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Field Emission SEM Studies on Softwood Tracheids and Hardwood Fibres – A Review of Activities at the EMPA Wood Laboratory

TANJA ZIMMERMANN and JÜRGEN SELL

Keywords: FE-SEM; fine structure; S2 layer; transverse fracture surfaces; softwoods; hardwoods; reaction wood; white rot fungi. FDK 81 : 945

ZIMMERMANN, T.; SELL, J.: Field Emission SEM Studies on Softwood Tracheids and Hardwood Fibres – A Review of Activities at the EMPA Wood Laboratory

1. Studies on fracture surfaces of softwoods

First studies on transverse fracture surfaces of spruce tracheids at the EMPA wood laboratory investigated the influence of temperature, wood moisture content and duration of load on the fine structure of tension fracture surfaces of bending-loaded spruce samples by means of a high-resolution SEM with a field-emission cathode (FE-SEM). In addition the aim was to detect weak elements in the wood structure that would be the first to break when tension-loaded (ZIMMERMANN *et al.* 1994).

The influence of the loading conditions, especially the load duration, on the tension strength and deformation of the microscopic fracture surface of the cell tissue, and of the cell wall was clearly visible. It was very easy to distinguish between brittle fractures of the cell wall and ductile fractures. Heat and moisture increased the ductile character of the fracture surface (cf. *Figures 1* and *2*).

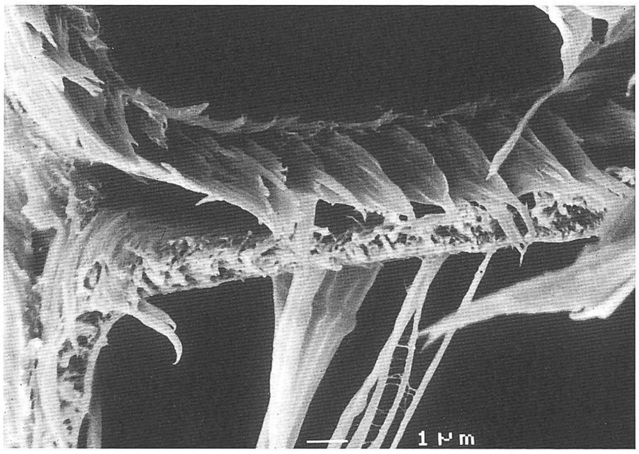

Figure 1: **FE-SEM micrograph: Transverse fracture surface of an earlywood tracheid, long-term bending-loaded, 60 °C/95% RH. Extremely ductile fracture with intense deformation of the entire cell wall tissue.**

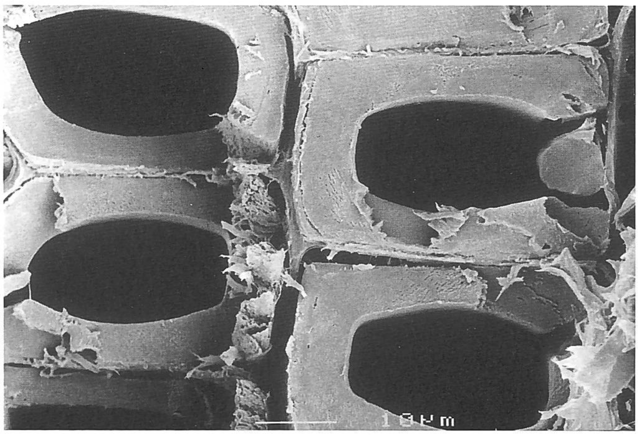

Figure 2: **FE-SEM micrograph: Transverse fracture surface of a latewood tracheid, impact bending at 20 °C/35% RH. Extremely brittle fracture with a clean surface of the S2, the fibril/matrix structure is not visible. Delaminations between S1 and S2.**

Figure 3: **FE-SEM micrograph: Transverse fracture surface (tension zone) of an earlywood cell of spruce, long-term bended at 20 °C/35% RH. The ductile fracture exhibits distinct radial agglomerations of cellulose fibrils (arrow). The cell wall structure resembles a sandwich.**

2. A modified model for the cell wall of softwood tracheids

When tension-loaded until fracture, the cell wall substance of softwood tracheids become loosened and the different wood components, cellulosic fibrils and lignin/hemicellulose matrix are separated. This effect allowed us to study the cell wall fine structure with the FE-SEM.

On transverse fracture surfaces by far the thickest secondary layer (S2) of cell walls of spruce and white fir shows an orientation of fibril agglomerations perpendicular to the middle lamella (radial relative to the longitudinal axis of the cell). The origin of these structures is explained by a higher packing density and stronger adhesion of the fibrils in the radial direction than in the circumferential direction. We can thus conclude that the strength of the bonds between fibril agglomerations of the S2 is greater in the radial than in the tangential direction. Concentric lamellae of the S2 fibrils (parallel to the ML) described in many studies could not be found. As far as we can tell with the FE-SEM, the single fibrils of the S2 are between 20 and 100 nm in diameter. The tangential width of the fibril agglomerations lies between 0.1 and 1 µm. In the radial direction the fibril agglomerations cover a considerable part of the S2 width, frequently extending from the S1 to the S3.

