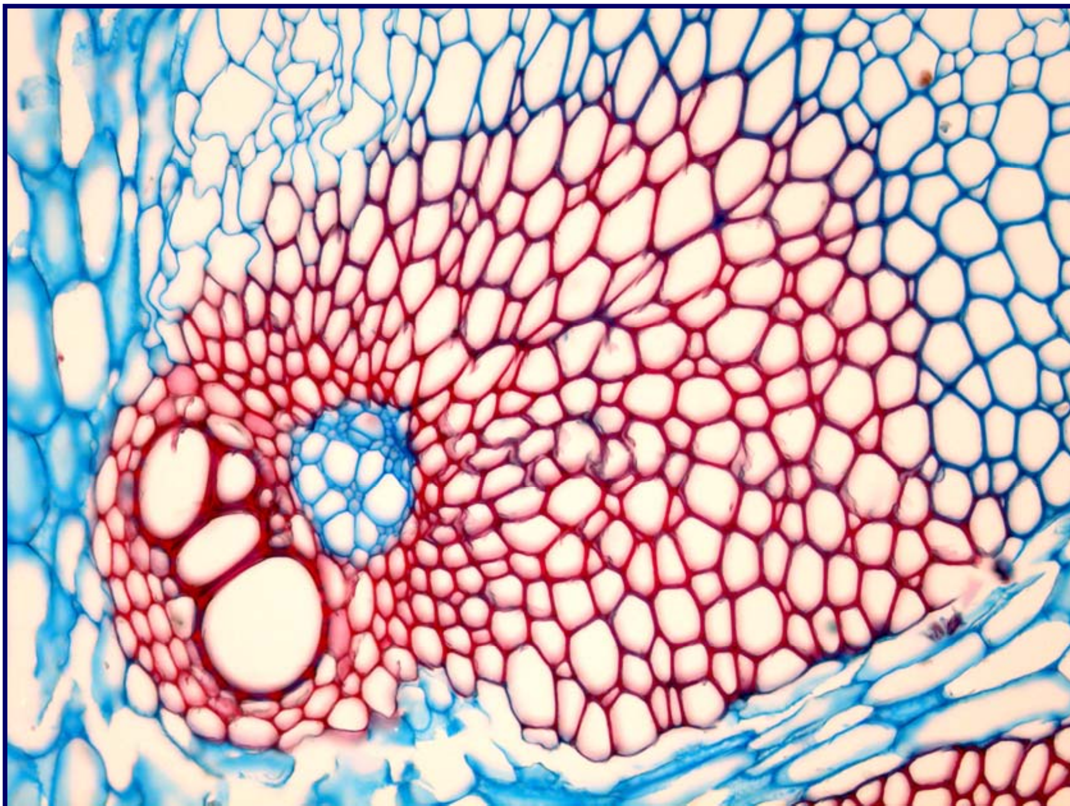
COST ACTION E50 CELL WALL MACROMOLECULES AND REACTION WOOD (CEMARE)

Workshop Potsdam, September 26th-28th, 2007

Structure and function of primary and secondary cell walls



Vascular bundle of a palm tree; courtesy of Markus Rüggeberg

Identification of cell wall mutants by a hydrolase screen

Markus Pauly, Sascha Gille, Ulrike Haensel and Mark Ziemann

MSU-DOE Plant Research Lab, Michigan State University, East Lansing, USA

The current goal of our research is to establish a collection of plant mutants with defined alterations in their wall structures. Such a collection of mutants would enable the establishment of correlations between wall structural features and e.g. mechanical properties of the wall. One focus are hemicelluloses, as they bind to cellulose microfibrils forming the major loadbearing network in plants.

One approach that we have developed to identify hemicellulose mutants takes advantage of growing seedlings in liquid culture. A specific hemicellulase is added to the culture medium, which has a strong effect on plant morphology. Therefore, when mutagenized seed populations are subjected to these growth conditions, mutants can easily be identified by their display of morphological differences when compared to the wild type grown under the same conditions. After selection of the mutants their walls are analysed in detail with particular emphasis on their hemicellulose structure. This approach has yielded a number of defined hemicellulose mutants. Identification of the mutated genes gives valuable insights into the mechanisms of hemicellulose biosynthesis, metabolism, and its regulation. Furthermore, this mutant collection represents a unique resource for the determination of structural/mechanical correlations.

Analysis of mutants with altered xyloglucan (axy) structures using Oligosaccharide Mass Profiling (OLIMP)

Lutz Neumetzler[1], Nicolai Obel[3], Kazuchika Yamauchi[2], Ingo Burgert[2], and Markus Pauly[1,4]

[1]*Max-Planck-Institute of Molecular Plant Physiology, Golm, Germany*
[2]*Max-Planck-Institute of Colloids and Interfaces, Department of Biomaterials, Golm, Germany*
[3]*European Patent Office, Munich*
[4]*Plant Research Laboratory, Michigan State University, East Lansing, USA*

Xyloglucan (XyG) is with approximately 25% the load bearing hemicellulose in the primary cell wall of dicots. According to the most accepted wall models XyG interlace cellulose microfibrils and keep them in spatial arrangement by tightening the network via hydrogen bonds as well as preventing cellulose microfibrils to adhere. Structural features of the side-chains and their distribution along the polymer influence physical properties of the wall and may alter corresponding biological functions during development. XyG is known to be targeted by cell wall loosening enzymes which are involved in cell wall remodelling. Variations in distribution of particular xyloglucan oligosaccharide (XyGO) and their ratios may play a role in biological processes such as cell expansion.

We used a semi-automated forward genetic approach (OLIMP, Lerouxel *et al*. 2002) to identify Arabidopsis thaliana mutants with altered XyG structures. So far, 48 mutants have been identified containing XyG with different degrees of substitution including patterns of O-acetylation as well as novel hitherto unknown structures. In order to elucidate the functional impact of alterations on cell walls we applied different techniques such as cell wall sugar analysis, test of mechanical properties using a microtensile device and immuno-labeling of various cell wall epitopes.

Lerouxel O, Choo TS, Seveno M, Usadel B, Faye L, Lerouge P, Pauly M (2002) Rapid structural phenotyping of plant cell wall mutants by enzymatic oligosaccharide fingerprinting. Plant Physiol 130: 1754-1763

Rotating microtubules and cellulose synthesis

Jordi Chan, Grant Calder, Samantha Fox, and Clive Lloyd

Department of Cell and Developmental Biology, John Innes Centre, Colney lane, Norwich, NR4 7UH, UK

The direction of plant cell expansion is thought to be controlled by the alignment of cellulose microfibrils in the cell wall with microtubules providing a spatial template for the cellulose synthesizing machinery. By growing Arabidopsis seedlings in microscopy chambers constructed using a breathable membrane layer we have been able to conduct long term time-lapse microscopy imaging of hypocotyl elongation. I will show that contrary to the 'text book' view of cell elongation, where microtubules exist in configurations transverse to the axis of elongation, we find that microtubules in hypocotyl cells undergo previously unseen clockwise or counter-clockwise rotary movements. Mapping the polarity of microtubules using the Arabidopsis microtubule-end binding protein, AtEB1a-GFP, reveals that rotation is driven by the slow plus-end migration of domains of polarised microtubules around the circumference of the cell. We show that cellulose synthase (CesA3/6-GFP) exhibits similar movements in living cells and matches the orientation of cellulose microfibrils along the inner cell wall of hypocotyl cells by FESEM. Rotary movements could explain how the angle of cellulose microfibrils can change from layer to layer in the polylamellate cell wall.

Pattern formation of cellulose microfibrils and cortical microtubules

Jelmer Lindeboom and Anne Mie Emons

Wageningen University, Laboratory of Plant Cell Biology, Arboretumlaan 4, 6703 BD Wageningen

In intercalary growing plant cells, microtubules and cellulose microfibrils have been observed to be in the same orientation. This fact has led to speculation of an interaction between cortical microtubules and cellulose synthase complexes. However, there is also experimental evidence which shows that an ordered pattern of cortical microtubules is not essential for an ordered cellulose microfibril pattern. For instance, in the absence of cortical microtubules cellulose synthases move in highly ordered patterns, and ordered textures of cellulose microfibrils are being produced without apparent interference of cortical microtubules.

In our research we assess the patterns in orientation of the fluorescently labelled cellulose synthases and microtubules at the same time in time lapse imaging. In addition, we look at the insertion and activation of cellulose synthases to establish if there are temporal and spatial patterns that control the formation of different patterns of cellulose microfibrils. By comparing both cellulose microfibril pattern formation, i.e. the movement of the cellulose synthases inside the plasma membrane, and cortical microtubule ordering we examine the existence and nature of the interaction between the two for different cell types and developmental stages. We will discuss the movement of cellulose synthases in the plasma membrane of root epidermal cells and the ordering of the cortical microtubule array from both a theoretical and experimental point of view.

Emergence and stability of textures of cellulose microfibrils in the plant cell wall

Kostya Shundyak[1] and Bela Mulder[1,2]

[1] *FOM Institute for Atomic and Molecular Physics (AMOLF), P.O.Box 41883, 1009 DB Amsterdam, The Netherlands*

[2] *Plant Cell Biology, Wageningen University, Arboretumlaan 4, 6703 BD Wageningen, The Netherlands*

Spatially regular textures of cellulose microfibrils (CMFs) in plant cell walls appear as the result of the collective space-time evolution of the cellulose synthesizing complexes in the plasma membrane. Many of the observed stationary textures are captured by the phenomenological geometrical model (Emons and Mulder 1998), based on the a posteriori assumptions of the optimal packing of CMFs and activated dynamics of the synthesizing complexes. We discuss how this "optimal packing" rule can emerge from the dynamics of interacting cellulose synthase complexes, which are propelled by the forces associated with the polymerization of cellulose, and explore several stochastic extensions of the previous model, which allows to estimate robustness of the constructed solutions.

Emons AMC, Mulder B (1998) PNAS 95, 7215

Microfibril width and structure in cellulose from primary walls of celery collenchyma and secondary walls of spruce wood

Andrew Parkin, Adriana Šturcová, J. Paul McLean, Clemens Altaner and Michael C. Jarvis

Chemistry Department, Glasgow University, Glasgow G12 8QQ, Scotland, UK

Native cellulose chains aggregate laterally to form microfibrils several nanometers thick and some microns in length. Cellulose microfibrils from wood and from the primary (growing) cell walls of plants are synthesised by different sets of CESA gene products. Primary-wall cellulose is generally considered to be less crystalline than wood cellulose but it has not been clear whether its microfibrils are similar or different in diameter, i.e. whether wood microfibrils and primary-wall microfibrils contain the same number of chains. We compared the wide-angle X-ray scattering (WAXS) patterns from Sitka spruce earlywood and from celery collenchyma cell walls, which contain cellulose microfibrils representative of softwood and primary-wall celluloses respectively but with highly uniform, axial orientation. Both materials were examined intact to avoid aggregation during isolation of cellulose, and identical conditions were used for collection and analysis of the data. The background correction for non-oriented matrix components of each material was based on the rotational minimum of the intensity at each value of 2theta. The width of the [200] reflection corresponding to the intersheet spacing in the crystal structure was used, through the Scherrer equation, to calculate the lateral dimensions of the microfibrils in the intersheet direction. This [200] Scherrer dimension was 2.68 + 0.07 nm for spruce earlywood cellulose and 2.59 + 0.11 nm for celery collenchyma cellulose. The absolute values were sensitive to the method used for background correction but it was concluded that the Scherrer dimension did not differ significantly between the two sources of cellulose. Assuming that the shape is similar, this implies that they were similar in diameter and in the number of chains that they contained. However there were clear differences in other features of the WAXS patterns. In that of celery collenchyma cellulose the [110] and [1-10] reflections merged and the [200] spacing was greater than for spruce earlywood cellulose, features characteristic of primary-wall celluloses that have been classed as ‘cellulose IV’. This primary-wall cellulose therefore differed more than did wood cellulose from the chain packing arrangements of crystalline cellulose Ibeta and Ialpha, without any evident difference in microfibril dimensions.

Formation and Structure of Lignin in Tree Xylem

Kazuhiko Fukushima

Graduate School of Bioagricultural Sciences, Nagoya University, Nagoya 464-8601, Japan

Lignin is mainly composed of three structual units, *p*-hydroxyphenyl (H), guaiacyl (G) and syringyl (S) moieties. These composition in lignin varies with the kind of species, tissues, cells and wall layers. The S/G ratio has the great effect on the quality of lignin, therefore many attempts to alter this ratio by genetic engeneering have been done. However, the manipulation of genes encoding enzymes of the conventional lignin pathway established by Higuchi (1990) has generated unexpected results to re-evaluate lignin biosynthesis. Therefore, our understanding of lignin biosynthesis has rapidly progressed in the last twenty years.

Many approaches have been employed to elucidate the structure of wood lignin. Most studies dealt with isolated lignins and utilized a number of different physical and chemical degradative methods. These methods provided important basic information on the structure of isolated lignin. However, these have afforded only limited information on the topochemical nature of lignin. Therefore the structure of protolignin in the cell wall is still an open question. Lignin is heterogeneous with respect to its macromolecular structure, morphological location and association with carbohydrates in its native state. This information is lost during its isolation from the cell wall. It is impossible to obtain a lignin sample which can be unambiguously considered to represent whole protolignin. In addition, it is impossible to depolymerize lignin quantitatively into monomeric or oligomeric building units by any known degradative methods. Therefore, it is very difficult to investigate the macromolecular structure of protolignin in the cell wall.

More detailed information about the macromolecular structure of protolignin can only be obtained by suitable non-degradative analyses, such as ultraviolet (UV) microscopic photometry (Fergus and Goring 1970ab, Musha and Goring 1975), bromination-SEM- and TEM-EDXA (Saka and Goring 1985) , Raman spectroscopy (Atalla and Agarwal 1985), and ToF-SIMS (Saito et al. 2005, 2006).

Gymnosperm lignin is believed to consist of almost G units. However, there are significant differences in reactivity and physical properties of protolignins found in the compound middle lamella and the secondary wall. According to the chemical characterization of tissue fractions corresponding to compound middle lamella and secondary wall of black spruce tracheids by Whiting and Goring (1982), the secondary wall lignin contains 1.7 times as much methoxyl per C_9 as the compound middle lamella lignin, indicating a substantial proportion of *p*-hydroxyphenylpropane moieties in the compound middle lamella. On the other hand, the compound middle lamella fraction of spruce normal wood gave only a small amount of *p*-hydroxybenzaldehyde by alkaline nitrobenzene oxidation (Westermark 1985). As H lignin seems to be highly condensed, it can not be estimated quantitatively by degradative methods (Faix and Schweers 1975, Terashima 1989). However, interestingly, most compression wood in gymnosperms gave considerable amount of *p*-hydroxybenzaldehyde by the oxidation.

