Morphology of Norway Spruce Tracheids with Emphasis on Cell Wall Organisation

Jonas Brändström
Department of Wood Science
Uppsala

Doctoral thesis
Swedish University of Agricultural Sciences
Uppsala 2002

Acta Universitatis Agriculturae Sueciae
Silvestria 237

ISSN 1401-6230
ISBN 91-576-6321-1

Tryck: SLU Service/Repro, Uppsala 2002

Abstract

Brändström, J. 2002. *Morphology of Norway Spruce Tracheids with Emphasis on Cell Wall Organisation.* Doctoral dissertation. ISSN 1401-6230, ISBN 91-576-6321-1

Wood from Norway spruce and other conifers consists mainly (>90%) of one cell type called tracheids. The morphology of these tracheids has a profound effect on wood industrial processes and products made from wood. A more in-depth understanding of tracheid morphology is therefore required in order to improve wood industrial processes and products derived from conifer wood.

The aim of this thesis was to study the morphology of Norway spruce (*Picea abies* (L.) Karst) tracheids with emphasis on cell wall organisation, especially microfibril orientation in the secondary cell wall. A literature review provided an overview of the micro- and ultrastructure of Norway spruce tracheids. The use of cavities from soft rot fungi was discussed and evaluated as a way of determining microfibril angles (MFA) in the secondary cell wall of tracheids. Soft rot fungi were shown to be a useful tool for ultrastructural research and results correlated well with polarisation confocal microscopy when measuring microfibril angles of the middle layer of the secondary cell wall (S2). Both methods showed a decreasing trend in S2 microfibril angle, from the beginning of earlywood (MFA ca. 30°) and towards the end of earlywood (MFA ca. 5°). The low microfibril angles continued into the latewood. No direct correlation was found between tracheid microstructure (*i.e.* cell length, cell width and cell wall thickness) and the microfibril angle in the S2 layer.

Several methods were used to investigate the structural organisation of the outer layer of the secondary wall (S1). It was concluded that the S1 layer is rather homogenous layer being primarily comprised of microfibrils oriented approximately perpendicular to the tracheid axis. No evidence could be found for a crossed fibrillar structure with microfibrils in alternate S and Z helices. The transition of microfibril orientation from the S1 to the S2 layer appeared abrupt.

Cell wall models illustrating microfibril orientation in the cell wall layers were discussed from a historical perspective. Three new cell wall models, based on results obtained in this thesis and from the literature were presented in order to visualise the microfibril orientation in the cell wall layers of Norway spruce tracheids.

Keywords: *Picea abies*, Norway spruce, tracheid, wood fibre, microstructure, ultrastructure, secondary cell wall, morphology, soft rot, cell wall model, microfibril angle

Author's address: Jonas Brändström, Wood Ultrastructure Research Centre, Department of Wood Science, Swedish University of Agricultural Sciences, P.O. Box 7008, SE-750 07 Uppsala, Sweden. E-mail: jonas.brandstrom@trv.slu.se

Wood Ultrastructure Research Centre (WURC)

This work was carried out within the framework of the Wood Ultrastructure Research Centre (WURC). WURC is a centre of competence established in co-operation with VINNOVA, six companies from the pulp and paper industry and one company from the chemical industry. The centre's main operations are at the Swedish University of Agricultural Sciences (SLU) in Uppsala. Research on wood fibre ultrastructure is performed through co-operation between SLU, Swedish Pulp and Paper Research Institute (STFI), Royal Institute of Technology (KTH), Chalmers University of Technology (CTH) and industrial partners (AssiDomän AB, EKA Chemicals AB, Korsnäs AB, M-Real, SCA AB, StoraEnso AB, Södra Cell AB). Website: *www-wurc.slu.se*

List of abbreviations and definitions

CLSM	Confocal Laser Scanning Microscopy
FE-SEM	Field Emission-Scanning Electron Microscopy
LM	Light Microscopy
MFA	Microfibril angle. Defined as the angle between the tracheid axis and the microfibril
ML	Middle lamella
P	Primary cell wall
S1	Outer layer of the secondary cell wall
S2	Middle layer of the secondary cell wall
S3	Inner layer of the secondary cell wall
SAXS	Small-angle X-ray scattering
SEM	Scanning Electron Microscopy
TEM	Transmission Electron Microscopy

Microstructure	Defined as features which are measured in micrometres and are visible by light microscopy (LM).
Morphology	Used in its general meaning of size, shape and structure.
Ultrastructure	Defined as features which are rendered visible by methods which are able to resolve structures at higher resolution than light microscopy. Ultrastructural features are measured in nanometres.