On transverse fracture surfaces therefore (in particular with ductile fractures), the structure of the entire secondary wall has the appearance of a sandwich, whereby the S1 and

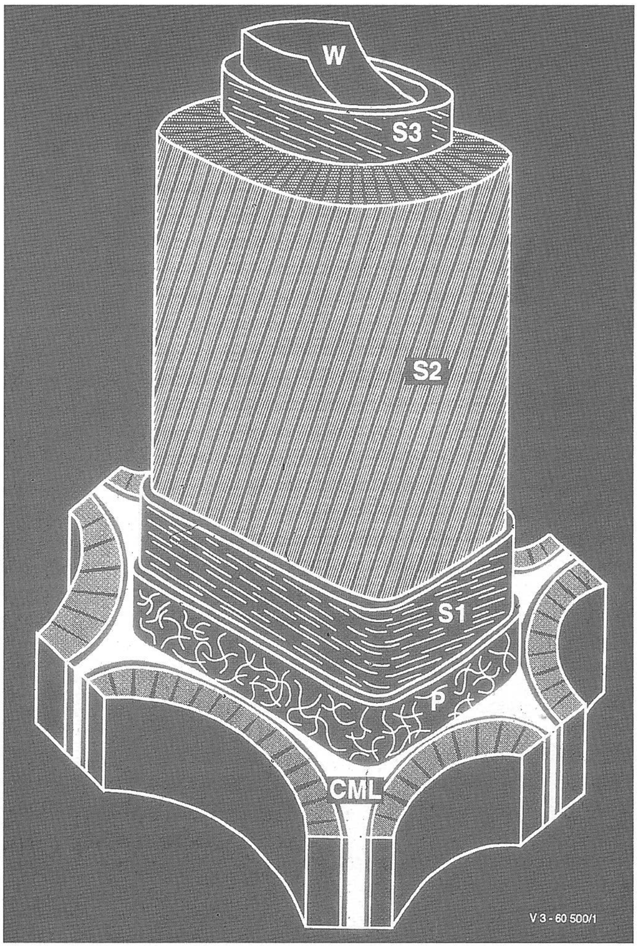

Figure 4: **Cell wall model (after Côté 1965 from CORE *et al.* 1979) with the different cell wall layers. The model is modified by (strongly schematised) radial agglomerations of fibrils on the cross section of the S2. Dimensions and character of the (white) intermediate zones are not identified.**

the S3 are on the outside and the S2 acts as the filling with fibril agglomerations perpendicular to the face layers *(Figure 3).*

These results are thoroughly described in SELL & ZIMMERMANN 1993 (a and b) and led to a modification of a model proposed by Côté 1965 (in CORE *et al.* 1979) *(Figure 4).*

3. Biomechanical aspects

Our main interest was not the nanostructure of the cell wall of wood itself but the functional advantages of such structures in a living tree. Obviously, the proposed radial fibril assembly within the S2 – or, in other words, fibril agglomerations perpendicular to the «cross banding faces» S1 and S3 – would be beneficial to the mechanical properties of the entire tree. The bending stiffness and thus the buckling resistance of such a sandwich-like cell wall under a longitudinal compressive load might be markedly higher than that of a cell wall of concentric lamellae. Therefore, the bending stiffness of the whole stem would be improved when loaded by wind pressure or by forces of gravity. This is particularly important for wood with moisture contents (MC) close to or above the fibre saturation point where the strength of wood is significantly reduced. This is the case for the compression zone of a living tree under bending load, which lies in the outer sapwood where the MC far exceeds the saturation point. The functional advantage of such a cell wall structure can be illustrated *(Figure 5)* in a model test (SELL 1994).

As to be expected there is no significant difference of the stiffness of the two sample types tested at normal temperature under dry conditions when the bond of a PVAC glue is not reduced. However, when an increased temperature – and particularly a high wood moisture content – markedly reduces the glue bond, the bending stiffness of the sample with «standing» veneers in the middle layer is only reduced by approximately 25 or 50%, respectively. On the other hand, the bending stiffness of the samples with «lying» veneers is reduced by approximately 75 to 100%, respectively. These results may illustrate the beneficial role of «standing» structure elements of the S2 layer to the stiffness of the wood cell wall.

This topic is discussed at length in an article by BOOKER & SELL 1998. The paper reviews findings on the nanostrucure of the cell wall of softwoods and discusses the probable relationship between microfibril directions in the secondary cell wall layers and potential threats to the survival of trees, such as excessive vibration and crack propagation.