Microautoradiographic studies strongly suggested the existence of this type of lignin in the compound middle lamella even in the normal wood, deposited in the early stage of cell wall differentiation both in hardwoods and in softwoods (Terashima and Fukushima 1988, Fukushima and Terashima 1990, 1991a, 1991b).

Hardwood lignins are considered to consist mainly of S units and G units, and the ratios of S to G units vary in different morphological regions of woody tissues. In Angiosperm xylem (*Betula papyrifera* Marsh.), the predominant accumulation of G units in vessel secondary walls, in cell corners and in middle lamellas has been generally confirmed with the corresponding accumulation of S units in fibre and ray cell walls (Fergus and Goring 1970ab, Musha and Goring 1975). This tendency was also shown by bromination-SEM, TEM-EDXA (Saka and Goring 1985), and by chemical characterization of various tissue fractions (Cho *et al.* 1980, Hardell *et al.* 1980, Eon *et al.* 1987, 1988). The structural differences of lignin between primary xylem and secondary xylem were also indicated in poplar (Fukushima *et al.* 1994). The proportion of cells rich in S lignin tended to increase at the location most distant from vessel elements, from the early wood vessels to the libriform wood fibers (Yoshinaga *et al.* 1992, 1997).

It is thus enough here to strongly underline that subcellular patterns of cell walls lignification change according the types of tissues, cells and cell wall layers. It is well accounted for in the comprehensive models of ultrastructure and formation of lignified plant cell wall by Takabe *et al.* (1989), Terashima *et al.* (1993), which emphasize the successive 'intercellular'- and 'intracellular'- space and time regulated lignin deposition in different cell walls. In the case of xylem, in 'normal' wood, lignification is initiated at cell corners then proceeding within the middle lamella compound, lignification proceed 'centripetally' through the secondary wall (Terashima and Fukushima 1989).

References

Atalla RH, Agarwal UP (1985) Science 227: 636-638

Cho NS, Lee LY, Meshitsuka G and Nakano J (1980) Mokuzai Gakkaishi 26: 527-533

Eon TJ, Mehitsuka G, Ishizu A and Nakano J (1988) Cell. Chem. Technol. 22: 211-219.

Eon TJ, Meshitsuka G and Nakano J (1987) Mokuzai Gakkaishi 33: 576-581

Faix VO and Schweers W (1975) Holzforschung 29: 48-55

Fergus BJ and Goring DAI (1970a) Holzforschung 24: 113-117

Fergus BJ and Goring DAI (1970b) Holzforschung 24: 118-124

Fukushima K and Terashima N (1990) J. Wood Chem. Technol. 10: 413-433

Fukushima K and Terashima N (1991a) Holzforschung 45: 87-94

Fukushima K and Terashima N (1991b) Wood Sci. Technol. 25: 371-381

Fukushima K, Imai T and Terashima N (1994) Mokuzai Gakkaishi 40: 958-965.

Hardell H-L, Leary GJ, Stoll M and Westermark U (1980) Svensk Papperstidn. 83: 71-74

Higuchi T (1990) Wood Sci Technol. 24: 23-63

Musha Y and Goring DAI (1975) Wood Sci. Technol. 9: 45-58

Saito K, Kato T, Tsuji Y and Fukushima K (2005) Biomacromolecules 6: 678-683

Saito K, Kato T, Takamori H, Kishimoto T and Fukushima K (2005) Biomacromolecules 6: 2688-2696

Saka S and Goring DAI (1985) Biosynthesis and Biodegradation of Wood Components, T Higuchi (ed) Academic Press Inc., New York, pp. 51-62

Takabe K, Fukuzawa K and Harada H (1989) Plant Cell Wall polymers, N Lewis, MG Paice (eds.) ACS Symp.Ser. n° 399, pp. 47-65 , Washington, ACS

Terashima N, Fukushima K, Tsuchiya S and Takabe K (1986) J. Wood Sci. Technol. 6: 495-504

Terashima N (1989) Plant Cell Wall Polymers, N Lewis, MG Paice (eds.) ACS Symposium Series 399, pp.148-159. American Chemical Society, Washington, DC.

Terashima N and Fukushima K (1988) Wood Sci. Technol. 22: 259-270

Terashima N and Fukushima K (1989) Plant Cell Wall Polymers, Biogenesis and Biodegradation, N Lewis, MG Paice (eds.) ACS Symp. Ser. 399, pp. 160-168 Washington, ACS

Terashima N, Fukushima K, He L-F and Takabe K (1993) Forage Cell Wall Structure and Digestibility, HG Jung, H.G. *et al*. (eds.) pp. 247-270, ASA-CSSA-SSSA.

Westermark U (1985) Wood Sci. Technol. 19: 223-232

Whiting P and Goring DAI (1982) Wood Sci. Technol. 16: 261-267

Yoshinaga A, Fujita M and Saiki H (1992) Mokuzai Gakkaishi 38: 629-637

Yoshianga A, Fujita M and Saiki H (1997) Mokuzai Gakkaishi 43: 384-390

Lignification and class III peroxidases of Norway spruce (*Picea abies*)

Kurt Fagerstedt[1], Kaisa Marjamaa[1], Eija Kukkola[1], Sanna Koutaniemi[3], Tino Warinowski[3], Teemu H. Teeri[3], Heidi Holkeri[4] and Taina Lundell[2]

[1]*Department of Biological and Environmental Sciences, University of Helsinki, P.O. Box 65, 00014UH, Finland;*
[2] *Department of Applied Chemistry and Microbiology, University of Helsinki, P.O. Box 56, 00014UH, Finland;*
[3]*Department of Applied Biology, University of Helsinki, P.O. Box 27, 00014UH, Finland;*
[4] *VTT Technical Research Centre of Finland, P.O. Box 1000, 02044 VTT, Finland;*

Class III plant peroxidases (POX) are heme-containing enzymes, which exist as large gene families and catalyze oxidoreduction between hydrogen peroxide and various phenolic substrates in plants. POXs are found from cell walls and vacuoles and they are implicated in many biological processes: e. g. defence, auxin catabolism, suberin synthesis and the oxidative polymerization of monolignols in lignin biosynthesis. Our group is studying participation of POX to lignin polymerization in Norway spruce (*Picea abies*). Over 12 peroxidase isoforms are found in protein extracts of developing stem xylem of Norway spruce (Marjamaa *et al*. 2003). Three peroxidase cDNAs, *px1*, *px2* and *px3*, were cloned RNA extracted from developing xylem of approximately 30-year old spruce using RT-PCR method and primers designed for conserved regions of known peroxidases. The translated amino acid sequences of these novel peroxidases share 40-60% sequence similarity and their isoelectric points vary from 9.43-5.15 (Marjamaa *et al*. 2006b). Localization of *px1*, *px2* and *px3* expression was studied using *in situ* hybridization for spruce seedlings, which showed that both *px1* and *px2* are expressed in lignifying tracheids in stems, whereas *px3* transcripts were not detected in spruce seedlings (Marjamaa *et al*. 2006b). Subcellular localization of PX1, PX2 and PX3 was studied by transiently expressing fusion constructs of putative localization directing signal peptides of PX1-3 and green fluorescent protein (GFP) in tobacco protoplasts. In tobacco protoplasts expressing spruce POX N-terminal putative secretion signal peptide-GFP constructs, GFP fluorescence was seen in ER-network like structures (Marjamaa *et al*. 2006b). In tobacco protoplasts expressing SS-GFP-CP constructs, GFP fluorescence was seen in various sheet-like, tubular and net-like structures. In order to study the protein products the novel POX cDNAs, *Catharanthus roseus* hairy root lines expressing *px1* were generated. In protein extracts from hairy roots expressing *px1*, an additional cationic peroxidase enzyme was detected. The isoelectric point of this putative protein product of *px1* was approximately 10, and by comparison to our earlier studies on partially purified spruce xylem peroxidases, it belongs to a group of basic peroxidases which efficiently oxidase coniferyl alcohol (Marjamaa *et al*. 2006a). The probable peroxidases taking part in lignin polymerisation are discussed.

References

Marjamaa K, Lehtonen M, Lundell T, Toikka M and Fagerstedt KV (2003) Developmental lignification and seasonal variation in β-glucosidase and peroxidase activities in xylem of Scots pine, Norway spruce and silver birch. Tree Physiol. 23: 977-986

Marjamaa K, Kukkola E, Lundell T, Karhunen P, Saranpää P and Fagerstedt KV (2006a) Monolignol oxidation by xylem peroxidase isoforms of Norway spruce (*Picea abies*) and silver birch (*Betula pendula*). Tree Physiol. 26: 605–611

Marjamaa K, Hilden K, Kukkola E, Lehtonen M, Holkeri H, Haapaniemi P, Koutaniemi S, Teeri TH, Fagerstedt K and Lundell T (2006b) Cloning, characterization and localization of three novel class III peroxidases in lignifying xylem of Norway spruce (*Picea abies*). Plant Mol. Biol. 61: 719-732

Development of cellulose-based model systems for the study of interactions among wall components

Véronique Aguie, Laurence Foulon, Miyuki Takeuchi, David Cronier, Anouk Habrant, Araik Hambardzumyan, Roger Douillard and Brigitte Chabbert

UMR 614, INRA-URCA Fractionnement des AgroREssources (FARE), Equipe Structures et Propriétés des Parois secondaires, 2 Esplanade R.Garros, BP 224, 51686 Reims, France

In order to describe the structure and the reactivity of secondary plant cell walls and to understand the underlying mechanisms, model approaches based on native cellulose were undertaken. Nanocrystals (whiskers) were obtained by mild hydrolysis of cellulose fibers and used as a suspension to study interactions between amorphous polymers and cellulose. Notably, sorption experiments using hemp glucomannans and poplar proteoglycan were carried out to examine their contribution to the wall network of highly cellulosics fibers, *i.e.* hemp bast fibers and gelatineous fibers that occur in poplar tension wood.

To get an in depth investigation of the interaction between cellulose and other wall macromolecule, model surfaces of native cellulose were prepared from nanocrystals. The cellulose whiskers were found to form stable monolayers at the air/water interface in the presence of an amphiphilic molecule and the film can be transferred onto silicon wafer using the Langmuir-Blodgett technique. In addition, the preparation of oriented films of cellulose similar to the successive cellulose planes of plant cell walls was explored. These films offer an opportunity to investigate at a nanometric scale interfacial properties of plant cell walls such as adsorption of other cell wall macromolecules (hemicelluloses and proteins). These properties were characterized by AFM, ellipsometry and chemical surface analysis.

New views of cell structure and lignin deposition during wood development

Lacey Samuels

Department of Botany, University of British Columbia, Vancouver, Canada

Secondary xylem (wood) formation in gymnosperms requires the tracheid protoplast to first build an elaborate secondary cell wall from polysaccharides, then reinforce it with lignin. The goal of this work was to track the spatial distribution of hemicelluloses and monolignols during development as they move from the symplasm to the apoplasm. For polysaccharides, this was done using immuno-fluorescence and immuno-gold techniques to localize the hemicelluloses during wood development in pine and poplar. For monolignols, this was done by feeding ^{3}H-phenylalanine to dissected cambium/developing wood from *Pinus contorta* var. latifolia seedlings, allowing uptake and metabolism, then rapidly freezing the cells with cryofixation and performing autoradiography to detect the locations of the phenylpropanoids using both light and electron microscopy. Chemical analysis of the developing wood following ^{3}H-phenylalanine incubation showed incorporation of radioactivity into lignin isolated by thioacidolysis as well as into a methanol soluble pool. With light microscopy, radiolabeled phenylpropanoids were detected in the rays as well as tracheids, with the two cell types showing differential sensitivity to inhibitors of protein translation and phenylpropanoid metabolism. At the subcellular level, secondary cell walls of developing tracheids were heavily labeled while cytoplasm and vacuoles of tracheids contained relatively little label. The question of whether monolignols are exported via Golgi-mediated vesicles or membrane-bound transporters is being addressed using quantitative autoradiography, inhibitors of vesicle transport and inhibitors of ABC transporters.

Application of scanning UV microspectrophotometry for the topochemical detection of aromatic compounds in the G-layers of tension wood fibres

Gerald Koch, Christian Lehringer and Uwe Schmitt

Institute for Wood Biology and Wood Protection, Federal Research Centre for Forestry and Forest Products, Leuschnerstr. 91, D-21031 Hamburg, Germany

The fibres of tension wood are characterised by a special wall layer, the so-called gelatinous layer or G-layer. Depending on the species and the intensity of tension stresses, the G-layer may be deposited instead of the S2 or the S3 wall layer or in addition to the normal wall layers. The G-layer consists of concentric lamellae of cellulose microfibrils aligned in direction of the fibre axis. The cellulose is highly crystalline, and the content of hemicelluloses is only a few percent. The occurrence of lignin in the G-layers is controversially discussed and not evidenced until now. In this context, scanning UV-microspectrophotometry was applied for the topochemical detection of aromatic compounds in the G-layers of tension wood fibers in beech, maple, and oak.

UV-scanning profiles recorded at a defined wavelength of 278 nm and a resolution of 0.25 µm x 0.25 µm revealed traceable UV-absorbances within the G-layers of all selected species (Fig. 1). The quantified absorbance values of the G-layers amount only few percent as compared with values for the normally developed S2 layers.

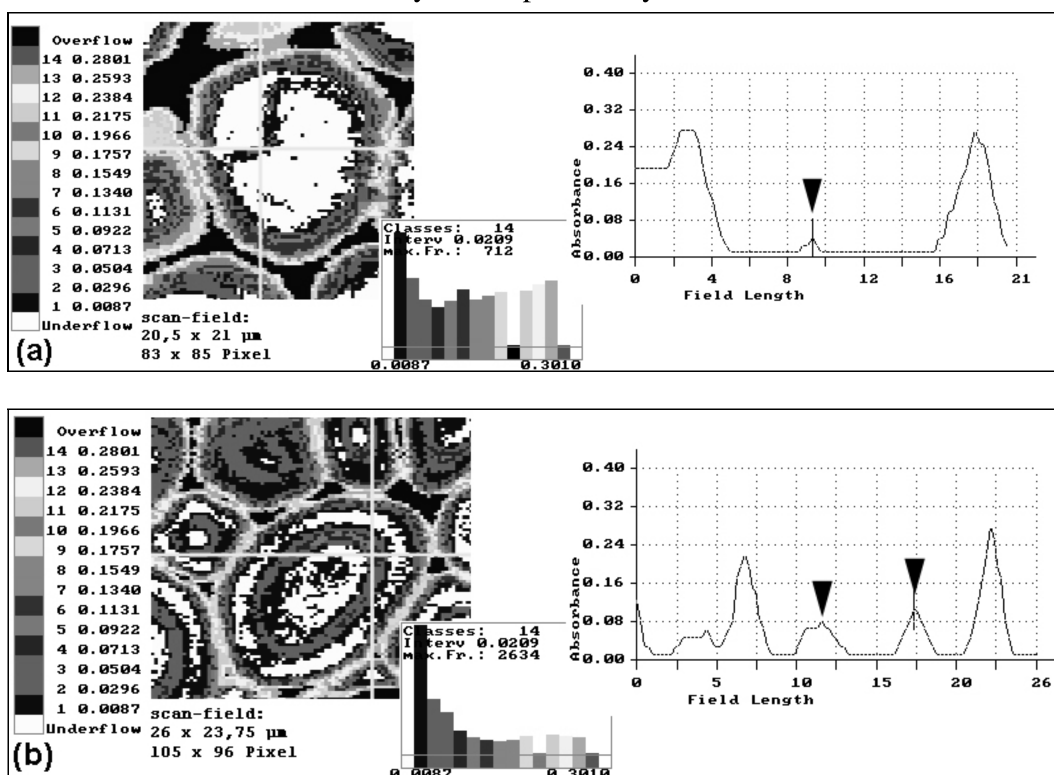


Figure 1. Representative images of UV-microspectrophotometric analysis of tension wood fibres in maple (a) and oak (b). The pixels represent the absorbance intensity at a wavelength of 278 nm. Aromatic compounds in the G-layer can be clearly visualized by line profiles (arrows)

For verification, UV-absorbance spectra in a wavelength range from 240 to 400 nm were recorded for individual G-layers and S2 layers of the selected species with a spot size of 1µm x 1µm. The UV-spectra of the G-layers also show traceable UV absorbances in the wavelength range of 272 to 278 nm, indicating the absorbance behaviour of hardwood lignin. In comparison to the UV-spectra of the S2 layers, the G-layers are characterised by a considerable lower UV-absorbance and a shift of the absorbance maximum towards lower wavelengths (range of 268 to 272 nm). The varying spectral behaviour may be explained by a different chemical composition of the lignin units or the deposition of precursors (phenylpropane-units) of the lignin molecules.