Contents

Introduction **7**

Background 7
Objectives of the study 8
General aspects of tracheid morphology 8
Orientation of microfibrils 11
Cell wall models 13

Materials and methods **15**

Use of soft rot cavities 15
Determination of the microfibril angle in the S2-layer by using polarisation confocal microscopy 15
Determination of the microfibril angle in the S2 layer by using X-ray diffraction 16
Determination of tracheid length, width and cell wall thickness 17
Shear forces applied to wood blocks and macerated tracheids 17
Chemical treatment of wood and macerated tracheids 17

Results and discussion **18**

Micro- and ultrastructure of Norway spruce tracheids 18
Soft rot fungi as a tool to determine microfibril angle 19
Variation of S2 microfibril angle within annual rings; trends, possible causes and consequences for the living tree 20
Variation of S2 microfibril angle within annual rings; consequences for wood fibre products 25
Relationship between tracheid microstructure and microfibril angle in the S2 layer 25
Microfibril orientation in the outer layer (S1) of the secondary wall 26

Conclusions **29**

References **33**

Acknowledgements **39**

Appendix

The present thesis is based on the following papers, which will be referred to by their Roman numerals:

I. Brändström, J. 2001. Micro- and ultrastructural aspects of Norway spruce tracheids: A review. IAWA Journal 22 (4) 333-353.

II. Brändström, J., G. Daniel and T. Nilsson. 2002. Use of soft rot cavities to determine microfibril angles in wood; advantages, disadvantages and possibilities. Holzforschung (*In press*).

III. Bergander, A., J. Brändström, G. Daniel and L. Salmén. 2002. Fibril angle variability in earlywood of Norway spruce using soft rot cavities and polarization confocal microscopy. Journal of Wood Science (*In press*).

IV. Brändström, J., S.L. Bardage, G. Daniel and T. Nilsson. 2002. The structural organisation of the S1 cell wall layer of Norway spruce tracheids.(Submitted to IAWA Journal).

Introduction

Background

The forest industry in Sweden uses about 64 million m³ roundwood every year, of which 51% is Norway spruce (*Picea abies* L. Karst) (National Board of Forestry 2001). Approximately 94% of the volume of Norway spruce wood consists of longitudinal cells called tracheids (Petrić & Šćukanec 1973). Knowledge about the morphology of tracheids is needed to enhance industrial processes and products derived from spruce wood. This is evident for both the pulp and paper industry (Varhimo & Tuovinen 1999; Gullichsen 2000) and the saw mill industry (Dinwoodie 2000). In addition, a detailed knowledge of tracheid morphology is important for understanding the processes involved in cell wall biosynthesis and degradation.

Not only morphological raw data are needed. The information has to be presented clearly to facilitate understanding of tracheid morphology. In this context, models can be helpful since models are usually employed to render theories more intelligible. Cell wall models (Fig. 1) have been presented for approximately one hundred years, but many of the models which are presented in modern literature are out of date since they do not take new research findings into account.

Different microscopical techniques are indispensable tools in morphological research, but it is important to realise that artefacts may always be present. Thus, correct interpretation is also required. It is also important to bear in mind that wood is a three-dimensional structure. For example, a two-dimensional micrograph represents a three-dimensional structure, which requires consideration when interpreting micrographs (Donaldson 2001).

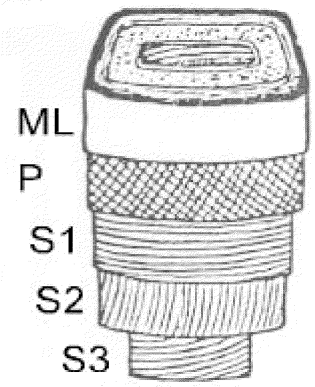


Fig. 1. Cell wall model of a Norway spruce tracheid according to Dolmetsch *et al.* (1944), based on Kerr and Bailey (1934). The labels in the model have been added afterwards and they indicate: the middle lamella (ML), primary wall (P) and the outer (S1), middle (S2) and inner (S3) layers of the secondary wall. Black lines in the layers indicate the microfibril orientation. Reproduced by the permission of Springer Verlag.