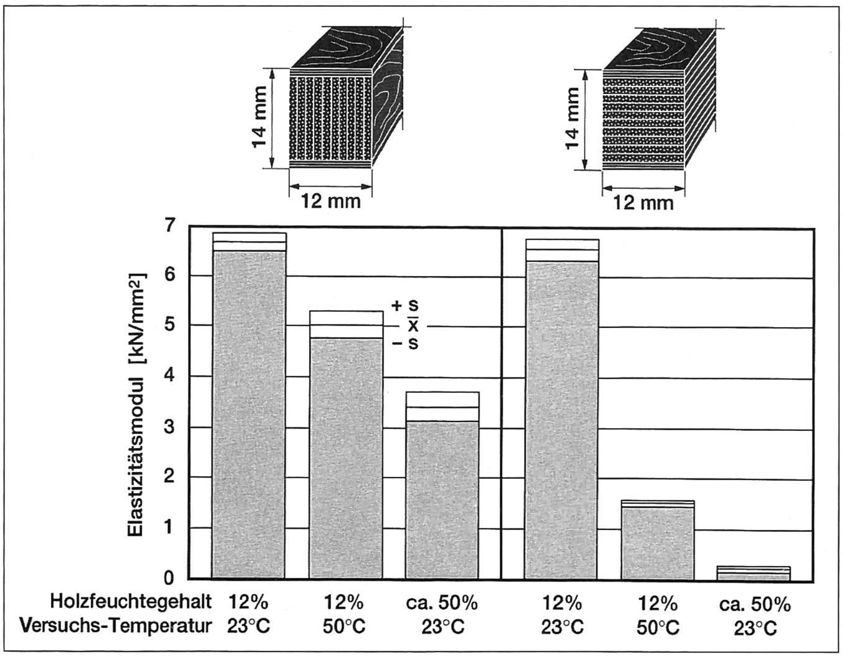

Figure 5: **Modulus of elasticity of glued veneer reproductions of the former and the modified cell wall model («standing» and concentric fibril orientations of the S2, respectively) at different wood moisture contents and temperatures.**

4. The fine structure of the cell wall of hardwoods

We were also interested in the question of whether the cell walls of hardwoods show radial structures like softwood tracheids. As in earlier works with softwoods, the morphological fine structure of the cell wall of beech *(Fagus sylvatica)* and oak *(Quercus robur, Quercus petraea),* and to a lesser extent of some other hardwood species, has been studied by means of a FE-SEM. To differentiate the cellulose fibrils (particular of the thick S2 layer) from the lignin matrix and the accompanying hemicellulose, we examined the transverse fracture surfaces of wood samples, which had been broken in longitudinal tension. Due to the fracture process the cell wall becomes loosened and its components can thus be distinctly observed (as opposed to smooth cross sections of cut wood).

Similar to softwood tracheids, we observed distinct fibril agglomerations of the S2 layer of the fibre cell walls of all hardwoods studied *(Figures 6 and 7).* The

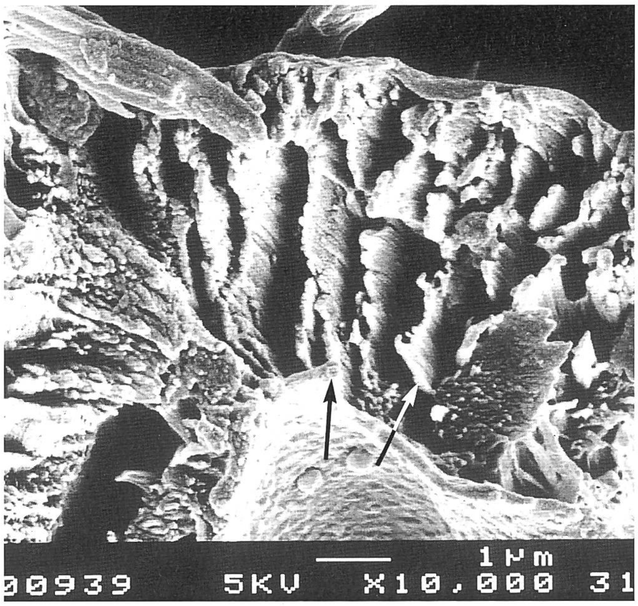

Figure 6: **Transverse fracture surface (tension zone) of a libriform fibre of beech; short-time static bending test at 20 °C/85% RH. Relatively intense deformed cell wall, the radial agglomerations (arrows) extend over the whole S2 cross section.**