Furthermore, deposits of phenolic extractives are locally detectable in G-layers of oak tension wood by using scanning UV-microspectrophotometry. The evaluation of the corresponding UV-absorbance spectra indicates the occurrence of low molecular phenolics, e.g., catechin derivates which are characterised by a bathochromic shift of the absorbance maximum to a wavelength of 300 nm (Fig. 2). This spectral behaviour can be explained by the presence of chromophoric groups, e.g., conjugated double bonds.

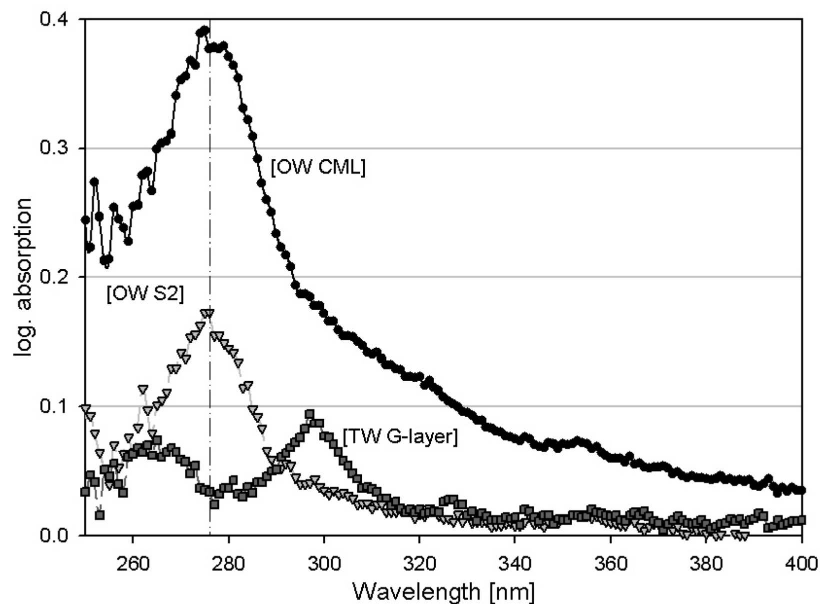


Figure 2. Representative UV-absorbance spectra of individual cell wall layers in oak [opposite wood compound middle lamella (OW CML), opposite wood secondary wall (OW S2) and tension wood gelatinous layer (TW G-layer)]

The current study demonstrates that UV-microspectrophotometry is ideally suited to study the topochemical distribution of lignin and phenolic extractives on a subcellular level. In particular, the application of the scanning technique enables a direct imaging of lignin distribution and provides fundamental information on the topochemistry of G-layers in tension wood fibres.

CO_2-mediated effects on biochemical properties of the cell wall and their influence on mechanical attributes of white Asparagus spears

Susanne Huyskens-Keil[1] und Werner B. Herppich[2]

[1] *Humboldt-Universität zu Berlin, Institute for Horticultural Sciences, Section Quality Dynamics/Postharvest Physiology, Lentzeallee 75, 14195 Berlin, Germany*

[2] *Leibniz-Institute for Agricultural Engineering Potsdam-Bornim e. V., Department of Horticultural Engineering, Max-Eyth-Allee 100, 14469 Potsdam, Germany*

Asparagus (*Asparagus officinalis* L.) is a long-lived, monocotyledonous herbaceous perennial growing from rhizomes. The spears of white asparagus are developmentally immature and rapidly growing subterraneous shoots. After harvest spears retain a high physiological activity leading to a rapid decline of respiratory substrates. On the other hand the unaltered continuation of shoot differentiation leads to a further thickening and increased lignification of the cell walls of the sclerenchyma sheath cells and those of the vascular bundles. The spears become fibrous and tough which is highly undesired in horticulture.

Elevated CO_2-concentrations are known to reduce the metabolic activity of harvested products, and therefore are used as suitable tools for maintaining e.g. textural properties of horticultural commodities. High CO_2-concentrations are assumed to retard respirational processes and hence, inhibit energy supply for cell wall synthesis. There is also evidence that high CO_2 might inhibit key enzymes for the lignin biosynthesis (PAL).

In the present study, high CO_2-mediated effects on chemical cell wall properties (cellulose, hemicellulose, pectic substances, lignin) and mechanical attributes (stiffness and tissue strength) in asparagus spears were investigated during a short-term storage at 10°C and 20°C under elevated CO_2-concentrations (10% CO_2, 17% O_2), and compared with those obtained on spears stored in normal atmosphere.

Respiration activity declined slightly during the entire storage period irrespective of the CO_2- and temperature regime. In addition, the elastic properties and tissue strength were not affected by increased CO_2-concentrations at both storage temperatures. In contrast, high CO_2-concentrations inhibited the degradation of soluble carbohydrates and the synthesis of both cellulose and lignin. The interaction of soluble and structural carbohydrates with biochemical and mechanical cell wall properties will be discussed.

From air-humidity driven actuators in plants to biomimetic micro-devices

Peter Fratzl and Ingo Burgert

Max Planck Institute of Colloids and Interfaces, Potsdam, Germany

Plants are known to have developed a multitude of actuation devices based on water swelling and de-swelling. Wheat awns are one example. As has recently been show, the swelling of the hemicellulose-rich matrix and a clever geometrical arrangement of cellulose fibrils allow these awns to perform swimming movements and to propel the seed along and into the ground (Elbaum *et al*. 2007). The driving force for the movement is just the daily change in humidity from the morning dew to the evening (Elbaum *et al*. 2007). Clever arrangement of cellulose fibrils in the cell wall of soft wood tracheids also seem to be responsible for generating tensile or compressive stresses as needed to correct the direction of branch or stem (Burgert *et al*. 2007). From a materials science point of view, it is quite interesting to note that these actuating movements are directly generated by changes in humidity without the need of an active metabolism for the movement itself, very unlike molecular motors in the human muscle, for example. This makes the active materials in plants particularly interesting for biomimetic materials research (Fratzl 2007). As an example for such a biomimetic active system, a device based on silicon needles joined by a hydrogel was generated (Sidorenko *et al*. 2007). This system is able to perform reversible translations or even microscopic gripping movement, triggered only by changes in air humidity (Sidorenko *et al*. 2007). Based on these first encouraging results, we believe that humidity-based actuation systems in plants are excellent models for the development of biomimetic active materials, which may have a wide range of applications.

Burgert I, Eder M, Gierlinger N, Fratzl P (2007) Tensile and compressive stresses in tracheids are induced by swelling based on geometrical constraints of the wood cell. Planta 226: 981-987

Elbaum R, Zaltzman L, Burgert I, Fratzl P (2007) The role of wheat awns in the seed dispersal unit. Science 316: 884 - 886

Fratzl P (2007) Biomimetic materials research: what can we really learn from nature's structural materials? J R Soc Interface 4: 637 - 642

Sidorenko A, Krupenkin T, Taylor A, Fratzl P, Aizenberg J (2007) Reversible switching of hydrogel-actuated nanostructures into complex micropatterns. Science 315: 487 - 490

Mechanical tests on microsamples of normal and tension wood of Poplar upon radial, tangential and longitudinal directions

Carole Assor, Tuan Dinh, Philippe Jacquin, Gilles Pilate and Patrick Perré

Laboratoire d'Etude et de Recherche sur le Matériau Bois, Equipe Anatomie et Physique du bois, 14, rue Girardet, 54 042 Nancy Cedex

In order to understand the influence of the cell wall structure on wood mechanical properties, mechanical tests were established on micro samples in radial, tangential and longitudinal wood directions.

Micro samples allow, first, to characterize homogeneous wood structure and, secondly, to compare mechanical properties of highly different wood structure. Three kind of samples were isolated from a bended Poplar that presented highly located tension wood: tension, opposite (180°C from the tension wood) and normal wood (90°C from the tension wood).

By longitudinal orientation, creep tests were performed on green and dry wood samples and, by radial and tangential directions, mechanical properties were determined using traction tests on both samples. Owing this direction, mechanical tests were performed in a room allowing relative humidity to be controlled. Relative humidity variations would permit to investigate the effect of wood water content on its mechanical properties (particularly in the case of the tension wood which the *G-layer* is well known to behave differently to the other cell wall layers during wood shrinkage).

The first experimental results, obtained from the longitudinal direction on green samples, showed that the tension wood exhibits a Young modulus (E) two times higher than the opposite and that the normal wood seems intermediate.

The rest of the mechanical tests are going to be carried out. They will be completed by anatomical and biochemical analysis in order to characterize the cell wall structure influence on mechanical properties.

The effect of growth conditions on the amount of dislocations in hemp fibres

Mohammad R. Asgharipour and Lisbeth G. Thygesen

Forest & Landscape Denmark, University of Copenhagen, Rolighedsvej 23, DK-1958 Frederiksberg C, Denmark

Dislocations (a.k.a. slip planes) are regions of the fibre cell wall where the fibril angle differs from the surrounding material. Dislocations may be induced in fibres by applying compressive stress in the longitudinal direction of the fibre. Various post-harvest treatments such as e.g. defibration processes or high-consistency mixing of fibre suspensions may result in the formation of dislocations, but they have also been found in fibres carefully removed from the living plant. The occurance of dislocations in living plants has been ascribed to wind damage (Koch *et al*. 1996), i.e. compression stress in the lee side of the plant stem. Even though this explanation is widely recognized as a fact, no data from a dedicated experiment set up in a controlled environment seem hitherto to have been published. The purpose of the present study was to test the hypothesis that wind introduces dislocations in living plants. A trial where plants were subjected to drought and less abundance of nutrients was also included for comparison.

Results diverge regarding the possible effect(s) of dislocations on the strength properties of plant fibres. Neither Baley (2005), nor Thygesen *et al*. (2007) found any relationship between single fibre tensile stress and the number or amount of dislocations for flax, respectively hemp single fibres. On the other hand, Terziev *et al*. (2005) subjected wood samples to longitudinal compression stress. This treatment introduced dislocations in the fibres, and the paper produced from these stem sections had inferior strength properties compared to paper prepared from untreated wood. In the present study the ultimate tensile stress of fibre bundles was determined in order to get an indication of the effect of growth conditions on the fibre tensile strength.

Hemp plants (*Cannabis sativa* L.) were grown in a controlled environment in a green house and subjected to three different growth conditions: optimal, windy or dry. Plants subjected to the optimal treatment were subjected to neither wind nor drought, and they were watered every day with water containing nutrients. Windy conditions were created by placing fans next to the plant bed from the time that the plant height reached about 30 cm and until harvest. Drought implied that from the time that the plant height reached about 30 cm, the plants were watered only enough to keep them alive, and no nutrients were added to the water.

Stem sections were stored at -18 C for up to about 3 months after harvest. For determination of the amount of dislocations fibres were removed from the stem sections using precision tweezers and about 100 fibre segments per treatment were analysed using Polarized Light Microscopy and image analysis according to the method described in Thygesen and Ander (2005).

In order to get an indication of possible differences between the three different treatments with regard to ultimate tensile stress, about 30 fibre bundles per trial were tested to failure using an Instron testing machine at zero span length. The cross-sectional area of the bundles was estimated from the weight of the bundles and assuming a cell wall density of 1.5 g cm^{-3}.

The amount of dislocations quantified as the percentage of the cell wall is given in Table 1 for all three treatments.

Table 1. The area of the dislocations in per cent of the fibre area as seen in PLM.

Growth conditions	***No. of fibres***	***Rel. dislocation area (%)***	
		mean	std.
Optimal	96	12.0[a]	8.5
Drought	114	21.3[b]	12.3
Wind	98	18.5[b]	12.8

Mean values marked by different letters are significantly different from each other on the 0.1 % level (***).

Qualitatively similar results were obtained when comparing the size of the largest dislocation in each fibre segment, i.e. the largest dislocation in each fibre segment from the optimal treatment was on average significantly smaller than for the two other treatments, which were not significantly different from each other (results not shown). The same trend was found when comparing the average longitudinal distance between dislocations. Distances were significantly longer for the optimal treatment than for the other two treatments, for which the results were not significantly different from each other (results not shown).

The tensile strength measurements showed that the average fibre bundle ultimate tensile stress was higher for the optimal treatment than for the two other treatments, which were not significantly different from each other.

In the present study, windy conditions introduced more dislocations than the wind-free environment, but so did the dry, wind-free conditions. Neither the amount of dislocations, nor their size or the distance between them showed any significant differences between windy and dry conditions. One interpretation of these results could be that it is not the wind itself that induces the dislocations. A hypothesis could be that it is the general stress-level of the plant during the biosynthesis of cellulose that initiates the formation of dislocations. With regard to zero span fibre bundle tensile strength, the results coincide with the dislocation results.

Acknowledgements

MRA thanks the Iranian Ministry of Science for financing his stay at Forest & Landscape Denmark.