Objectives of the study

The aim of the work in this thesis was to study the morphology of Norway spruce (*Picea abies* (L.) Karst.) tracheids with emphasis on cell wall organisation, especially microfibril orientation in the secondary cell wall. Microfibril orientation was studied due to the importance for the mechanical properties of tracheids and since microfibril orientation is always shown in cell wall models.

Morphology is used in its general meaning of size, shape and structure. Microstructure is defined as features which are measured in micrometres and are visible by light microscopy (LM). Ultrastructure is defined as features which are rendered visible by methods which are able to resolve structures at higher resolution than light microscopy, for example by scanning and transmission electron microscopy (SEM and TEM). Ultrastructural features are measured in nanometres. In practice, these definitions are not clear since it is possible to observe ultrastructural features indirectly, such as microfibril angle using light microscopy. Cell wall features at atomic or supramolecular level are not included. The thesis focuses on the morphology of spruce tracheids, and not on their physiology, physics or chemistry.

The main objectives of this thesis are:

- To review current knowledge of the micro- and ultrastructure of Norway spruce tracheids, in order to obtain an overall view of tracheid morphology;
- To discuss and evaluate the orientation of soft rot cavities as a tool for studies of microfibril angle in the cell walls of tracheids;
- To study the variability of microfibril angle in the middle layer (*i.e.* S2) of the secondary wall within annual rings;
- To study the ultrastructure of the outer layer (*i.e.* S1) of the secondary wall of Norway spruce tracheids with special interest on microfibril orientation;
- To discuss visualisation of cell wall ultrastructure in models and present cell wall models based on data generated within the project and recent findings presented in the literature.

General aspects of tracheid morphology

Wood or secondary xylem of conifers consists mainly of longitudinal (axial) tracheids, radially oriented ray tracheids and ray parenchyma cells. Tracheids are often called fibres in pulp and paper research. This can be misleading since the term fibre is a general term of convenience for any long, narrow wood (plant) cell, and can also include cell types such as ray tracheids and ray parenchyma cells. This thesis deals only with longitudinal tracheids from stem wood of Norway spruce (*Picea abies* L. Karst.).

Tracheids are produced in the vascular cambium by periclinal division of fusiform initials. After division the cells enlarge and just before cessation of cell enlargement, deposition of the secondary cell wall is initiated (Abe *et al.* 1997). The secondary wall deposits on the inner surfaces of the existing primary wall

thereby reducing the cell lumen. Completion of cell enlargement and differentiation is followed by cell death. Tracheid morphology has been found to be dependent on both the rate and duration at which the developing cambial derivatives expand and form their secondary walls (Wilson & Howard 1968; Skene 1969; Wodzicki 1971; Skene 1972; Denne 1974; Dodd & Fox 1990).

The cell wall of tracheids is divided into several layers according to the generally accepted terminology proposed by Kerr and Bailey (1934). The layers have varying microfibril orientation (Fig. 1) and chemical content (Fig. 2). Tracheids are interconnected through pits. Bordered pit pairs are found between tracheids (axial or radial) and half-bordered pit pairs are found between tracheids (axial or radial) and parenchyma cells. The half-bordered pit pairs are also called cross-field pits.

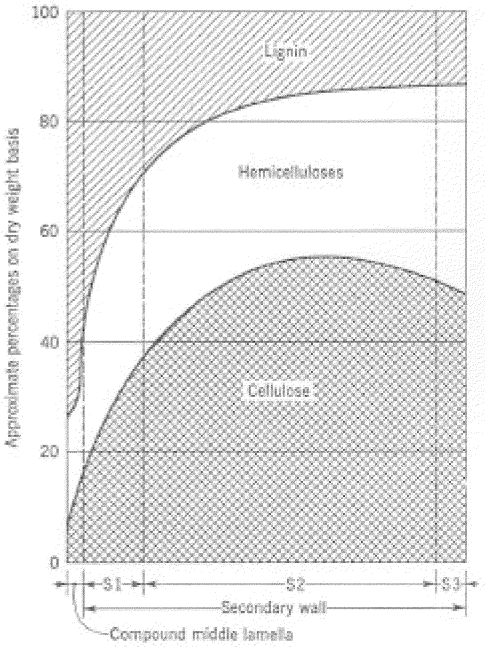


Fig. 2. Distribution of the principal chemical constituents within the layers of the cell wall in conifers, according to Panshin and de Zeeuw (1980).