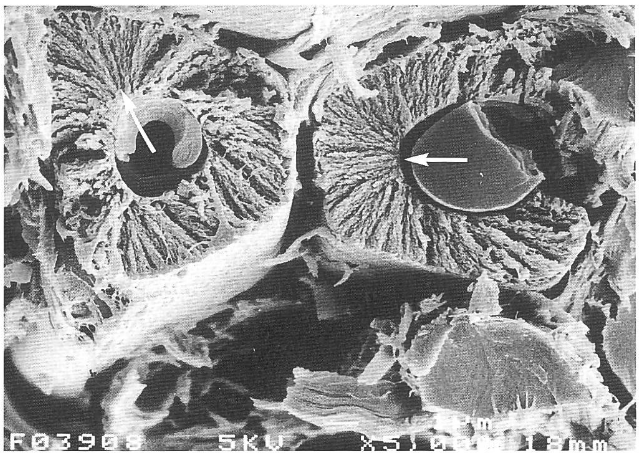

Figure 7: **Transverse fracture surface (tension zone) of fibres of oak; impact bending at 20 °C/35% RH. Distinct preferentially radial orientations of the cellulose fibrils (arrows); deposited extractives in the cell lumen.**

fibril agglomerations are oriented in a perpendicular or partly perpendicular direction to the middle lamella and to the S1 and S3 layer, respectively. In other words, they are oriented (mostly) radially with respect to the longitudinal axis of the cell wall. Sporadically, concentric (tangential) structures («lamellae») of the S2 are also exhibited. In a few cell walls there were even mixed radial and tangential fibril orientations on transverse fracture surfaces within a single S2 layer. However, only fibre cells (libriform and tracheid fibers) showed the radial or partially radial fibril orientations of the S2, which we did not find with parenchyma cells and vessels. The latter exhibited only a polylamellar (concentric) structure of the transverse fracture surface of the entire cell wall (*Figures 8* and *9).*

These results on the S2 structure, which differ from the majority of numerous earlier studies published over the past decades, have been discussed at length in (Zimmermann & Sell 1997, Sell & Zimmermann 1998) regarding the possible influence of artefacts. Especially the tension-fracture conditions could create a distinct radial crack propagation in the S2 layer. However, the more or less pronounced radial orientation of fibril agglomerations – and therefore implicitly of the lignin matrix of the cell wall – has been confirmed in the meantime by several independent investigations using various methods. For instance, transmission electron microscopic investigations (Pöhler 1995, Singh *et al.* 1998) and decomposition studies of the cell wall of hardwoods with fungi (Schwarze & Engels 1998) provide additional confirmation of a radial arrangement of lignin and/or cellulose of the S2 layer.

The results support the hypothesis: The radial assembly of the cellulose fibrils (and probably of the lignin matrix) of the S2 layer forms, together with the S1 and S3 layers acting as cross-banding faces, form a sandwich-like structure of the cross-section of the cell wall. This might be beneficial for the bending stiffness and buckling resistance of the cell wall and for the longitudinal compression strength of the wood in the standing tree.

However, it has still not been conclusively explained why different microscopical, chemical, and other studies used in numerous former investigations have exhibited both concentric and/or radial arrangements of the cellulose fibril/lignin matrix structure of the S2 of the cell wall. It is, therefore, quite plausible that these structures coexist.

5. The fine structure of the cell wall of reaction wood

As in the earlier investigations of normal wood, the arrangement of the cellulose fibrils on the tensile fracture surfaces of the cell walls of white fir, spruce and pine *(Pinus sylvestris* and *Pinus radiata)* compression wood and of beech tension wood was investigated with a high resolution FE-SEM (Zimmermann & Sell 2000). To a lesser extent the tension wood of other hardwood species was also investigated. The fine structure of the tracheid walls in *Pinus radiata* mild compression wood was also examined by transmission electron microscopy (TEM) (Singh *et al.* 1998).

The examined compression wood exhibits the fibril orientation of the thick S2 layer of the cell wall observed in normal wood, likewise with a markedly preferential orientation approximately transverse to the compound middle lamella (CML) *(Figure 10)*. The TEM observations of ultra-thin sections of samples from *Pinus radiata* showed the S2 layer with radial striations, corresponding in their direction to the radial structures seen with the FE-SEM (Singh *et al.* 1998) *(Figure 11).* While the S2 and G layers of beech fibres of mild tension wood

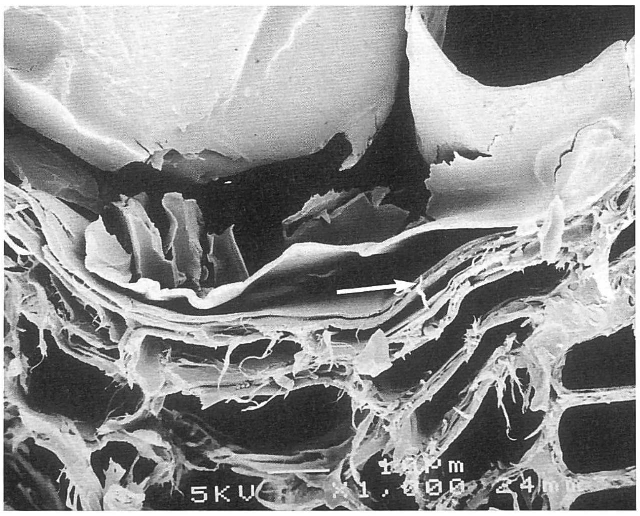

Figure 8: **Transverse fracture surface of a cell wall area of an earlywood vessel of chestnut; in contrast to the fibres no radial structures are visible. On the contrary, the cell wall seems to consist of numerous concentric lamellas.**