References

Baley C (2004) J Mater Sci 39:331

Koch G, Bauch J, Dünisch O, Seehann G, Schmitt U (1996) Holz Roh Werkst 54:243

Terziev N, Geoffrey D, Marklund A (2005) Holzforschung 59:163

Thygesen LG, Eder M, Burgert I (2007) J Mater Sci 42:558

Thygesen LG, Ander P (2005) Nordic Pulp Paper Res J 20: 64

Characteristics of the thigmorphogenetic response in the xylem of F5H over-expressed *Populus tremula* x *P. alba*, Clone 717

Frank W. Telewski[1], Lothar Koehler[2], J. Al-Haddad[2], Kyu-Young Kang[3], Shawn D. Mansfield[3] and Frank W. Ewers[4]

[1]*W.J. Beal Botanical Garden, Department of Plant Biology, Michigan State University, East Lansing, MI, U.S.A.*
[2]*Department of Plant Biology, Michigan State University, East Lansing, MI, U.S.A.*
[3]*Department of Wood Science, University of British Columbia Vancouver, BC, Canada*
[4]*Biological Sciences, California State Polytechnic University, Pomona, CA, U.S.A.*

Wind-induced sway or dynamic mechanical flexing are known to induce alterations in xylogenesis resulting in modifications in the mechanical properties, cellular properties, anatomy, chemistry and morphology of wood in a number of tree species. In a previous study in the conifer, *Abies fraseri*, the term 'flexure wood' was coined to distinguish wood formed in response to a swaying motion from that of 'normal wood' and 'compression wood'. In conifers, flexure wood has characteristics which are intermediate between normal and compression wood, including shorter tracheids, higher wood density, and an increased microfibrilar angle (MFA). In porous wood angiosperms, tension wood is the characteristic reaction wood formed in the gravitropic response and is characterized by fibers containing a gelatinous cell wall component which is higher in glucose content and lower in lignin content. MFA also decreases, approaching 0°. In this study, wild type and C4H::F5H/Cald5H transformed hybrid poplar (*Populus tremula* x *P. alba*, clone 717) were used to explore the effect of simulated wind sway on wood formation and chemical composition. The flexure wood of these hybrids poplar is characterized in this study by no significant change in total lignin, no significant change in sugar content, a significant increase in both syringyl content, and in MFA. Although some gelatinous fibers were observed in the stems of both control and flexed trees, the appearance of these fibers does not appear to significantly alter the total wood chemical composition. However, vessel and fiber lumens are significantly smaller and fiber wall are significantly thicker in flexed trees. In the case of this diffuse porous species, flexure wood appears to share more in common with flexure wood in conifers than with tension wood. The C4H::F5H/Cald5H transformed hybrid poplars exhibit a normal thigmorphogenetic response. However, with the exception of a significant increase in S:G, which translates to an increase in acid-soluble lignin, increased elastic modulus and height-to-diameter ratio, over expression of the C4H::F5H/Cald5H gene does not significantly impact wood anatomical features.

This research was supported by the National Research Initiative of the USDA CSREES grant number 2005-35103-15269.

Modelling approaches to three-dimensional hygroelastic behaviour of compression wood and their tracheids

Kristofer Gamstedt[1, 2] and Stig. L. Bardage[3]

[1] *Department of Fibre and Polymer Technology, Royal Institute of Technology (KTH), Teknikringen 56-58, S-100 44 Stockholm, Sweden*
[2] *STFI-Packforsk AB, Division of Packaging and Logistics, P.O. Box 5604, S-114 86 Stockholm, Sweden*
[3] *Wood Ultrastructure Research Centre, Swedish University of Agricultural Sciences, Department of Wood Science, P.O. Box 7008, S-750 07 Uppsala, Sweden*

The objective of this work is to improve the understanding of hygroelastic properties of compression wood using an analytical model based on three-dimensional composite framework. The influence of the ultrastructure has been analysed with Hashin's (1983) micromechanical model and a three-dimensional helically orthotropic model of concentric cylinders developed by Tarn (2002). The geometry is presented in Figure 1. Comparison with finite element results confirms the accuracy of the analytical approach. Based on input from literature on the lignin content, dimensions and layerwise structure of typical conifer compression wood tracheids, the elastic and hygroexpansion properties of the constrained tracheids (as in wood) or free fibres (as in pulp) was simulated. The influence of the microfibril angle was varied to show salient differences with normal wood. With increasing microfibril angle, it was found that the stiffness in the axial direction decreases, whereas the axial shrinkage and twist increase on drying (cf. Figure 2). These phenomena explain the high degree of warpage and fracture of dried wooden boards containing both compression and normal wood. Furthermore, the results indicate that absence of the S3 layer has little effect on the mechanical properties of compression wood. Analogies are made to failure mechanisms observed in fibre composite laminates, as a quantitative explanation of the optimized shape and ultrastructure of compression wood. Although the literature is relatively scarce on the hygroelastic deformation of compression wood tracheids, similar trends are shown as a result from the composite model, albeit in a quantitative mechanics framework which may be used in simulations for a wide variety of natural fibres.

Analysis of the effects of an uniaxial transverse stress of any axisymmetric structure is a difficult task, and no closed form solution of the transverse Young's modulus can probably therefore be obtained directly. Alternative approaches to estimate the transverse stiffness are therefore discussed. These include self-consistent approaches of a transversely isotropic material with uniform radial stresses, and cellular mechanics where the cell-wall stiffness is determined by classic laminate theory. Homogenization to a material based Cosserat's micropolar elasticity theory is also exemplified to provide a link from the cell-wall ultrastructure to cellular wood material.

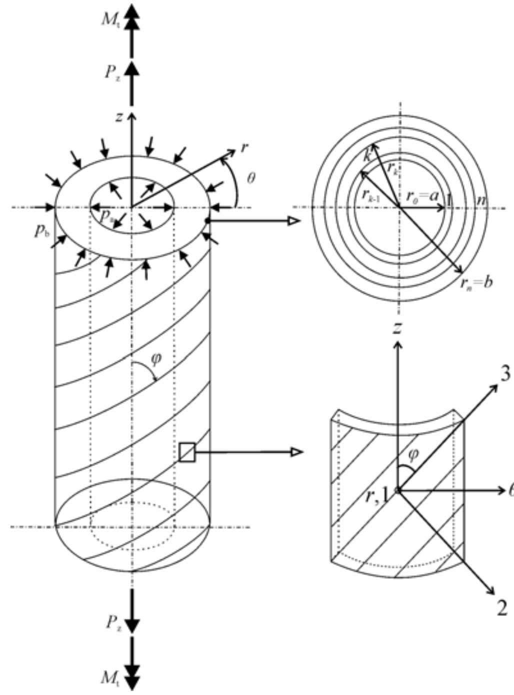


Figure 1. Geometry and loads on the wood fibre composed of coaxial orthotropic cylinders with helical orientation of the main material axes.

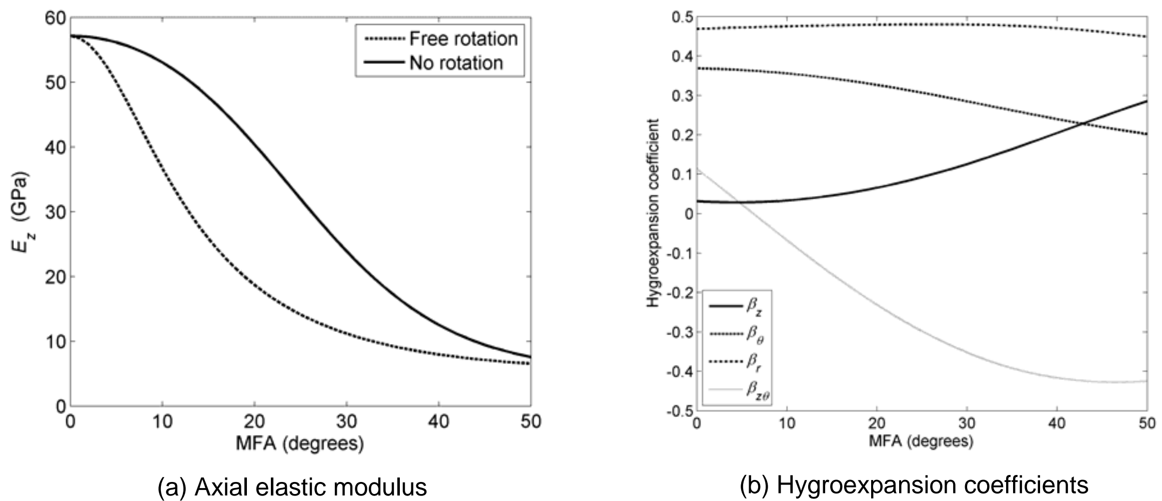


Figure 2. Simulation results of the influence of MFA on (a) the axial stiffness of free and constrained fibres, and on (b) the hygroexpansion coefficients in the cylindrical coordinate system (Neagu and Gamstedt 2007).

Hashin Z (1983) Analysis of composite materials – A survey. Journal of Applied Mechanics 50: 481-505

Neagu RC and Gamstedt EK (2007) Modelling of effects of ultrastructural morphology on the hygroelastic properties of wood fibres. Accepted for publication in J. of Materials Science.

Tarn JQ (2002) A state space formalism for anisotropic elasticity. Part II: Cylindrical anisotropy. International Journal of Solids and Structures 39: 5157-5172

Interaction among polymers in the primary cell wall of Norway spruce (*Picea abies* (L.) Karst.)

Jasna S. Stevanic and Lennart Salmén

STFI-Packforsk, Box 5604, SE-114 86 Stockholm, Sweden

The primary cell-wall is a complex multipolymer system whose composite structure has been mostly determined from chemical and biochemical studies. It is known that lignin can make covalent cross-links with both protein and pectin (Harrak *et al*. 1999). It is also suggested that xyloglucan is hydrogen bound to cellulose and covalently bound to pectin (Bacic *et al*. 1988, Whitney and Gidley 1999). Numerous other studies showed that pectin and protein are linked by ionic bonds and that cellulose and pectin build covalent bonds (Ishii and Shimizu 2001). However, the knowledge about the interactions among the polymers when it comes to mechanical properties is very limited. The physical properties of the polymers, i.e. their elastic and viscous deformations, as well as the ultrastructure of the polymers, i.e. interaction among polymers in the outer fibre wall layers that lead to this behaviour, are still not fully understood.

The aim of this study was to examine how the different wood polymers, viz. lignin, protein, pectin, xyloglucan and cellulose, interact in the outer fibre wall layers of the spruce wood tracheid. The initial objective was to separate an enriched primary cell-wall material from a first stage TMP, by screening and centri-cleaning. From this material, consisting of the primary cell-wall (P) and outer secondary cell-wall (S_1) materials, thin sheets were prepared and analysed using a number of different analytical methods. The major measuring technique used was dynamic FT-IR spectroscopy in combination with dynamic 2D FT-IR spectroscopy. This spectroscopy technique has been shown to be very applicable for studying of the interactions among polymers and their ultrastructural organization in complex materials (Åkerholm *et al*. 2004, Hinterstoisser and Salmen 1999, 2000). The technique is based on the detection of the small changes in molecular absorption that occur due to a sinusoidally stretched sample under low strain. The molecular groups affected by the stretching will respond in a specific way, depending on their environment, while the unaffected molecular groups will provide no response to the dynamic spectra, by giving no elastic or viscous signals. Moreover, the dynamic 2D FT-IR spectroscopy provides useful information about various intermolecular and intramolecular interactions, which influence the reorientability of functional groups in polymer material. A synchronous correlation spectrum shows the correspondence for the vibrational changes with the same phase. Positive cross-peaks indicate that the changes in two wavenumbers are in phase. Negative cross-peaks indicate that molecular vibrations do not correlate. Cross-peaks in an asynchronous spectrum usually signify absence of strong chemical interactions. Additionally, the measurements under humid conditions at a defined temperature provide an improved estimation regarding the viscoelasticity of the polymers reflecting their softening behaviour.

This study was made in order to later assess how components may be affected by different chemimechanical and/or enzymatic treatments. The properties of the primary cell-wall differ from the properties of the inner fibre wall layers, mainly due to the occurrence of pectin and protein. Dynamic Fourier Transform Infra-Red (FT-IR) spectroscopy indicates that strong interactions exist among lignin, protein and pectin, as well as among cellulose, xyloglucan and pectin in this particular layer (Figure 1). This is in contrast to the secondary cell-wall, where interactions of cellulose with glucomannan and of xylan with lignin are dominant. The presence of the strong interactions among the polymers in the primary cell-wall and,

especially, the relatively high content of pectin and protein, give a very good possibility to selectively attack these polymers in the primary wall (Salmén and Petterson 1995, Westermark *et al*. 1987). The first selective reaction chosen was the low degree sulfonation applied by an impregnation pretreatment of chips with a very low charge of sodium sulfite (Na_2SO_3). The question to be answered was whether chemical and structural changes in lignin cause a weakening of the lignin; pectin and lignin; protein interactions, thus changing the softening of pectin and protein in the primary wall.

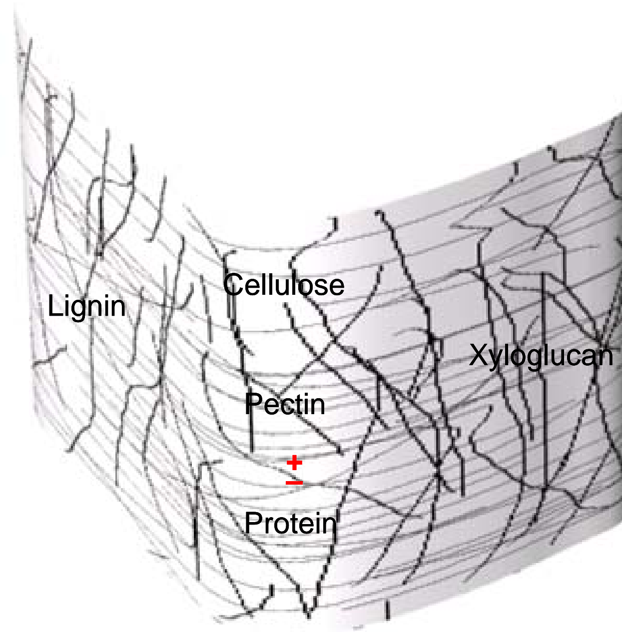


Figure 1. Schematic chard illustrating the suggested ultrastructure of the primary cell-wall

Cellulose is a semicrystalline polymer with both crystalline and amorphous regions. The most abundant native crystalline cellulose is the cellulose I. It is composed of two allomorphs, i.e. cellulose I_α and cellulose I_β where the cellulose I_β is a more thermodynamically stabile form (Atalla and VanderHart 1984). It was found that the presence of xyloglucan decreases the content of cellulose I_α that further corresponds to an improved crystallization of cellulose I_β (Yamamoto and Horii 1994, Yamamoto *et al*. 1996). The same authors suggested that the more stabile crystalline form, cellulose I_β, may be produced as result of less stressed or stress-free crystallization. The dynamic FT-IR spectroscopy has been presented as a potential measuring technique for studying the cellulose I allomorphs, both qualitatively and quantitatively (Åkerholm *et al*. 2004). The advantage of this technique is a possibility in resolving the overlapping absorption bands of the hydrogen bonds in cellulose, since the major difference between two cellulose allomorphs lies in the difference in their hydrogen bonding pattern. The aim of this work was also to invastigate a relative content of the respective cellulose allomorphs as well as a cellulose I_α/I_β ratio in the primary cell-wall material.