In temperate regions, tracheids developed during the first part of the growing season are called earlywood tracheids. These tracheids have thin cell walls and large lumina (Fig. 3), and are heavily pitted since their major role is to provide water and nutrient transport to the crown. Later in the growing season, latewood tracheids are developed. These tracheids are radially narrow, have thick cell walls and small lumina (Fig. 3). Their major role is to give mechanical support to the stem. Early- and latewood tracheids produced during one growing season represents an annual ring.

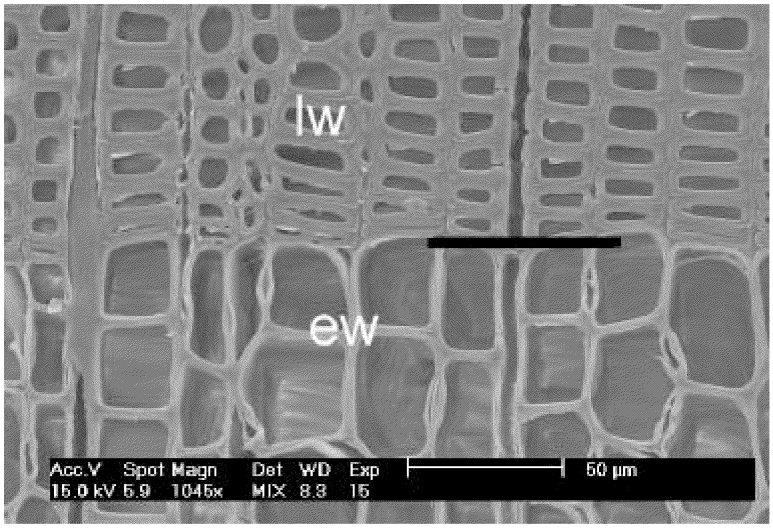


Fig. 3. Transverse section of Norway spruce wood. The latewood (lw) is comprised of radially narrow, thick-walled tracheids having small lumina. Earlywood (ew) is comprised of thin-walled tracheids having large lumina. The black bar indicates the border between two consecutive annual rings. SEM micrograph.

Wood formed near the pith is called juvenile wood, or sometimes core wood. Tracheid morphology changes progressively in the juvenile wood towards the mature wood which develops after about 5-20 years (Haygreen & Bowyer 1989). Tracheid morphology changes relatively little in the mature wood. If a tree stem is leaning, reaction wood is formed. Reaction wood is called compression wood in conifers and can be found on the underside of a leaning stem, *i.e.* the side that is subject to compressive force.

The cell wall of tracheids consists mainly of cellulose, hemicellulose and lignin (Fig. 2). The cell wall also contains smaller amounts of pectic substances, proteins (Westermark *et al.* 1986) and inorganic compounds, *e.g.* metal ions (Berglund *et al.* 1999). Cellulose is a linear homopolysaccharide composed of glucose monomers linked by ß-1,4 linkages with cellobiose as the repeating unit. Reviews on the structure and biosynthesis of cellulose have been published by Brown (1996; 1999). Several chains of cellulose form cellulose aggregates. The terminology for cellulose aggregates is not clear. In the literature; fibrils (*e.g.* Preston 1934), microfibrils (*e.g.* Hodge & Wardrop 1950) and macrofibrils (*e.g.* Bogomolov & Berenzon 1971) are used to describe aggregates of cellulose in wood. The term microfibril will be used in this thesis but the term fibril was used in Paper III.