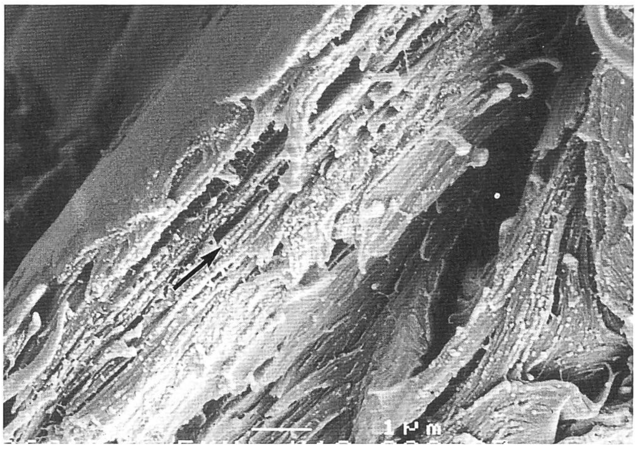

Figure 9: Transverse fracture surface of a cell wall area of an earlywood vessel of oak; the cell wall seems to consist of numerous concentric lamellas (arrow).

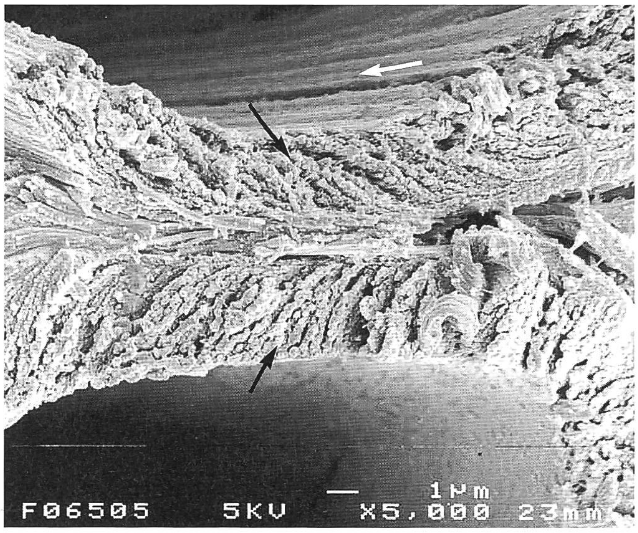

Figure 10: Transverse fracture surface (tension zone) of the cell wall of a tracheid of mild compression wood of pine *(Pinus radiata)*. There is a discernible preferential orientation of the cellulose fibrils rather transverse to the CML (black arrows). Indicatively, slight deep (radial) fissures are discernible transverse to the CML (white arrow).

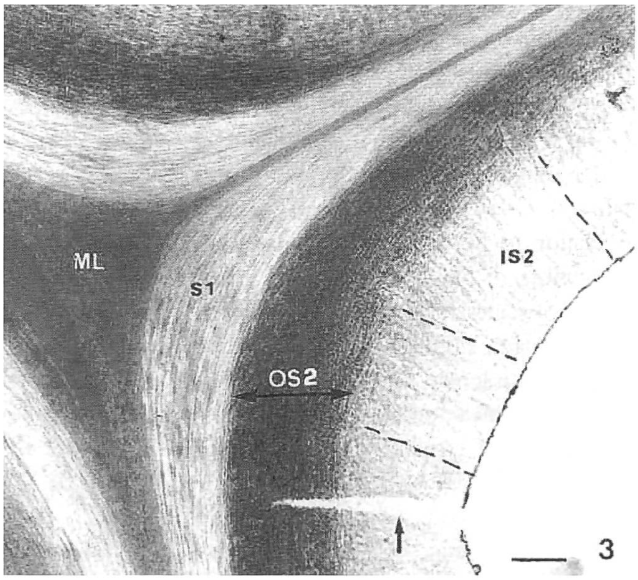

Figure 11: Transverse section through a corner region of mild compression wood tracheids. The secondary wall is divided into 3 morphologically different regions, the S1, the OS2 and IS2. The IS2 wall appears to be striated in a direction largely perpendicular to the plane of the middle lamella (ML) (stippled line). TEM micrograph, from Singh *et al.* 1998.

