and lignin monomer impregnation (coniferyl alcohol). Determination of the amount of lignin and its characterisation will be done with micro-Klason, thioacidolysis, UV-microscopy of thin wood sections and FTIR/NIR-analysis.

Degradation of the lignocellulose complex in wood was studied by using a white-rot fungus *(Coriolus versicolor*) which is able to degrade all cell wall components including lignin; and brown-rot fungus (*Poria placenta*) which breaks down polysaccharides but by which lignin degradation is limited. The amount of lignin was analysed by micro-Klason and the samples were further used in the calibration of FTIR and NIR analyses. A baseline shift in IR spectra during the time of exposure to *Poria placenta* could be clearly observed.

The association between a Fourier transformed mid infrared (FTIR) spectrum and the amount of lignin in a sample was modelled by principal component regression (PCR). In this method, the spectral information is encapsulated in a small number of principal components, which are then used as the explanatory variables in a linear model with the amount of lignin as the response variable. Preliminary results suggest that it be possible to come up with very accurate models with good prediction capability. Similar calibrating models will be built for predicting the amount of cellulose and carbohydrates in a sample.

Microscopical and cytochemical observation on hemp stems with emphasis on fibres

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Fibres in hemp (*Cannabis sativa L.*) are long cellulose rich fibres with potential as reinforcing fibres in high strength composites. The fibres should be used as the main reinforcing component. In order to characterise strength and binding between the fibres and the matrix material, the distribution of lignin and pectin in unretted hemp fibres was investigated by cytochemistry and scanning electron microscopy. The Wiesner reaction was applied to stain for lignin followed by image analysis to observe the lignin distribution within the stem section investigated. Pectin was stained using ruthenium red.

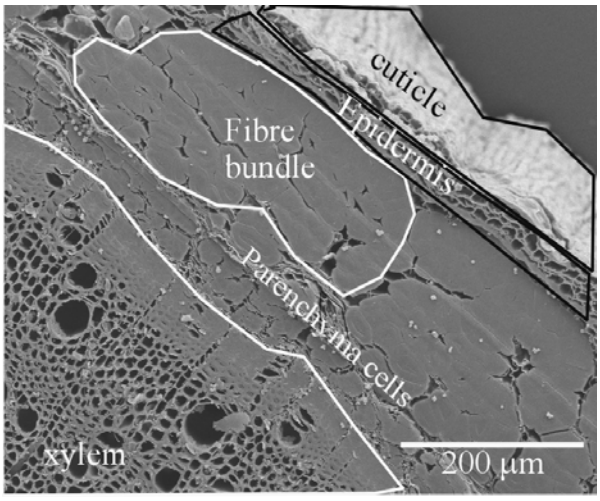

Figure 1. Scanning electron microscopy image of a hemp stem section, showing the xylem, parenchyma cells, fibre bundles, epidermis and cuticle from the centre of the stem outward.

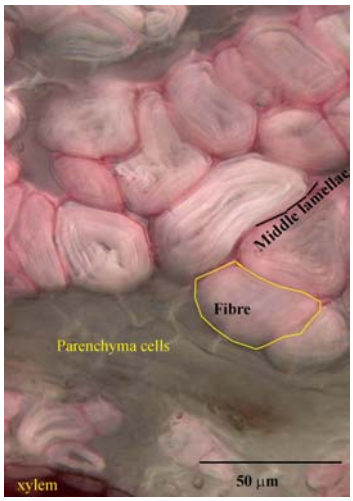

Figure 2. Light microscope image: fibre bundle, parenchyma cells and xylem after the Wiesner reaction. Lignin is stained dark red.

The stem section consisted of xylem, surrounded by parenchyma cells, fibre bundles and epidermis (Figure 1). The xylem was stained dark red and had a uniform colour and normally accepted to contain 15-20 % lignin (Figure 2). The fibre cells had no visible lumen and were tightly packed. The fibre bundles formed a 0.2 mm thick layer between the xylem and the epidermis, consisting of 5-40 fibres separated by middle lamellae. A single fibre secondary wall consisted of 1-4 major concentric layers of which the outer layer was most reactive to the Wiesner stain for lignin (Figure 2).