References

Åkerholm M, Hinterstoisser B and Salmén L (2004) Carbohydrate Research 339: 569

Atalla RH and VanderHart DL (1984) Science 223: 283

Bacic A, Harris P J and Stone BA (1988) Structure and function of plant cell walls, In: The Biochemistry of Plants 14, Carbohydrates (ed.) J. Preiss, Academic Press, New York, pp 297

Harrak H, Chamberland H and Plante M (1999) Plant and Cell Physiology 121: 557

Hinterstoisser B and Salmén L (1999) Cellulose 6: 251

Hinterstoisser B and Salmén L (2000) Vib. Spectrosc. 22: 111

Ishii T and Shimizu K (2001) Chemistry of Cell Wall Polysaccharides, In: Wood and Cellulosic Chemistry, David N-S, Hon NS (eds.) Marcel Dekker, Inc., Basel, Switzerland

Salmén L and Petterson B (1995) Cellulose Chemistry and Technology 29: 331

Westermark U, Samuelsson B, Simonson R and Pihl R (1987) Nordic Pulp & Paper Research Journal 2: 146

Whitney SEC and Gidley M J (1999) Plant Physiology 121: 657

Yamamoto H and Horii F (1994) Cellulose 1: 57

Yamamoto H, Horii F and Hirai A (1996) Cellulose 3: 229

Towards understanding compression wood

Brian Butterfield

School of Biological Sciences, University of Canterbury, Christchurch, New Zealand

Between 15 and 30% of the timber cut from vertical trunks of New Zealand grown radiata pine comprises compression wood. The higher than normal shrinkage in compression wood leads to distortion and instability in machined timber, and inferior paper making qualities for its separated fibres. The problem is particularly serious in fast grown plantation trees. Despite the volumes that have been written on the subject, there is still a disappointing tendency for plantation growers and timber millers to look upon compression wood as some sort of "defect", and to assume that it occurs only in branches or leaning stems. As a consequence, the reality of the large amounts of compression wood in millable vertical trunks has been seriously underestimated.

Compression wood forms in stems of gymnosperms in response to asymmetric loading. Its primary function is to bear a higher compressional load than the opposite or "normal wood" side of the stem. Compression wood formation is known to be regulated by plant growth hormones. In order to try and understand how and why woody plants develop compression wood, we have grown radiata pine seedlings in controlled environments, tied and growing free, angled and vertical, rocked and rotated intermittently and continuously, and subjected to various auxin and ethylene treatments. We have also successfully established stem explants in culture, both partial and entire stem portions. These cultures have responded to experimental manipulation and produced compression wood under a number of experimental conditions including different gravistimuli, rotation, and rocking. We have also subjected the explants to various auxin and ethylene treatments.

We have confirmed that biophysical stress induced by gravistimulus and asymmetric loading due to branches or prevailing winds stimulates the formation of compression wood. We have also found that compression wood forms in gravity free explants in response to hormone application.

Identifying compression wood in logs prior to milling is of critical importance to but has been hampered by the lack of a consistently but reliable grading system for defining compression wood, and the absence of any simple mechanized tool for identifying it. There is a need for a reliable and definitive compression wood grading system and the development of a non destructive tool to predict potential wood instability caused by compression wood.

Functional genomics of fibre differentiation in Populus

Gilles Pilate[1], Miyuki Takeuchi[1,3], Dominique Arnaud[1], Annabelle Dejardin[1], Françoise Laurans[1], Marie-Claude Lesage-Descauses[1], Régis Fichot[1,2], Franck Brignolas[2] and Jean-Charles Leple[1].

[1]*UR INRA "Amélioration, Génétique et Physiologie Forestières", BP 20619, Ardon, 45166 Olivet Cedex, France;*
[2]*Laboratoire de Biologie des Ligneux et des Grandes Cultures, UPRES EA 1207, Université d'Orléans, Rue de Chartres, BP 6759, 45067 Orléans Cedex 2, France;*
[3]*Present address : INRA-UMR Fractionnement des AgroRessources et Emballage (FARE), Equipe Structures et Propriétés des Parois secondaires, 2 Esplanade R.Garros, BP 224, 51686 Reims, France*

In response to a gravitational stress, hardwood trees are able to reorientate their axes thanks to the differentiation of tension wood. In poplar, tension wood fibres are mainly characterized by an additional secondary layer, named G-layer mostly constituted of highly crystalline cellulose microfibrils orientated almost parallel to the fibre axis. Thus, tension wood is an attracting model for the study of fibre differentiation.

We developed functional genomic studies on wood formation. Using such an approach, we have sequenced more than 25000 EST of wood-expressed genes, performed transcript profiling studies and identified a set of genes likely to be important in tension wood formation. Further in depth studies are presently carried out on several of these genes: I will present and discuss our most recent results on this.

Structural response and moisture alterations in wounded tissues of beech

Primož Oven[1], Maks Merela[1], Igor Serša[2]

[1]*University of Ljubljana, Biotechnical Faculty, Department of Wood Science and Technology, Ljubljana, Slovenia;*
[2]*Jožef Stefan Institute, Ljubljana, Slovenia*

Tissues of trees actively respond to wounding and/or colonization by formation of boundaries, which tend to minimize the extent of the damage. Existing cells and tissues, occurrence of new cells, cell wall alterations, and deposition of phytoalexin-like antifungal compounds constitute compartmentalization boundaries or increase disease resistance (Pearce 1996, Oven *et al*. 1999, Frankenstein *et al*. 2003). Structural and biochemical response is relatively slow and lags behind the initial dehydration and aeration of compromised tissue (Pearce et al. 1994). However, dynamics of dehydration process and its possible relationship to structural response of wounded tissues are still imperfectly understood. True dynamic studies of response in tissues of living trees are limited by the technical difficulties of monitoring events nondestructively deep within the tissue. Nuclear magnetic resonance imaging (MRI) would appear to be suitable technique to overcome such obstacles (Pearce *et al*. 1997, Merela *et al*. 2005, Kuroda *et al*. 2006). The aim of this research was to employ the 3D magnetic resonance imaging technique (3D MRI) complemented by light and UV-microscopy to visualize dynamic of wound response and to reveal structural features and moisture distribution in pruned branch of beech (*Fagus sylvatica* L.).

A three meter high healthy beech (*Fagus sylvatica* L.) was transplanted in a portable pot one month before the study began and the top of branch with a 5 mm in diameter was cut off on May 25th. The remaining 80 cm long stub of branch (still part of the tree) was imaged immediately after pruning and by 3rd, 8th, 14th, 21st, 28th, 35th and 168th day after wounding. Before every imaging experiment pruned part of the branch was inserted into a 10 mm saddle shaped Bruker RF coil for MR microscopy and positioned in the centre of a 2.35 T horizontal bore Oxford superconducting magnet. Images were acquired by the proton density weighted 3D spin-echo method at parameters: field of view 25 mm by 12.5 mm by 12.5 mm, imaging matrix 256 by 128 by 128, echo time 2.4 ms, repetition time 600 ms, and at 8 averages; the total imaging time was 22 hours. A pilot scan was done before and after completing every high-resolution scan. MRI data were processed and analyzed by ImageJ computer software. A Nikon E800 was used for conventional microscopy and observations at UV excitation (mercury burner HBO 100W, filter: UV-2A, EX 330-380, DM 400, BA 420). Presence of wound-induced lignin and suberin was visualized by using a polychromatic stain acrydin-chrysoidin/astrablue and an autofluorescence quenching technique based on the selective use of phluoroglucinol+HCl and Sudan Black B.

Within 22 hours after wounding, 0.9 mm thick layer of tissues on the pruned surface showed low MR signal, probably due to dehydration. Dehydrated xylem area enlarged significantly within 3 days after wounding. 3D MR imaging revealed cone-shaped dehydrated tissue reaching 4.7 mm deep in the central part of the branch.

After 8 days, increase of MR signal was detected in 2.4 mm thick inner layer of the previously dehydrated tissue. Volume rendered MR image clearly demonstrate that the increase of MR

signal was most conspicuous in rays. MR signal of this tissue was up to 1.9 times higher than the signal of the healthy xylem.

Within 14 days after wounding further changes in the intensity and distribution of MR signal were detected. Only 1.2-1.5 mm thick inner layer of the "re-wetted" tissue kept high MR signal, while the outer part exhibited loss of MR signal. At the edge of the pruning wound new tissue formed, probably representing callus.

Differences in intensity of MR signal became more pronounced in the further course of the experiment. The MR image obtained on 28 day after wounding showed that three zones of tissue with clear differences in MR signal became differentiated. Tissue of high MR signal is separating dehydration from sound wood.

Later in the experiment (day 35), dehydrated cone-shaped area extended to the depth of 3.4-4.0 mm and had approximately five times lower MR signal than the healthy wood. Dehydrated tissue was delimited from the healthy wood with 0.3-1.5 mm thick tissue layer, exhibiting 1.9-3.7 times higher MR signal than underlying healthy wood.

Five months after the wounding, 3D MRI revealed dieback of the previously formed wound-wood and the cambium as well as retreat of a high-MR-signal-boundary deeper in the branch.

Light microscopy revealed that the tissue of a higher MR signal corresponded to typical reaction zones formed in beech, characterized by tyloses in vessel, coloured deposits in lumina of parenchyma, fibre tracheids and vessels as well as by intracellular suberization of tylsoses and parenchyma cells. Our observations on changes in MR signal intensity are in accordance with observation of Pearce *et al.* (1994, 1997) on sycamore. They demonstrated that these changes were due to moisture content redistribution in the developing reaction zone.

In conclusion, we propose a model of response of beech to pruning wounds. Immediately after pruning the tissues dehydrate. Inner parts of dehydrated tissue rewet after approximately 8 days. Outer parts of rewetted tissue dehydrate again, whereas inner parts keep up to 3-folds higher moisture content than that of healthy wood. Moisture boundary corresponds in position to reaction zone and is delimiting dehydrated tissue and sound wood. Structural characteristics as well as high moisture content of reaction zones contribute to the efficient compartmentalization of mechanical wounds in beech.

References

Frankenstein C, Schmitt U, Waitkus C, Eckstein D (2005) Wound callus formation – a microscopic study on poplar (*Populus tremula* L. x *Populus tremuloides* Michx.). J. Appl. Bot. 79: 44-51

Kuroda K, Kanbara Y, Inoue T, Ogawa A (2006) Magnetic resonance micro-imaging of xylem sap distribution and necrotic lesions in tree stems. IAWA 27: 3-17

Merela M, Sepe A, Oven P, Serša I (2005) Three-dimensional *in vivo* magnetic resonance microscopy of beech (*Fagus sylvatica* L.) wood. Magma 18: 171-174

Oven P, Torelli N, Shortle WC, Zupancic M (1999) The formation of a ligno-suberised layer and necrophylactic periderm in beech bark (*Fagus sylvatica* L.). Flora 194: 137-144

Pearce RB (1996) Antimicrobial defences in the wood of living trees. New Phytol. 132: 203-233

Pearce RB, Fisher BJ, Carpenter TA, Hall LD (1997) Water distribution in fungal lesions in the wood of sycamore (*Acer pseudoplatanus*), determined gravimetrically and using nuclear magnetic resonance imaging. New Phytol. 135: 675-688

Pearce RB, Sümer S, Doran SJ, Carpenter TA, Hall LD (1994) Non – invasive imaging of fungal colonization and host response in the living sapwood of sycamore (*Acer pseudoplatanus* L.) using nuclear magnetic resonance. Physiol. Plant Pathol. 45: 359–384

Plant AGC protein kinases: a compass that orients auxin-dependent plant growth and –development

Fang Huang, Marcelo K. Zago, Helene Robert, Ab Quint,
Carlos S. Ampudia and Remko Offringa

Dep. of Molecular & Developmental Genetics, Institute of Biology, Leiden University, Wassenaarseweg 64, 2333 AL Leiden, The Netherlands

A major determinant in plant development is the polar transport of the plant hormone auxin, which generates vectorial gradients that direct growth and provide positional cues for patterning, organogenesis and vascular differentiation. The directional auxin flow depends on the activity and polar sub-cellular localisation of the PIN auxin efflux carriers. We have identified the PINOID (PID) protein kinase as regulator of the apico-basal polar localization of PIN proteins (Benjamins *et al*. 2001, Friml *et al*. 2004). PID is a membrane-associated kinase that belongs to the plant-specific AGCVIII family of protein kinases. Recently, we obtained evidence that PID may influence PIN polarity by phosphorylating the PIN central hydrophilic loop (Michniewicz *et al*. 2007). In Arabidopsis, three PID-like kinases cluster to the same clade as PID, and we are currently testing the hypothesis that PID and PID-like kinases provide plants with a compass that integrates both external (e.g. gravity, touch) and internal (e.g. calcium) signals (Benjamins *et al*. 2003), and directs auxin transport to determine the proper growth orientation or lateral organ position.

Benjamins R, Quint A, Weijers D, Hooykaas P, and Offringa R (2001) The PINOID protein kinase regulates organ development in Arabidopsis by enhancing polar auxin transport. Development 128: 4057-4067

Benjamins R, Ampudia CS, Hooykaas PJ, and Offringa R (2003) PINOID-mediated signaling involves calcium-binding proteins. Plant Physiol 132: 1623-1630

Friml J, Yang X, Michniewicz M, Weijers D, Quint A, Tietz O, Benjamins R, Ouwerkerk PB, Ljung K, Sandberg G, Hooykaas PJ, Palme K, and Offringa R (2004) A PINOID-dependent binary switch in apical-basal PIN polar targeting directs auxin efflux. Science 306: 862-865.

Michniewicz M, Zago MK, Abas L, Weijers D, Schweighofer A, Meskiene I, Heisler MG, Ohno C, Huang F, Weigel D, Meyerowitz EM, Luschnig C, Offringa R, and Friml J (2007) Phosphatase 2A and PID kinase activities antagonistically mediate PIN phosphorylation and apical/basal targeting in *Arabidopsis*. Cell *In press*

Structural characterisation of G-layer allows to explain longitudinal shrinkage in chestnuts tension wood

Bruno Clair[1], Joseph Gril[1], Pierre Cabrolier[1], Francesco Di Renzo[2], Hiroyuki Yamamoto[3] and Françoise Quignard[2]

[1] *Equipe Mécanique de l'Arbre et du Bois - Laboratoire de Mécanique et Génie Civil (LMGC) UMR 5508, CNRS - Université Montpellier 2 - Place E. Bataillon, cc 048, 34095 Montpellier Cedex 5, France*

[2] *Laboratoire des matériaux catalytiques et catalyse en chimie organique UMR 5618-ENSCM-CNRS UM1 - 8 rue de l'Ecole Normale - 34296 Montpellier Cedex 5*

[3] *Laboratory of Bio-material Physics, School of Bio-agricultural Sciences, Nagoya University, Japan*

Wood behaviour is characterized by a high sensibility to humidity since it shrinks during drying. Longitudinal drying shrinkage, typically 0.1 % in normal wood, can amount to 10 times more in reaction wood and be a problem for end-users. It can be explained and modelled according to fibre structure. The angle formed by the cellulose microfibril helix is the determinant factor; the higher the angle, the higher the shrinkage. However, usual models cannot explain the behaviour of tension wood since it contains the so called G-layer characterised by a high cellulose content and a very low microfibril angle, and exhibits a very high shrinkage.