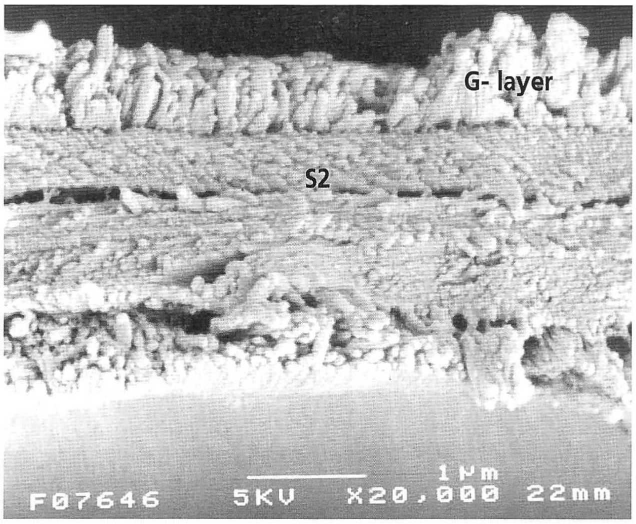

Figure 12: Transverse fracture surface (tension zone) of elm fibre cell walls. No preferential orientation of the cellulose fibrils in the S2 or G layer is discernible.

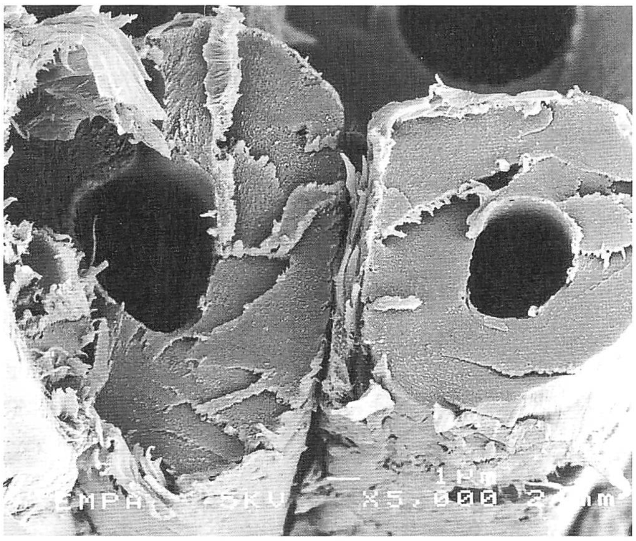

Figure 13: Transverse fracture surface (tension zone) of two beech fibre cells. The fracture of the thick G layer is so brittle that no fibril orientation is discernible.

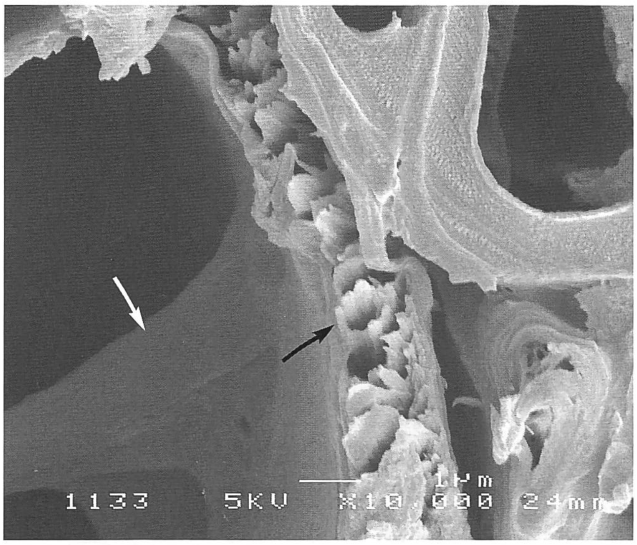

Figure 14: TS of two adjacent fibres of sycamore wood, artificially inoculated with *Flammulina velutipes* at an early stage of delignification. A hypha is growing within the lumen on the S3 layer (white arrow). This layer shows no structural alterations, whereas the underlying S2 layer has been partially degraded. Preferential delignification of the S2 results in the exposure of fibril agglomerations, arranged perpendicular to the CML (black arrow).

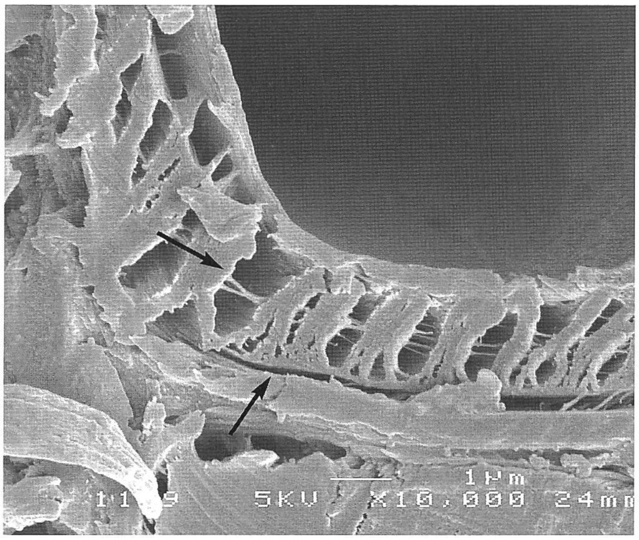

***Figure 15:* FE-SEM micrograph: TS of a fibre cell wall of sycamore artificially inoculated with *Flammulina velutipes*. In addition to radial structures the S2 layer also shows concentrically oriented fibrillar elements (arrows).**

exhibit transverse structures in opposing directions in some cases, there is no discernible preferential orientation of the cellulose fibrils in the S2 or G layer of pronounced tension wood cell walls (*Figure 12* and *13).*

These findings were interpreted to mean that the cell walls of normal wood, but especially of compression wood, are stiffened against longitudinal compression and buckling by the observed compressed arrangement of the fibrils of the S2 layer transverse to the CML. On the other hand, however, in tension wood fibres of hardwood trees exposed solely to longitudinal tension, this compression stiffening of the cell walls is not required and the radial fibril agglomerations are consequently absent.