Orientation of microfibrils

Microfibrils form the reinforcing material in the cell wall, analogous to steel rods in concrete. The angle between the tracheid axis and microfibrils is called the microfibril angle (MFA) and the helix of microfibrils is denoted either as S for left-handed helices or Z for right-handed helices. The microfibril angle in the S2 layer of tracheids influences mechanical properties to a large extent, such as shrinkage (Barrett *et al.* 1972), longitudinal tensile strength (Page *et al.* 1972) and longitudinal modulus of elasticity (Cave 1968; Page *et al.* 1977; Salmén & de Ruvo 1985). The S1 and S3 layers are important for transverse elasticity of tracheid cell walls (Bergander & Salmén 2000). Since the S2 layer represents about 80% of the total cell wall thickness (Fengel & Stoll 1973), the microfibril angle in the S2 layer is often considered synonymous to the microfibril angle of the whole tracheid.

Roland *et al.* (1987) reviewed the concept of plant cell walls organised in a helicoidal pattern. In tracheid cell walls the helicoidal expression was explained as "arrested motion", which means that cellulose deposition is arrested within the S1, S2 and S3 layers – but between those layers a shift in microfibril orientation occurs (*i.e.* transition zones) (Fig. 4). These transition zones have a semi-helicoidal appearance (Roland *et al.* 1987).

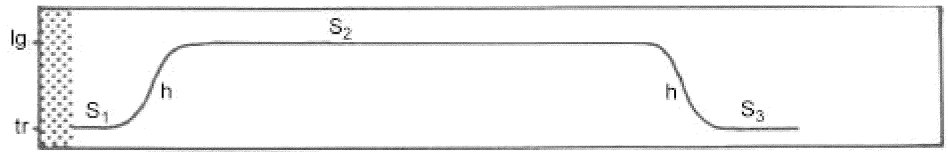


Fig. 4. Helicoidal cell wall expression in tracheids according to Roland *et al.* (1987). Cellulose microfibrils are deposited longitudinally (lg) in the S2 layer, transversely (tr) in the S1 and S3 layers and the angle shifts between them (h). Reproduced by the permission of Springer Verlag.

It is accepted that cellulose microfibrils are synthesised in so-called "terminal complexes" or "TCs" embedded in the plasma membrane of living cells. The complexes move in the plasma membrane as a result of poly-merisation/crystallisation reactions (Brown 1996). However, consensus has not been reached on what controls the direction of movement of the terminal complexes. The question posed is: what mechanism is responsible for the microfibril angle? The two major hypotheses on cellulose deposition are: the microtubular hypothesis (Ledbetter & Porter 1963; Wymer & Lloyd 1996; Chaffey *et al.* 1999; Funada *et al.* 2000); and the geometrical model (Emons 1994; Emons & Mulder 1998, 2000). The former hypothesis suggests that cortical microtubules direct cellulose deposition since a parallelism has been observed between microtubules and microfibrils (Barnett 1981; Abe *et al.* 1995a; Abe *et al.* 1995b; Chaffey *et al.* 1999). Although convincing studies on such parallelism have been reported, it is not agreed whether this is causal or coincidental. Baskin (2001)

recently discussed the relationship between microfibrils and microtubules and concluded that microtubules sometimes orient microfibrils, but that microfibrils do not necessarily require microtubules for alignment. Baskin (2001) proposed a model for microfibril deposition and organisation termed "templated incorporation" which postulates that microfibrils are incorporated into the cell wall by a scaffold (the template) which is built up and oriented around already deposited microfibrils or membrane proteins, or both. The role of microtubules is to bind and orientate components of the scaffold at the plasma membrane.

Emons and co-workers (Emons 1994; Emons & Mulder 1998, 2000) did not consider that microtubules direct cellulose deposition. According to their hypothesis, "the geometrical model", microfibril deposition is controlled by the density of active cellulose synthases in the plasma membrane, the distance between individual microfibrils within a wall lamella and the geometry of the cell. The model is expressed by the formula:

$$\sin \alpha = Nd / \pi D \qquad (1)$$

where α is the helical angle (which is 90° - the commonly defined microfibril angle, MFA), N is the number of microfibrils, d is the distance between the centres of neighbouring microfibrils and D is the cell diameter.