A correlation between lignin content and Wiesner staining intensity was made based on the uniform xylem staining, by light microscopy and image analysis and using the accepted value of 15-20 % lignin in the xylem as calibration. The parenchyma cells were not stained and therefore contained no lignin. The fibre compound middle lamella was almost as stained as the xylem and estimated to contain 10-18 % lignin. The fibre cell walls contained 1-3 % lignin. Pectin was present in the parenchyma cells and in the compound middle lamellae between the fibres, based on its reactivity with ruthenium red.

Quantification of dislocations in hemp fibres

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Dislocations are local misalignments of cellulose microfibrils in the cell wall of natural fibres such as flax or hemp. Dislocations are formed as a result of subjecting a fibre to compression stress in the longitudinal direction – either already in the living plant due to for example wind load or growth stress, or during processing. When flax fibres have many dislocations their tensile strength is reduced compared to less damaged fibres, and for composites reinforced with hemp fibres, dislocations have been found to be sites for crack initiation and/or debonding between the fibre and the surrounding matrix. A method for the quantification of the amount of dislocations in natural fibres would therefore be a valuable tool, both when evaluating different fibre resources, and when evaluating the damaging effect of a given processing step.

If light microscopy is combined with two crossed polarization filters, the fibre itself can be made to appear dark, while dislocations can be made bright by rotating the object stage. Polarized light microscopy has been used for decades to visualize dislocations in wood fibres and a number of studies have employed manual counting of the dislocations made visible in this way as a means of quantification.

In the present study we have developed a semi-automatic method for registration of the proportional dislocation area in hemp fibres as seen using polarised light microscopy. The method is based on digital images and uses simple image analysis tools. Two images per fibre are needed: One where the fibre edges are made to light up, and one where only dislocations are seen. The former is used for determination of the fibre area, the latter for determination of the dislocation area. The dislocation area relative to the fibre area is the proportional dislocation area. The light intensity of the microscope and the fibre angle relative to the first polarization filter turned out to be important for the results, and strict standardization was consequently found to be vital to the method.

X-ray micro densitometry and microscopical analysis of compression wood in relation to an image analysis method

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When quantifying the extent and grade of compression wood in stem cross sections, it is important that the method used is rapid and accurate. In this study, x-ray microdensitometry, and fluorescence microscopy were used for verification of an image analysis method [1]. The content of severe and mild compression wood in six discs from two Norway spruce trees, were analysed using an image analysis software. X-ray microdensitometry measurements were conducted along radii of the discs in order to study density profiles for different grades of compression wood (Fig.1.) [2]. From the discs, 30 samples, 10x10x20 mm, containing normal wood and different grades of compression wood were chosen for the microscopy analysis. Features typical for compression wood tracheids such as roundness, cell wall thickness, the presence or absence of intercellular spaces and lignin distribution were used to distinguish between normal wood and severe and mild compression wood (Fig.2.). The lignin redistribution was studied using fluorescence microscopy and the auto fluorescence of lignin. The microscopy studies and X-ray microdensitometry generally confirmed the image analysis estimations of presence and grade of compression wood. However within most rings containing compression wood, the compression wood features followed a gradient within the ring. Also the ring density of severe compression followed a gradient as it, within the same ring, decreased from 0.93 at stem base to 0.75 at 2.4 m height. According to our results image analysis is a reliable method for detection of compression wood in Norway spruce.