6. Fungi as a tool to determine the arrangement of wood cell wall components

The fine structure of transverse sections of hardwood cell walls (sycamore, small-leafed lime and common beech), that had been degraded at an early stage by white rot fungi, was studied with FE-SEM. The structural features observed were compared with the cell wall structure typically found on transverse fracture surfaces of sound wood.

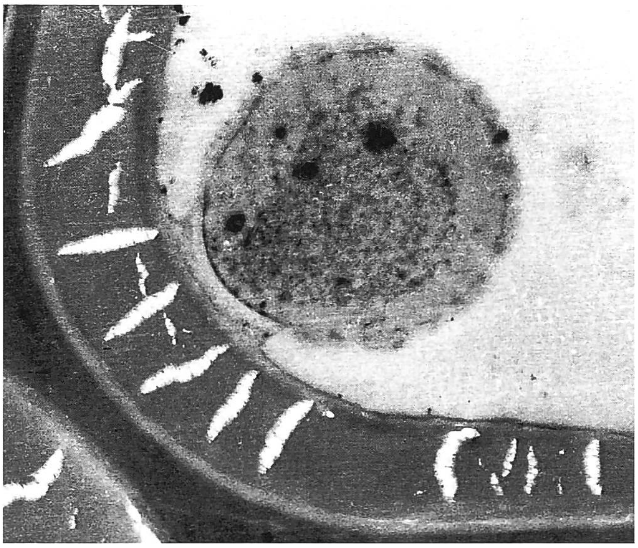

***Figure 16:* TEM-picture out of a study published by SCHWARZE & FINK 1999: At an early stage of fungal degradation radial and concentric clefts became visible.**

Transverse sections (TS) of partially delignified hardwood fibre and vessel cell walls exhibited characteristic structural features, which were also observed on the fracture surfaces of sound wood. This was particular evident in the radial arrangement (i.e. perpendicular to the middle lamella) of the fibrils within the S2 layer *(Figure 14).* Similar to sound wood, the partially delignified samples also showed tangential (concentric) structural features within the S2, which were however, less distinct than the radial structures *(Figure 15)*. This result corresponds with findings of (SCHWARZE & FINK 1999). The authors published a light microscopic and TEM study, showing characteristic radial and concentric clefts in transverse sections of Norway spruce secondary walls during early stages of decay caused by the white rot fungus *Stereum sanguinolentum (Figure 16).*

During incipient stages of lignin degradation the greatest loss of cell wall substance was initially observed within the S2. This layer was often extensively degraded, while the S3 layer and often also the S1 and CML appeared to be more or less intact.

7. Future activities

We are planning to apply different analytical (chemical), microscopical and spectroscopical methods to obtain new findings about the fine structure of the wooden cell wall. The aim is a convincing model of the cell wall that combines different investigations showing both concentric and/or radial arrangements (perpendicular to the other cell wall layers) of the cellulose fibril/ lignin matrix structure of the S2 of the cell walls of soft- and hardwoods.

Summary

Over the past ten years transverse fracture surfaces of tension-loaded softwoods, hardwoods and reaction wood have been studied using a high resolution FE SEM. Due to the fracture process the cell wall becomes loosened and its components can be distinctly observed (as opposed to smooth cross sections of cut wood). Softwood tracheids (compression wood, too) and hardwood fibres showed predominantly radial orientations (perpendicular to the other layers) of the cellulosic fibrils of the secondary wall layer 2 (S2). Similar structures were found on sections of different hardwoods degraded by white rot fungi. Contrary to that finding there is no discernible preferential orientation of the cellulose fibrils in the S2 or G layer of pronounced tension wood cells.

In our opinion functional advantages of such structures in the strengthening tissue are quite clear. The sandwich-like structure is important for the buckling resistance of the tracheids and fibre cell walls and therefore also to the bending stiffness of the whole tree. Tension wood fibres of hardwood trees solely exposed to longitudinal tension do probably not need a compression stiffening, explaining why transverse fibril agglomerations are absent here. In many other studies a polylaminated concentric arrangement of the cellulose/ lignin-hemicellulose matrix in the S2 layer is postulated and clearly documented.

Taking our results into account, we conclude that concentric and/or radial arrangements (perpendicular to the CML) in the S2 of softwood tracheids and hardwood fibres coexist for good ontogenetic, physiological, and mechanical reasons.