Several methods are available for measuring the microfibril angle of the S2-layer of tracheid cell walls. Examples of methods for measuring microfibril angle are: X-ray diffraction (Cave 1966; Sahlberg *et al.* 1997), small-angle X-ray scattering (SAXS) (Kantola & Kähkönen 1963; Jakob *et al.* 1994; Lichtenegger *et al.* 1999), orientation of cross-field pit apertures (Hiller 1964; Huang *et al.* 1998), polarised light (Page 1969; Leney 1981; Donaldson 1991; Ye & Sundström 1997), iodine staining (Bailey & Vestal 1937a; Senft & Bendtsen 1985), ultrasonic checking (Huang 1995; Wang *et al.* 2001), confocal microscopy using birefringence (Batchelor *et al.* 1997; Long *et al.* 2000), confocal microscopy using difluorescence (*i.e.* polarisation confocal microscopy) (Jang 1998), orientation of soft rot cavities (Bailey & Vestal 1937b; Anagnost *et al.* 2000; Khalili *et al.* 2001) and high-resolution techniques such as field emission microscopy (FE-SEM) (Abe *et al.* 1991) and transmission electron microscopy (TEM) (Kataoka *et al.* 1992).

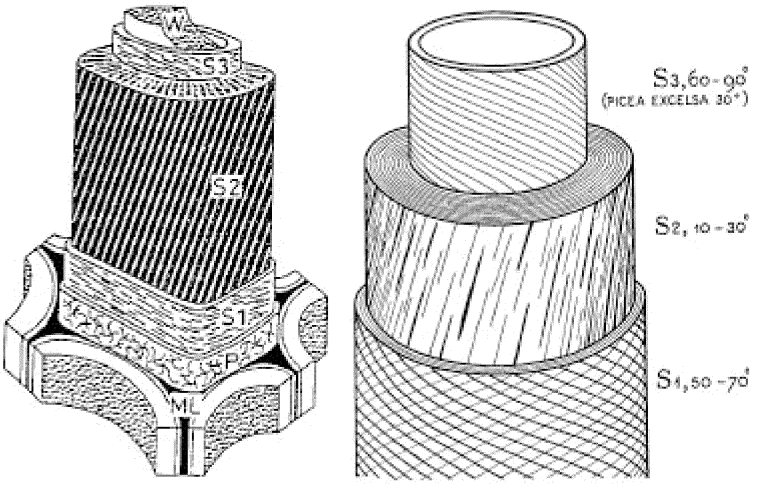
Batchelor *et al.* (2000) compared several techniques in a review and concluded that in general, different techniques give similar results but individual measurements are often subject to large variation. This discrepancy is attributed to both the methods used and to the intrinsic variation of microfibril angle, within and between tracheids. Since the methods have different advantages and disadvantages, the choice of method is dependent on the purpose of the study.

Cell wall models

The ultrastructure of the wood cell wall has been visualised in models since the beginning of the 20th century. There have also been models predicting mechanical properties of the cell wall (Salmén & de Ruvo 1985; Persson 2000; Bergander & Salmén 2002) but such mechanical models will not be discussed in this thesis.

Models which aim to show the morphology of wood cell walls often show one or a few cells in transverse section where the cell wall layers have been pulled out like a telescope (Figs. 1 and 5). Cell shape is rectangular (Fig. 5a) or circular (Fig. 5b) in transverse section. The latter can be misleading since the circular shape is a characteristic of compression wood tracheids. In order to visualise all cell wall layers, the thickness of the cell wall is large, which implies that S1 and S3 layers are exaggerated in thickness and the model looks like a latewood cell. Due to the importance of microfibrils for the mechanical properties of tracheids, microfibril orientation (*i.e.* S- and/or Z helices and angle) is always shown in the layers of cell wall models (Figs. 1 and 5).