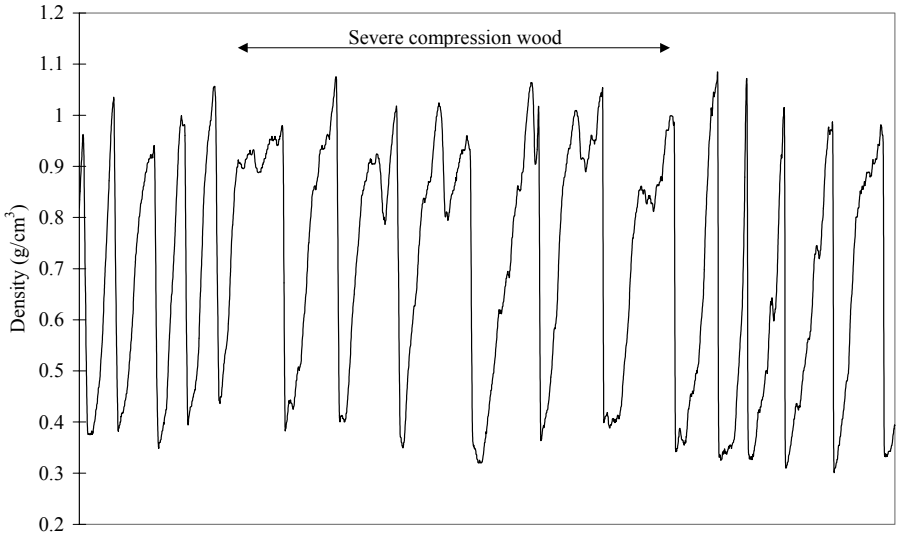

Fig. 1. Density profile showing rings with severe compression wood

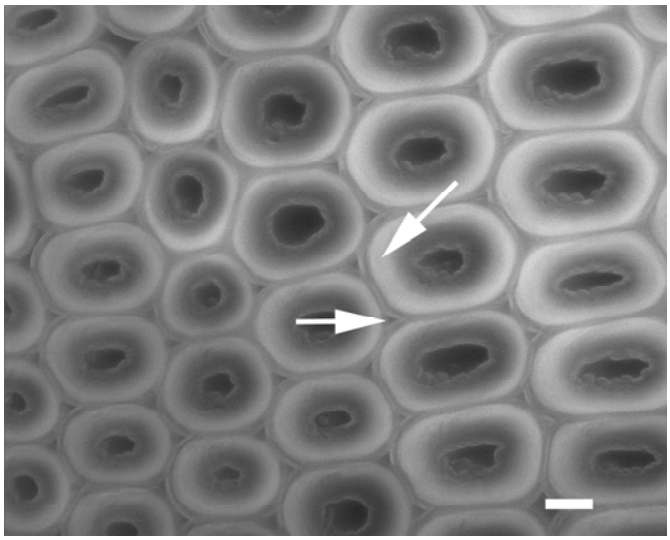

Fig. 2. Transverse section of severe compression wood. The outer part of the middle layer of the secondary cell wall (i.e. S2L) appears bright due to the high lignin content (upper right arrow). The tracheids have a rounded outline and intercellular spaces are present (lower left arrow). Scale bar 10µm.

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A small-angle x-ray scattering study on the morphology of kraft lignin

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Kraft pulping is one of the major pulping processes, thus the efficiency of the process is of great importance. The control of the redeposition of lignin onto cellulose fibres during all stages of pulping process has become an important issue for the overall pulping process efficiency and the papermaking quality of the fibres, in particular, with the introduction of the displacement batch cooking and the water circle closure.

In this study the morphology of kraft lignin in aqueous solutions and in the solid state were determined. The lignin solutions were prepared by dissolving dry pure kraft lignin in 0.1 M NaOH. Ionic strength and pH were adjusted to levels of 0.1 M NaCl and 7, respectively, by adding NaCl and HCl. The shape of the kraft lignin particles in the solution was determined to be more prolate than oblate in contradiction to previous studies with pulsed field gradient NMR [1]. The study of dry lignin aggregates supports the studies according to which fractionated kraft lignin in aqueous solution tends to aggregate as a fractal network from 100 nm up to 1 – 2 μm scale [2]. According to our study the smallest unit of the fractal was determined to be about 6 nm or more in size, while the basic units in the solutions had a maximum diameter ranging from 5 to 10 nm.

Cationic starches (CS) are widely used in papermaking to improve the dry strength, retention and dewatering. To gain a more fundamental understanding of lignin/CS complexes' adsorption onto cellulose fibres, we studied the morphology of lignin/CS complexes by small-angle x-ray scattering both as dissolved in a solution and as centrifuged samples of the solution.

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