Zusammenfassung

Feldemissions-Rasterelektronenmikroskop-Studien zu Nadelholztracheiden und Laubholzfasern – eine Übersicht über Forschungsarbeiten am Holzlaboratorium der Empa

In den vergangenen zehn Jahren wurden Bruchflächen von zugbelasteten Nadel-, Laub- sowie Reaktionshölzern mit einem hochauflösenden Feldemissions-Rasterelektronenmikroskop (FE-REM) untersucht. Durch den Bruchprozess wurde die Zellwandsubstanz aufgelockert und ihre Bestandteile und deren Anordnungen konnten dadurch beobachtet werden (im Gegensatz zu glatten Querflächen von geschnittenem Holz). Nadelholztracheiden (auch Druckholz) und Laubholzfasern zeigten vorzugsweise radiale Orientierungen (quer zu den anderen Zellwandschichten) der Cellulosefibrillen in der dicken Sekundärwand 2 (S2-Schicht). Ähnliche Strukturen wurden auf Dünnschnitten verschiedener Laubhölzer gefunden, bei denen die Zellwände von Weissfäulepilzen teilweise abgebaut worden waren. Im Gegensatz zu diesen Ergebnissen konnte keine erkennbare Vorzugsorientierung von Cellulosefibrillen in der S2- oder G-Schicht von ausgesprochenen Zugholzfasern gefunden werden.

Wir sind der Meinung, dass funktionale Vorteile der im Festigungsgewebe gefundenen Strukturen offensichtlich sind: Der Sandwich-ähnliche Aufbau der Zellwandschichten ist wichtig für die Beulsicherheit der Tracheiden- und Faserzellwände und dadurch für die Biegesteifigkeit des ganzen Baumes. Vermutlich benötigen Zugholzfasern, die lediglich Längszugbelastung ausgesetzt sind, keine Druckaussteifung. Quer orientierte Fibrillenagglomerationen sind konsequenterweise in diesem Fall nicht vorhanden. In vielen anderen Untersuchungen wurde eine mehrschichtige, konzentrische Anordnung der Cellulose/Lignin-/Hemicellulosematrix in der S2-Schicht vorgeschlagen und klar dokumentiert.

Unter Berücksichtigung dieser Ergebnisse kommen wir zum Schluss, dass konzentrische und radiale (quer zur Mittellamelle) Anordnungen von Zellwandkomponenten in der S2-Schicht von Nadelholztracheiden und Laubholzfasern aus ontogenetischen, physiologischen und mechanischen Gründen koexistieren müssen.

Résumé

Etudes des trachéides de bois résineux et des fibres de bois feuillus à l'aide du microscope électronique à balayage. Aperçu des travaux de recherche du laboratoire du bois de l'EMPA

Au cours des dix dernières années, le microscope électronique à balayage (MEB), disposant d'une résolution élevée, a permis d'étudier les surfaces de rupture de bois résineux et feuillus soumis à des phénomènes de tension ainsi que de bois de réaction. Le processus de rupture a assoupli la substance de la paroi cellulaire, rendant possible de ce fait l'observation des composants et de leur disposition (contrairement aux sections lisses du bois coupé). Les trachéides des bois résineux (y compris le bois de compression) et les fibres des bois feuillus ont montré une orientation préférentielle de type radial (perpendiculairement aux autres couches de la paroi cellulaire) des fibrilles cellulosiques dans l'épaisse paroi secondaire (S2). Des structures similaires ont été observées sur les sections de différents feuillus dont les parois cellulaires étaient partiellement décomposées par la pourriture blanche. Contrairement à ces résultats, aucune orientation préférentielle n'a été constatée pour les fibrilles cellulosiques dans les couches S2 ou G des fibres de bois de tension prononcé.

Nous pensons que les avantages fonctionnels des structures découvertes dans le tissu de consolidation sont évidents: la structure en sandwich des couches de la paroi cellulaire est importante pour la résistance à la déformation de la paroi cellulaire des trachéides et des fibres, et par conséquent pour la rigidité de l'arbre entier. Les fibres de bois de tension, exposées uniquement à une force longitudinale, n'ont probablement pas besoin d'un renforcement pour résister à la compression. Cela explique pourquoi on ne rencontre pas, dans ce cas, d'agglomération de fibrilles orientées transversalement. De nombreuses autres études admettent et décrivent clairement la présence d'une structure multicouche concentrique de la matrice cellulose-lignine-hémicellulose dans la couche S2.

Compte tenu de ces résultats, nous parvenons à la conclusion que des structures concentriques et radiales (perpendiculairement à la couche intercellulaire) des composants de la paroi cellulaire dans la couche S2 des trachéides des bois résineux et des fibres des bois feuillus doivent coexister pour des raisons ontogénétiques, physiologiques et mécaniques.

Traduction: Claude Gassmann

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