New insights on the organisation of this peculiar cell wall layer allows to explain how it could drive the axial shrinkage.

Nitrogen adsorption-desorption isotherms of aerogels show that tension wood cell wall has a gel-like structure characterised by a pore surface more than 30 times higher than in normal wood. According to argued hypothesis on the amount of G-layer, we propose a new description of the layer as a hierarchical structure of 50 nm-diameter aggregates forming super-aggregates separated from each other by more than 50 nm. SEM and adsorption volumetry indicate that this wide spacing would be filled by the liquid phase of a polysaccharidic gel.

Our results allow explain the paradoxical shrinkage of tension wood. Potential applications in biomechanics and biomimetics are worth investigating, considering that tension wood produces tensile growth stresses 10 times higher than normal wood in living trees.

Enzymatic removal of the G-layer - New insights into its mechanical role

Luna Goswami[1], Karin Jungnikl[1], Catherine Coutand[2], George Jeronimidis[3], Peter Fratzl[1], Ingo Burgert[1]

[1]Max-Planck-Institute of Colloids and Interfaces, Department of Biomaterials, Am Mühlenberg 1, 14476 Potsdam, Germany
[2]Institut National de la Recherche Agronomique (INRA) umr Physiologie Intégrative de l'Arbre Fruitier et Forestier (PIAF), 234 av. du Brézet, 63100 Clermont-Ferrand, France
[3]Centre for Biomimetics, Composite Materials Engineering, School of Construction Management and Engineering, University of Reading, Whiteknights, Reading RG6 2AY, UK

There is a great interest of biologists as well as materials scientists to understand the role of the G-layer for the stress generation of tension wood. In most tension wood fibre types parts of the secondary cell wall layers are substituted by the G-layer. This layer consists almost exclusively of cellulose and can completely fill the lumen of the cell. The orientation of the cellulose microfibrils is parallel to the cell axis (MFA ~0°). In our current study we eliminated all the G-layer from poplar tension wood (*Populus nigra x Populus deltoids*) by using cellulase onozuka RS enzyme. We performed mechanical tests on tissue slices of tension wood with and without G-layer and established a significant alteration of the mechanical behaviour. These findings are interpreted with regard to the crucial role of the G-layer in tensile stress generation.

Mechanical stimuli and vascular tissue differentiation

Véronique Douet, Mustapha Tiouabi, Eliane Abou-Mansour, Brigitte Pollet, Catherine Lapierre and Pia A Stieger

Physiologie végétale, Institute of Biology, University of Neuchâtel, Rue Emile Argand 11, CH-2009 Neuchâtel, Switzerland

The vascular system connects all parts of the plant body and serves as the transport system for nutrients, hormones and water, as well as in the physical stabilization of the plant body. Plants develop a primary vascular system during embryogenesis, which is connected to vascular bundles emanating from newly developing organs in the vegetative and reproductive phases. The vasculature is composed of the cambium (meristematic cells) and the two differentiated units, phloem and xylem. In a first phase of vascular differentiation, defined cells are recruited within a growing organ to form continuous files – the procambium. In the second phase, cells deriving from the procambium differentiate into xylem, phloem and fibers (Sieburth and Deyholos 2006). Many plant species form a secondary cambium, from which wood and bark are produced. Wood is generated by the meristematic tissue cambium during xylem differentiation and secondary cell wall formation. In this process, lignins are incorporated into the cell wall composed of cellulose microfibrils, hemicellulose and pectins. Cambium activity and the chemical and physical properties of wood vary during different growth seasons and are influenced by external factors, such as wind, light and temperature. *Arabidopsis* has previously been shown to undergo secondary growth (Busse and Evert 1999, Chaffey *et al*. 2002), but the molecular controls regulating the formation of a secondary cambium and secondary xylem, phloem and interfascicular fibers are unknown. Several hormones, among them auxin and ethylene were proposed to trigger secondary cambium formation (Eklund and Little 1996, Sundberg *et al*. 2001). In addition, it was proposed that weight carried by a stem was a primary signal for the induction of cambium differentiation and that the plant hormone auxin was a downstream carrier of the mechanical signal (Ko *et al*. 2004).

The goal of our work was to unravel regulatory mechanisms of vascular cambium formation and xylem differentiation that underlie wood formation. In particular we were interested in the role of mechanical signals in this process. As a model system we used the plant species *Arabidopsis thaliana*. To this end we developed a system, in which pressure on the plant body is created by centrifugal force resulting from the low-speed centrifugation of growing plants. The strength of the mechanical stimulus is given by regulating the speed of centrifugation, whereas the direction of the mechanical stimulus is determined by the plant's orientation with respect to the centrifugal vector.

Inflorescence stems of *Arabidopsis thaliana* were submitted to compression by centrifuging the plants horizontally at 11 ms^{-2} with the inflorescence tips pointing to the centre of the centrifuge. The treatment was initiated, when stems started to bolt and was maintained for 10 days. In order to differentiate between effects of a mechanical load and effects of vibration on plant growth and morphology, plants were also grown on a shaker at 100 rpm for 10 days. Centrifugation induced the formation of a secondary cambium and secondary growth at the base of inflorescences, but reduced longitudinal stem growth, whereas vibration had no effects on stem morphology.

In a next step, we analysed the lignin contents and composition in the inflorescence stems of centrifuged plants, to elucidate the influence of a mechanical signal on secondary cell wall formation. Soluble phenols and low-Mr phenolics were slightly increased in the inflorescence stems upon centrifugation, whereas vibration had no influence on the contents of soluble phenols and low-Mr phenolics. The composition of lignin polymers was analysed with the method of thioacidolysis (Lapierre *et al*. 1995). Syringyl monomeric products (S-units) were decreased, whereas guaiacyl (G-units) and p-hydroxyphenyl monomeric products (H-units) were increased in centrifuged plants compared to controls and vibrated plants.

Secondary growth induction and a change in the amount of free phenols and low-Mr phenolics were not observed, when a centrifugal force was applied to the ethylene insensitive mutant ein2. Our results so far suggest that mechanical signals are involved in secondary growth induction and the regulation of cell wall composition and that ethylene plays a role in the transduction of the mechanical signal. We are currently analysing changes in gene expression profiles upon a short time of mechanical pressure on inflorescences of wild type and ethylene mutant plants, in order to elucidate primary regulators of mechanically induced secondary growth.

References

Busse J, Evert RF (1999) Vascular differentiation and transition in the seedling of Arabidopsis thaliana. Int J Plant Sci 160: 241-251

Chaffey N, Cholewa E, Regan S, Sundberg B (2002) Secondary xylem development in Arabidopsis: a model for wood formation. Physiol Plantarum 114, 594-600

Eklund L, Little CHA (1996) Laterally applied Ethrel causes local increases in radial growth and indole-3-acetic acid concentration in Abies balsamea shoots. Tree Phys 16: 509-513

Ko JH, Han KH, Park S, Yang J (2004) Plant body weight-induced secondary growth in Arabidopsis and its transcription phenotype revealed by whole-transcriptome profiling. Plant Physiol 135; 1069-1083

Lapierre C, Pollet B, Rolando C (1995) New insights into the molecular architecture of hardwood lignins by chemical degradative methods. Res Chem Intermed 21: 397-412

Sieburth LE and Deyholos MK (2006) Vascular development: the long and winding road. Curr Opin Plant Biol 9: 48-54

Sundberg B, Uggla C, Tuominen H (2001) Cambial growth and auxin gradients. In Cell and Molecular Biology of Wood Formation (Savidge RA, Barnett JR, Napier R eds.) Oxford, UK: Bios Scientific Publ Ltd, 169-188

Rapid measurement of the microfibril angle of single wood fibres by spectroscopic transmission ellipsometry

Tero Kesti and Chun Ye

University of Oulu, Measurement and Sensor Laboratory,
Technology Park 127, 87400 Kajaani, Finland

A method based on spectroscopic ellipsometry has been developed for measuring the microfibril angle φ of single, untreated wood or pulp fibres. The setup consists of a polarization microscope equipped with a pair of polarizers and quarter-wave plates. The spectrum of light transmitted through a selected part of the fibre is recorded with a spectral camera. The optical arrangement is such that the transmission spectrum depends only on the microfibril angle and phase retardation Δ proportional to the cell wall thickness, irrespective of the orientation of the fibre. Then, φ and Δ are obtained by least-squares fitting according to a mathematical model. The method requires no sample pre-treatment and is very fast. Each measurement only takes a fraction of a second, so that a large number of fibres can be measured in a reasonable time.

References

Chun Ye (2006) Applied Optics 45: 9092

Chun Ye (2006) Nordic Pulp & Paper Research Journal 21: 520

Chemical characterization of cell walls from transgenic tobacco overexpressing *PtaRHE1* and *PtaERF1*, two genes linked to vascular development in aspen

Johnny Mukoko Bopopi[1], Olivier Vandeputte[1], Mondher El Jaziri[1], Brigitte Chabbert[2], Marie Baucher[1]

[1]*Laboratoire de Biotechnologie Végétale, Université Libre de Bruxelles (ULB), 8 rue Adrienne Bolland, B-6041 Gosselies, Belgium*
[2]*INRA Parois Végétale et Matériaux Fibreux UMR FARE, 2 Esplanade Roland Garros, BP 224, 51686 REIMS, France*

Previously, we have identified *PtaRHE1* and *PtaERF1* as two genes which expression seems to be linked to the setting up of secondary growth in aspen (*Populus tremula* x *P. alba*) (van Raemdonck et al., 2005). *PtaRHE1* codes for a RING-H2 type protein. RING-H2 finger proteins are potentially involved in targeted proteolysis as ubiquitin E3 ligases. In plants, protein degradation by the ubiquitin/proteasome system is important for various vital processes including development, cell division, hormonal responses, and biotic and abiotic stress responses. More than 5% of the predicted proteome in *Arabidopsis thaliana*, for example, is postulated to be involved in the ubiquitination/26S proteasome pathway, and about 1300 genes are predicted to encode the E3 components (Smalle and Viestra 2004). Database searches have identified 469 RFD (Ring Finger Domain)-containing proteins in the Arabidopsis genome (Stone et al., 2005) and 218 cDNA clones in the rice full-length cDNA database (Katoh *et al*. 2005). *PtaRHE1* had a pronounced expression in ray initials and their derivatives within the cambial zone (van Raemdonck *et al*. 2005). The expression of *ATL2*, the closest Arabidopsis homologue to *PtaRHE1* was detected in the area around the shoot apical meristem, in the vascular tissues of the inflorescence stem and in all tissues of stems undergoing secondary growth.

PtaERF1 codes for a putative transcription factor belonging to Ethylene Responsive Factor (ERF) sub-family comprising 65 members in *Arabidopsis thaliana* which are categorized in four classes (Sakuma *et al*. 2002, Tournier *et al*. 2003). Based on its MCGGAII/L N-terminal signature, *PtaERF1* belongs to the Class IV ERF. For most of the ERF genes, the function is unknown but members of Class IV have been shown to be induced by various hormones (including ethylene, JA and ABA) as well as by a number of biotic and abiotic stresses (Nakano *et al*. 2006). The expression of *PtaERF1* has been localized by *in situ* RT-PCR specifically in the phloem of 1 month old aspen stems (van Raemdonck *et al*. 2005).

To further characterize these 2 genes, transgenic tobacco lines overexpressing *PtaRHE1* or *PtaERF1* have been established. *PtaRHE1* overexpressing (OE) lines are strongly affected in their growth and development and exhibit reduced stem growth and a curled leaf phenotype. In addition, necrosis was observed on the leaves and the flowering was delayed. This phenotype is similar to plants with inactivated ubiquitin (Bachmair *et al*. 1990). Transgenic *PtaERF1* OE lines exhibit a reduced apical dominance and a smaller stem

diameter as compared to non transgenic lines. These phenotypes suggest an alteration in vascular system development.

Therefore, the chemical characterization of cell wall components of these transgenic plants was investigated. Cell walls from two *PtaERF1* and 2 *PtaRHE1* transgenic lines as well as from two wild type (WT) plants were extracted, ground and freeze-dried for cell wall components analysis. Acid hydrolysis of cell wall residues (CWR) was performed on 10 mg samples using 12 M H_2SO_4 (125 µl, 2h at room temperature) and then brought to 1M of H_2SO_4 by adding deionized water. Soluble fractions (1,5 ml) recovered from chemical fractionation were hydrolyzed for 2 h at 100°C (Searman *et al.* 1954). The lignin content of cell wall residues was estimated by the Klason procedure (Effland 1977) as modified by Monties (1984). The monomer composition of the labile ether-lignin structure was determined by thioacidolysis which specifically disrupts the ether linkages (8-O-4' intermonomeric linkages).

Several differences in carbohydrates and lignin content and/or composition between wild type and transgenic plants were observed. Preliminary results indicate that sugar content of *PtaERF1* tobacco plants is slightly higher than that of WT. In addition several differences in relative composition can be observed such as decrease in galacturonic and glucuronic acids and increase in galactose, glucose and mannose. *PtaRHE1* OE lines have a slightly higher sugar content than WT plants. A decrease in glucuronic acid and a slight increase in galactose, glucose and mannose were also observed. A slightly lower lignin content was measured for both *PtaERF1* and *PtaRHE1* OE lines. The lignin composition of *PtaERF1* and *PtaRHE1* OE lines was characterized by a low amount of G units and an increased amount in S units yielding a slightly higher S/G ratio.

Preliminary anatomical analysis revealed differences in xylem tissue organization between the WT and the transgenic plants, as evidenced by Phloroglucinol-HCl staining of lignin. These differences have to be confirmed with more material, lines and lignin specific staining such as the Maüle reaction.

References

Bachmair A, Novatchkova M, Potuschak T, and Eisenhaber F (2001) Ubiquitylation in plants: a post-genomic look at a post-translational modification. Trends Plant Sci. 6: 463-470

Effland MJ (1977) Modified procedure to determine acid-insoluble lignin in wood and pul. Tappi J 60:143-144.

Katoh S, Tsunoda Y, Murata K, Minami E, and Katoh E (2005) Active site residues and amino acid specificity of the ubiquitin carrier protein-binding RING-H2 finger domain. J. Biol. Chem. 280: 41015-41024

Monties B (1984) Dosage de la lignine insoluble en milieu acide: influence du prétraitement par hydrolyse acide sur la lignine Klason de bois et de paille. Agronomie 4: 387-392

Nakano T, Suzuki K, Fujimura T and Shinshi H (2006) Genome-Wide Analysis of ERF Gene Family in Arabidopsis and Rice. Plant Physiol. 140: 411-432.

Sakuma Y, Liu Q, Dubouzet JG, Abe H, Shinozaki K, Yamaguchi-Shinozaki K (2002) DNA-binding specificity of the ERF/AP2 domain of Arabidopsis DREBs, transcription factors involved in dehydration- and cold-inducible gene expression. Biochem. Biophys. Res. Commun. 290: 998-1009

Searman J, Moore W, Mitchell R, Millet M (1954) Techniques for determination of wood constituents by quantitative paper chromatography. Tappi J.: 336-343

Smalle J, and Viestra RD (2004) The ubiquitine 26S protéasome proteolytic pathway. Annu. Rev. Plant Biol. 55: 555-590

Stone SL, Hauksdottir H, Troy A, Herschleb J, Kraft E, Callis J (2005) Functional analysis of the RING-type ubiquitine ligase family of *Arabidopsis*. Plant Physiol. 137: 13-30

Tournier B, Sanchez-Ballesta MT, Jones B, Pesquet E, Regad F, Latché A, Pech J-C, Bouzayen M (2003) New members of the tomato ERF family show specific expression pattern and diverse DNA-binding capacity to the GCC box element. FEBS Letters 550: 149-154

van Raemdonck D, Pesquet E, Cloquet S, Beeckman H, Boerjan W, Goffner D, El Jaziri M, and Baucher M (2005) Molecular changes associated with the setting up of secondary growth in aspen. J. Exp. Bot. 56: 2211-2227.

Acknowledgements

MB is a Research Associate of the Belgian “Fonds National de la Recherche Scientifique” (FNRS).

Effect of nutrient optimisation on Norway spruce wood properties

Kaakinen Seija[1], Piispanen Riikka[2], Lehto Satu[2], Pohjanen Johanna[1], Nilsson Urban[3], Linder Sune[3], Saranpää Pekka[2], Vapaavuori Elina[1]

[1]*Finnish Forest Research Institute, Suonenjoki Research Unit, Juntintie 154, FI-77600 Suonenjoki, Finland*
[2]*Finnish Forest Research Institute, Vantaa Research Unit, P.O. Box 18, FI-01301 Vantaa, Finland*
[3]*SLU, Southern Swedish Forest Research Centre, P.O. Box 49, SE-230 53 Alnarp, Sweden*

Norway spruce is both economically and ecologically important forest species in Scandinavia, where, however, low nutrient, especially nitrogen, availability is a limiting factor to forest growth. Increases in stem volume growth by forest fertilisation in Scandinavia are well known, and in spite of low temperature, the boreal climate allows considerably higher stem wood production than the current production if water and nutrients are not limiting. This study was carried out to measure how the wood and fibre characteristics of Norway spruce (wood density, fibre length and wood chemistry) would be affected by a nutrient optimisation treatment in experimental research forest in Asa in southern Sweden. Annual growth ring width increased while wood density decreased in nutrient optimised trees as compared to control trees with no treatments. The results also suggest that nutrient optimisation effects on wood chemistry were only slight while clear changes in vertical and radial directions of the stem were detected. The changes in wood properties are discussed in relation to the physiological function and the growth increase and how the changes can affect the suitability of wood for different end-use purposes.

Degradation of spruce pulp fibres by HCl and Cellulases reflects different action on the fibre cell walls

Paul Ander and Geoffrey Daniel

WURC, Wood Ultrastructure Research Centre, SLU (Swedish University of Agricultural Sciences), Dept. of Forest Products/Wood Science, PO Box 7008, SE-75007 Uppsala, Sweden.

The new pulp fibre testing method called the HCl-method can be used to compare different spruce, pine and other pulp fibres and calculate number of fibre cleavages in dislocations and other weak points. This method has been compared with treatment of spruce kraft pulp fibres using different cellulase mixtures. The results show some interesting features:

HCl-treatment can distinguish between mill and laboratory made spruce kraft pulp fibres from the same wood batch. The sugar release is characterized by release of xylose and other hemicellulose sugars and little glucose. This is in contrast to cellulases, which despite strong fibre cleavage, did not distinguish between mill and laboratory made pulp fibres and released much glucose from the fibres. Similarity in pore sizes between the presently used mill and laboratory pulp fibres probably contribute to this result.

It seems as if hemicellulose degradation by HCl and deep penetration of the acid into the primary and secondary fibre cell walls at 80°C is of major importance for the differentiation between mill and laboratory pulp fibres. Cellulases on the other hand act mostly on the fibre surfaces and deeper penetration is only taking place in amorphous parts of dislocations. HCl swelled the fibres by 0.7-1.7 μm, while cellulase (EG + CBH) decreased fibre width by 1-3 μm.

Using polarized light microscopy it was seen that after cellulase treatment there was a strong erosion and degradation of earlywood fibre surfaces in addition to fibre cleavage. For latewood fibres this type of erosion/degradation was not discerned and instead typical dislocations appeared together with the rainbow colours of crystalline cellulose in the thick S2 cell wall. For HCl, many cracks but less erosion were seen in the earlywood fibres. In latewood fibres, dislocation cleavage was common, and the general appearance was rather similar as for cellulase treatment.

References

Ander P and Daniel G (2006) Dislocation counting and comparison of pulp fibre properties after HCl-treatment and fibre length determination. Proc. 5th Plant Biomechanics Conference, Stockholm, Vol. I. August 28 – September 1, 2006. Ed.: L. Salmén. STFI-Packforsk, Stockholm, Sweden, ISBN 91-86018-12-4, pp. 169-174.

Ander P and Daniel G (2007) Pulp fibre testing with the HCl-method and comparison with cellulase activity and phosphoric acid swelling. Proc. COST Action E54 “Characterisation of the fine structure and properties of papermaking fibres using new technologies”. April 25-27, 2007. Riga, Latvia, p. 24-28.

Ander P, Hildén L, and Daniel G (2007) Differences in Cleavage of Softwood Kraft Pulp Fibres by HCl and Cellulases as Determined by Fibre Length Measurements, Sugar Analyses, Polarized Light and Scanning Electron Microscopy. Proc. 10th Int. Congress Biotechnol. Pulp Paper Ind., Madison, Wisconsin, June 10-15, 2007, p. 71-73.

Cell wall chemistry and mechanical strength of the peduncle of cut roses

Wolfgang Graf[1], Werner B. Herppich[3], Susanne Huyskens-Keil[2] and Heiner Grüneberg[1]

[1]*Humboldt-Universitsität zu Berlin, Institute of Horticultural Sciences, Section of Floriculture;*

[2]*Section of Quality Dynamics/Postharvest Physiology, Germany,*

[3]*Leibniz-Institute of Agricultural Engineering Potsdam-Bornim, Department of Horticultural Engineering, Germany*

Bending of the flower buds (bent-neck) is a widespread problem damaging millions of cut roses well before they achieve their genetically fixed vase-life. Structural weakness, reduced cell wall strength or disturbed stem water status are assumed to cause the bent-neck phenomenon. However, the exact reason for this disorder is still unknown. Hence, four rose cultivars, largely differing in their bend-neck susceptibility were used to comprehensively evaluate the possible relative contribution of vascular bundle structure and cell wall biochemistry, tissue strength, and water relation to this phenomenon.

The roses were grown in a greenhouse from early May to end of June and from early November to end of December under controlled conditions. All roses were harvested at the flower development stage 2, to determine the physiological age.

Cutting energy, indicating the mean tissue strength, and the content of structural carbohydrates were significantly higher in 'Red Giant' roses than in samples of the three other cultivars, as was tissue stiffness (i.e. the apparent quasi static elastic modulus). In 'Red Giant', 'Aloha' and 'Akito' the duration of vase life (23.7 d, 12 d and 10.3 d, respectively) correlated well with bent neck susceptibility (low in 'Red Giant' and high in 'Akito' and Aloha'). In contrast, stems of 'Milva' had the lowest content of all structural carbohydrates, a low tissue strength but a long vase-life (23.7 d).

Strength and structural carbohydrate content were highly correlated. While the relative contribution of the structural carbohydrate components lignin, cellulose and hemicellulose was similar in plants of all varieties their total amounts differed largely. This might be explained be a progressed maturity stage of the peduncle tissue of 'Red Giant' roses. The high correlation (r^2 = 0.8445) between the dry matter content and the content of structural carbohydrates may confirm this assumption. A higher dry mass content might result from a slower stem development from flower induction to harvest. However, investigations (March to Mai 2007) indicated that the period of development was identical in 'Milva' (40.3 d) and 'Red Giant`' (40.1 d). The higher dry mass contents could result from a different and genetically fixed stem structure of the respective cultivars.

In fact, the anatomical inspection of the peduncle tissue revealed significant cultivar-specific differences in structure and formation of vascular bundles. The overall structure of vascular bundles in plants of the cultivar 'Milva' was least developed, mostly forming no clear ring structure in the peduncle (79%). Most of these vascular bundles were arranged disjunctively, i.e. each bundle was clearly separated from the other. Only in some cases the configuration showed a loose wavy structure. In contrast, 'Red Giant' samples showed the progressed developed vascular bundle structure. The bundles form an almost completely closed ring with

many (54%) to few openings (46%) and with a strongly wavy form like a corrugated iron sheet. The structure of the ring from the vascular bundles in ‘Aloha’ is nearly that in ‘Red Giant’. ‘Akito’ has only slightly waved rings with many openings. The bundles were arranged solitarily in most cases (79%). A significant difference in the vascular bundle structure was found only between the cultivar ´Milva` and the three other cultivars. These results were highly correlated with the mean tissue strength in ‘Milva’ and ‘Red Giant’ but less clear in ‘Akito’ and ‘Aloha’. Stems structure of these cultivars closely reflects that of ‘Red Giant’ but not that of ‘Milva’. On the other hand, their content of structural carbohydrates was similar to ‘Milva’. In ‘Aloha’ and, though less obvious, in ‘Akito’, structural carbohydrates have to be more important for the tissue strength than the structure and formation of vascular bundles.

All the above results were well correlated with vase life and bent-neck susceptibility of 'Akito', 'Aloha' and 'Red Giant' plants but not for Milva. In 'Milva' plants, the relatively low bend-neck susceptibility might be due to a well balance peduncle water status with a lower water potential in the upper leaves. In addition, the difference between flower and leaf water potential was always significantly lower in ‘Milva’ than in 'Akito', 'Aloha' and 'Red Giant'. The magnitude of the water potential difference between flower bud and leaves might be explained by a safety function of the abscission zone at the base of the peduncle, or simply by the extent of leaf transpiration. It is well known that ‘Milva’ reveal a stable water relation also under postharvest stress.

Physical aging and its impact on the characterisation of viscoelastic properties of green wood

Jana Dlouha [1, 2], Joseph Gril[1], Bruno Clair[1]

[1] *UMR Laboratory of Mechanics and Civil Engineering (LMGC), Montpellier, France*
[2] *MZLU Mendel University of Agriculture and Forestry in Brno, Czech Republic*

One of partial goals of the project Woodiversity is to characterise long-term viscoelastic properties of green wood and use these data as an input for a biomechanical model simulating the up-righting process of a tree. Wood material from straight growing and tilted trees is used to investigate viscoelastic properties of normal and reaction tissues. To identify the parameters of a rheological model, a set of creep tests are carried out at different temperatures.

While analysing creep results, we could note that it was impossible to apply time-temperature superposition principle to predict long-term behaviour of green wood from our data. However this principle is commonly used in the world of polymers and was also successfully applied to predict thermally activated wood behaviour (Passard and Perré 2005). Relaxation of locked-in strains during creep tests at elevated temperature may induce an error leading to a misinterpretation of our data so that we decided to anneal the specimen by heating it and perform the creep test once it has reached new equilibrium state of its molecular structure.

Preliminary tests revealed that there is no rapid stabilisation of molecular mobility and that we are confronted with a physical aging phenomenon as observed for many polymers (Struik 1976). Thus we have applied Struik's procedure to analyse the effect of aging time on creep properties of green wood and we could note that this effect was systematic. It was possible to build a master curve from the creep data at different aging times and establish a linear relation between the log of retardation factor a and log of aging time te. Double logarithmic shift rate $\mu = d \log a / d \log t_e$ was close to that observed by Struik in semi-crystalline polymers (Levita and Struik 1983). To describe the phenomenon of physical ageing in wood, we tried to fit a model of the following form:

$$J(t) = J_0[1+(t/\tau)^k],$$

τ being function of t_e:

$$\log[\tau(t_e)] = \log[\tau_0] + \mu[\log(t_e) - \log(t_{e0})].$$

The correspondence between the experimental data and modelled values was quite satisfying for individual tests however it was impossible to generalize it to the whole of specimens.

As the aging phenomenon seems to persist during a long period and its general description is difficult, the practical conclusion for the characterisation of viscoelastic properties was to avoid the quench of the specimen. Therefore the above-mentioned problem of the recovery of locked-in strains during the creep test is still remaining. Hence we decided to elaborate new test procedure consisting of creep cycles without any quench between the tests at increasing temperature levels (going from 30 to 70°C). Stabilisation period preceding creep test at a given temperature allows the measurement of possible recovery. Extrapolation of the recovery

signal for the duration of the following creep test will be used to correct creep measurements. Using this procedure, we hope to measure correctly viscoelastic properties and determine all parameters of a rheological model allowing the prediction of a green wood behaviour at a long time scale.

References

Levita G, Struik LCE (1983) Physical ageing in rigid chain polymers. Polymer.Volume 24, Issue 8: 1071-1074.

Passard J and Perré P (2005) Viscoelastic behaviour of green wood across the grain. Part II. A temperature dependent constitutive model defined by inverse method. Annals of forest science 62(8): 823-830

Struik LCE (1976) Physical aging in amorphous glassy polymers. Annals of the New York Academy of Sciences 279: 78-85

Peroxidases and lignification in flax stem

Frédérique Nolin[1], Anouk Habrant[1], Godfrey Neutelings[2], Simon Hawkins[2], Brigitte Chabbert[1]

[1]*UMR 614, INRA-URCA Fractionnement des AgroRessources (FARE), Equipe Structures et Propriétés des Parois secondaires, 2 Esplanade R.Garros, BP 224, 51686 Reims, France*
[2]*UMR 1281, USTL-INRA Stress abiotiques et différenciation des végétaux cultivés, UFR de Biologie, Université des Sciences et Technologies de Lille, 59655 Villeneuve d'Ascq, France*

During the last step of lignification, phenolic radicals are polymerised in a non-enzymatic step. These radicals are probably generated through the action of peroxidases (PODs) that, *in vitro*, can oxidise monolignols using H_2O_2 (Piontek 2002). Several anionic and cationic isoforms have been reported in plants as soluble and wall-linked POD (Tsusumi *et al*. 1998). Although the contributions of cell-wall peroxidases have been repetitively reported, the precise role of the various POD isoforms in lignification is still unsolved. Notably, studies based on *in vitro* synthesis of model lignins as dehydrogenative polymers (DHP) are controversial. Some studies have reported that anionic PODs are involved in lignification (Christiansen *et al*. 2001, Li *et al*. 2003) while others authors have proposed that cationic PODs would be mainly responsible for cell wall assembly and lignification (Sasaki *et al*. 2004, Koutaniemi *et al*. 2005). In this case, the preferential location of cationic PODs in the cell corners and the intercellular layer supports the involvement of these isoforms in the initiation of lignification (Sasaki *et al*. 2006).

In flax stem tissues, lignin represents nearly 25% of cell wall polymers in the inner core (xylem) whereas only very low amounts are deposited in bast fiber cell walls. Generally, flax lignin is characterized by the predominance of type G condensed lignin (Day et al. 2005). In order to gain a better understanding of the lignification process in flax, cell wall peroxidases were obtained from stem tissues (bast fibers or xylem) of plants harvested at the flowering stage (McDougall *et al*. 1992), and isolated by gel chromatography for *in vitro* synthesis of DHPs. Further separation on DEAE ion exchange chromatography allowed the isolation of anionic and cationic forms (Fernandes *et al*. 2006). These isoforms were compared with regards to they ability to oxidize monolignols and to form polymers *in vitro*. Data are discussed regarding the possible role of POD isoforms in the deposition of the guaiacyl-rich lignin characteristic of flax xylem and bast fibers.

References

Christensen *et al*. (2001) Plant Molecular Biology 47 : 581-593
Day *et al*. (2005) Planta 222 : 234-245
Fernandes *et al*. (2006) Journal of Plant Physiology 163 : 1040-1048
Li *et al*. (2003) J. Plant. Res. 116: 175-182
McDougall *et al*. (1992). Phytochemistry 31: 3385-3389
Koutaniemi *et al*. (2005) Plant Molecular Biology 58: 141-157
Piontek K (2002)New insights into lignin peroxidase. 0376-4710, 41: 46-53
Sasaki *et al*. (2004) FEBS Letters 562 : 197-201
Sasaki *et al*. (2006) Plant Molecular Biology 62: 797-807
Tsusumi *et al*. (1998) Holzforschung 52: 275-281

Mechanical characterization of wood at the submicrometre scale: a prospective study

Karl Bytebier[1], Oliver Arnould[1], R. Arinero[2]

[1] *Laboratoire de Mécanique et Génie Civil, Université Montpellier 2*
[2] *Institut d'Electronique du Sud, Université Montpellier 2*

Measuring the elasto(plastic) properties of an homogeneous/isotropic material is now usually available at the microscopic scale by using nanoindentation and AFM techniques. Some studies show the possibility to measure elastic properties of anisotropic materials, some approaches try to determine the elastic properties of multi-layered isotropic material at the nano or micrometre scale and local measurements by nanoindentation on wood have already shown that mechanical properties of each cell wall layer can be estimated if these layers are sufficiently thick. The goal here is to extend these techniques to the case of any type of cell-wall layer using reverse identification of the mechanical properties. As a first step, the measurement of elastic properties on reference materials like sulfur monocristal or polymers using different AFM techniques has been tried. Preliminary tests on wood specimens are under way.

Ozone alters cellulose and lignin biosynthesis in tension wood of poplar

Mireille Cabané[1], Nicolas Richet[1], Rana El Zein[1], Françoise Huber[2], Brigitte Pollet[3], Jacques Banvoy[1], Pierre Dizengremel[1], Catherine Lapierre[3], Patrick Perré[2], Dany Afif[1]

[1]*Equipe Ecophysiologie Cellulaire et Moléculaire, UMR 1137 INRA-UHP Nancy 1, Ecologie et Ecophysiologie Forestières, BP 239, 54506 Vandoeuvre les Nancy, France*
[2]*Laboratoire d'Etudes et de Recherche sur le Matériau Bois (LERMAB), Unité Mixte de Recherche ENGREF-INRA-Université Nancy I (Henri Poincaré), 14 rue Girardet, CS 4216, 54042 Nancy CEDEX, France*
[3]*Laboratoire de Chimie Biologique, UMR 206 INRA-INA-PG, Institut National Agronomique, 78850 Thiverval-Grignon, France.*

Because of the changing climate, the concern for the future of forest ecosystems is increasing. Ozone is the third most important greenhouse gas and its concentration have increased continuously since the Pre-industrial Era. Tropospheric ozone is a potent atmospheric pollutant that causes widespread damages to plants. Damage estimates based on current ozone concentrations indicate billions of dollars in losses for agricultural crops annually and significant impacts on forest tree productivity. However, ozone impacts on wood are not well documented. The aim of this work was the study of the effect of ozone concentration on the biosynthesis of two main wood components, lignin and cellulose.

Poplar trees (*Populus tremula x alba*) (3 month-old) were fumigated for 46 days at different ozone levels (50, 100, 200, 300 ppb) during the light period in phytotronic chambers,. Trees were bended at an angle of 45° in order to control tension wood formation.

Lignification and cellulose biosynthesis were analysed in tension and opposite wood at different stem heights. Different enzymes involved in lignin biosynthesis pathway or in upstream supply pathways were estimated at activity and RNA levels. Cinnamyl alcohol dehydrogenase (CAD), which catalyses the synthesis of monolignols, phenylalanine ammonia-lyase (PAL), the first enzyme of the phenylpropanoid pathway, and shikimate dehydrogenase (SHDH) were studied. Different enzymes of the cellulose biosynthesis pathway were analyzed, sucrose synthase (SuSy), uridine diphosphate glucose pyrophosphorylase (UGPase), producing the UDPG the substrat of cellulose synthase, and uridine diphosphate glucose dehydrogenase (UGD) utilizing the UDPG to produce glucuronic acid. Ozone reduced the activity of enzymes involved in lignin and cellulose pathways. Cellulose content was decreased but lignin amount was increased under ozone fumigation. These effects were more pronounced in tension than in opposite wood suggesting that tension wood formation was more ‘ozone sensitive’.

Mechanical properties of *Arabidopsis* hypocotyls treated with XEG

Kazuchika Yamauchi[1], Willie Abasolo[1,2], Lutz Neumetzler[3], Markus Pauly[3,4], Ingo Burgert[1]

[1]*Max-Planck-Institute of Colloids and Interfaces, Department of Biomaterials, Potsdam, Germany*
[2]*College of Forestry and Natural Resources, University of the Philippines Los Baños, Philippines*
[3]*Max-Planck-Institute of Molecular Plant Physiology, Potsdam, Germany*
[4] *Plant Research Laboratory, Michigan State University, East Lansing, USA*

The primary cell wall is composed of cellulose, hemicelluloses, pectin, and structural proteins building a load-bearing cell wall network. The molecular structures of the components are well-known, but the interaction and bonding characteristics are not yet fully understood. To study the interaction of polysaccharides in primary cell walls, xyloglucan was partially removed by a specific cell wall polysaccharide hydrolyzing enzyme and the mechanical response of the hypoctoyls were investigated. Hypocotyls of 4-day-old etiolated *Arabidopsis* plants were harvested and treated with *endo*-β-1,4-glucanase (XEG) solution. The stiffness and strength of the hypocotyls were examined in microtensile tests. XEG treated hypocotyls showed lower strength and stiffness than the hypocotyls without enzyme treatment. The results are interpreted with regard to a tethering function of xyloglucan in the cell wall.

Tensile properties of single Norway spruce fibres with different amounts of dislocations

Michaela Eder[1*], Nasko Terziev[2], Geoffrey Daniel[2], Ingo Burgert[1]

[1] *Max-Planck-Institute of Colloids and Interfaces, Department of Biomaterials, Germany.*
[2] *Wood Ultrastructure Research Centre, Department Forest Products/Wood Science, Swedish University of Agricultural Sciences, Uppsala, Sweden.*

Axial compressive stresses can cause deformations within the wood cell wall, so-called dislocations, microcompressions, slip planes etc. These deformations are thought to occur already in the living tree or develop during wood processing. It has been shown recently that pulp & paper and other fibre-based products are strongly influenced by fibres that contain dislocations (Terziev *et al*. 2005). For characterizing the influence of dislocations on the mechanical properties of the cell wall, single fibres with different amounts of dislocations were subjected to tensile tests and cyclic loading experiments. A comparison between micromechanical properties of reference fibres and fibres that were artificially loaded in compression revealed the importance of dislocations for the mechanics of both earlywood and latewood. High amounts of dislocations resulted in a decreased initial stiffness of the fibres but a kind of re-stiffening was observed during ongoing stretching. Tensile strength (decrease ~ 19% for earlywood and ~ 26% for latewood) was less affected than one would expect from structural observations of the pre-compressed zones.

Terziev N, Daniel G, Marklund A (2005) Dislocations in Norway spruce fibres and their effect on properties of pulp and paper. Holzforschung 59:163-169

Fibre-matrix-transitions in palm trees – structure and mechanics

Markus Rüggeberg[1,2], Ingo Burgert[2], Thomas Speck[1]

[1] *Plant Biomechanics Group Freiburg, Botanic Garden of the University*
[2] *Max-Planck-Institute of Colloids and Interfaces, Department of Biomaterials, Potsdam, Germany*

Plants have developed a range of mechanisms to vary physiological and mechanical properties of their tissues and cells. Here, we investigate alterations in mechanical properties, structural parameters and the amount of lignification in the transition area of stiff fibres and soft parenchymatous tissue. The aim of our study is a better understanding of structure-function-relationships of gradients in biological systems.

We have taken palm tree species as model organisms, as they have extended fibre caps accompanying the vascular bundles. These fibre caps are responsible for the overall stiffness of the plant stem and protect the water and nutrition conducting tissues. Microtensile tests have been performed with series of sequential fibre strips across the fibre cap. The cellulose microfibril orientation was measured with synchrotron microbeam X-ray scattering and the amount of lignification was investigated with spectroscopic methods. The results show gradual transitions in the elastic modulus, structural parameters and amount of lignification across the fibre cap. We will compare these findings with a second model organism from a different plant family.

Investigations on root contraction in red clover (*Trifolium pratense*)

Nicole Schreiber[1,2], Notburga Gierlinger[2,3], Ingo Burgert[2], Peter Fratzl[2], Norbert Pütz[1]

[1] *Institute of Didactics of Science, Biology, University of Vechta, Germany*
[2] *Max Planck Institute of Colloids and Interfaces, Research Campus Golm, Potsdam, Germany*
[3] *APART (Austrian Programme for Advanced Research and Technology)*

One of the most impressive plant movements is the phenomenon of root contraction which is known for a wide variety of Mono- and Dicotyledons. Root contraction is of vital ecological importance for geophytes, because it enables the plant to regulate its depth in the ground. This protects buds from frost and supports the stability of the plant. In addition the contraction facilitates seedling establishment in plants with contractile roots.

The underlying mechanisms of root contraction at the cellular level are not yet fully understood – especially in Dicotyledons. In this project contractile roots of red clover (*Trifolium pratense*) were structurally analysed. Light and Raman microscopy was used to analyse thin sections of the main roots of plants of different ages.

Predominantly the proximal parts of the *Trifolium* roots showed highly deformed tissues. Vessels were bent around the surrounding parenchyma or even kinked. Clusters of tension wood fibres were evenly spread over the entire cross-section. The classical mechanism of root contraction is attributed to radial growth of cells. To find tension wood fibres rather suggests that they may enable the plant to generate the high tensile stresses needed for achieving a severe root contraction.

Chemical imaging of tension wood in tropical rain forest species by Confocal Raman microscopy

Notburga Gierlinger[1], Puneet Singla[1], Julien Ruelle [2] and Bruno Clair[3]

[1]*Max-Planck-Institute of Colloids and Interfaces, Department of Biomaterials, Potsdam, Germany*
[2]*UMR "Ecologie des Forets de Guyane" (ECOFOG) CIRAD DNRS ENGREF INRA UAG, BP 316, 97379 Kourou, Guyane Francaise, France*
[3]*Laboratoire de Mecanique et Genie Civil 8lmgc9, UMR 5508, DNRS, Universite Montpellier 2, Place E.Bataillon, cc 048, 34095 Montpellier Cedex 5, France*

Within tension wood of differentent tropical rainforest species the distribution and occurrence of gelatinous fibres varies from species to species and learning more about the chemistry in context with cell wall structure will help to understand growth stress phenomena. Confocal Raman microscopy allowed to follow lignification and cellulose orientation with a high spatial resolution (<0.5µm) on the cell wall level. Spectral maps were acquired from cross-sections of tension and opposite wood and by integrating over defined wavenumber areas, images based on chemical changes within and between the cell wall layers were calculated. For temporal hardwood species (poplar, oak) lignin accumulation was already reported within the G-layer. In these species lignification was always restricted to a layer towards the lumen and to low concentrations (max 50% compared to the normal secondary cell wall content). In the tropical species *Symphonia globulifera* and *Laetia procera* high levels of lignification were found on the tension wood side. *Symphonia globulifera* showed again the tendency of higher lignification towards the lumen and in *Laetia procera* a multilayered structure with the occurrence of small layers with higher lignification was imaged. So on contrary to more uniform lignification in normal secondary cell walls, in tension wood an inner layer of higher lignin content is typical and for the family of the *Flacourtiaceae* an alternate layering with different lignin content.

Lignocellulose feedstocks for cell wall biorefining

Ralf Möller[1], Markus Pauly[2], Sarah Hake[3], Marcel Toonen[4], Jan B. van Beilen[5], Elma Salentijn[4], David Clayton[6] and Dianna Bowles[6]

[1] *Max Planck Institute of Molecular Plant Physiology, Germany*
[2] *Plant Research Laboratory, Michigan State University, USA*
[3] *Plant Gene Expression Center, USDA, USA*
[4] *Plant Research International, The Netherlands*
[5] *Université de Lausanne, Switzerland*
[6] *CNAP, University of York, UK*

Crop platform for cell wall biorefining were analysed within the EPOBIO project – a Science to Support Policy Project funded by the European Commission. The key objective of EPOBIO is to design new generations of bio-based products derived from plant raw materials that will reach the market place 10-15 years from now and our aim is that the knowledge gained from EPOBIO will underpin the development of bio-renewables in the emerging knowledge-based bio-economy of this new century. The cell walls research area within EPOBIO is aimed specifically at decreasing the current economic risks associated with cell wall biorefining, which include the expense and continuing difficulties of efficient fractionation of biomass. Here we present the key findings of our latest report, which evaluated biological, agronomical, environmental and economic aspects of crop platforms providing lignocellulose biomass for biorefining. Four sources of biomass of relevance to Member States of the EU were considered as case studies and their strengths, weaknesses, opportunities and threats (SWOT) were analysed. These were poplar and willow, *Miscanthus* and wheat straw, which have been chosen as representative of woody species, grass and a co-product from arable crop cultivation.