One of the first cell wall models was presented by Lüdtke (1931). He showed a generalised cell wall model that was as equally valid for tracheids as for wood fibres in general, bast fibres and plant hairs. This general approach was later criticised by Bailey (1938) who suggested different cell wall models for different cell types. However, models continued to be general although they were most often valid for one plant cell type, *e.g.* conifer tracheids. Morphological research involved light microscopy during the thirties and forties, but nevertheless the models showed commanding accuracy. Several new cell wall models were presented during the fifties and sixties through the development of electron microscopy and methods for preparing ultrathin sections of tissues. These models were often derived from studies on conifers and thus most valid for them, but hardwood cell wall models were also presented (Meier 1955). At that time, there were several reports claiming that the outer layer (S1) is composed of lamellae with alternate S- and Z microfibril helices, the so-called "crossed fibrillar structure" (*e.g.* Hodge & Wardrop 1950; Frei *et al.* 1957; Wardrop 1957). The crossed fibrillar structure was visualised in several cell wall models (*e.g.* Fig. 5b) but there were still many reports which regarded the S1 layer as not having crossed microfibrils (Svensson 1956; Liese 1970; Berenzon & Bogomolov 1972). Another area of controversy has been the lamellation of the S2 layer, a discussion of whether it is radial or concentric. Consequently, this have been presented in cell wall models (Fig. 5).



a) © Springer Verlag b) © Gebrüder Borntraeger

Fig. 5. Cell wall models. a) cell shape is rectangular, S1 is shown without crossing microfibrils and S2 has radial lamellation (reproduced from Sell & Zimmermann 1993). b) cell shape is circular, S1 is shown with crossed microfibrils and S2 has concentric lamellation. Note, *Picea excelsa* = *Picea abies*. (reproduced from Roelofsen 1959). Fig. 5a is reproduced by the permission of the authors and Springer Verlag and Fig. 5b is reproduced by the permission of Gebrüder Borntraeger.

Despite the fact that it is well known that tracheid morphology varies to a large extent within the tree (*e.g.* from pith to bark and within annual rings), this is seldom considered in cell wall models. The morphology also varies along the tracheid (*e.g.* within or distant to pit regions), which is also not considered in cell wall models. Since there is a risk that cell wall ultrastructure is considered as constant or fixed due to the generalised models it would be advantageous to develop cell wall models for different types of tracheids, *e.g.* tracheids from juvenile wood, early/latewood and compression wood. It would also be relevant to produce models representing different parts of tracheids such as cell corners, pit regions and tracheid tips.

Materials and methods

Use of soft rot cavities

When soft rot fungi degrade the cell wall of wood fibres, they reveal the orientation of microfibrils by the development of cavities (Bailey & Vestal 1937b; Levi & Preston 1965; Nilsson 1974a, b; Khalili 1999; Anagnost *et al.* 2000) which are readily seen in light microscopy (Fig. 1; Paper II). The soft rot method was compared with X-ray diffraction and polarisation confocal microscopy (CLSM) for measuring the microfibril angle in the S2 layer (Paper III). In addition, soft rot cavities were used to study the intra-ring variation of microfibril angles in the S2 layer, and correlation between microfibril angle in the S2 layer and tracheid microstructure (Paper III). Soft rot was also used as an ultrastructural tool for studying the microfibrillar orientation of the S1 layer (Paper IV).

Small sterile Norway spruce wood blocks were placed in sterile soil and inoculated thereafter with the soft rot fungus *Phialophora mutabilis* (van Beyma) Schol Swartz (Paper III). Sterile macerated Norway spruce tracheids were placed on sterile soil and inoculated thereafter with the soft rot fungus *Humicola alopallonella* (Meyers and Moore) strain CBS 207.60 (Paper IV). Unsterile soil containing soft rot fungi was also used to introduce soft rot to wood blocks which were macerated afterwards (Paper IV).

Measurements of soft rot cavities located in the S2 layer were carried out on micrographs of thin radial sections using image analysis software (Paper III). Approximately ten randomly selected tracheids were measured per section, and three sections per annual ring were studied. The angle between the soft rot cavity and the tracheid axes was measured by first drawing a reference line parallel to the tracheid axis, and then a line along the longitudinal axis of the soft rot cavity (Fig. 1 Paper II). The average of three cavities was recorded as the microfibril angle of each tracheid. Measurements were conducted in areas of the cell walls lacking pits. In order to compare soft rot- and polarisation CLSM measurements on the same tracheids, some soft rot measurements were also conducted on CLSM images that had been measured previously using this technique.

Determination of the microfibril angle in the S2-layer by using polarisation confocal microscopy

Since one aim of this thesis was to evaluate the soft rot method as a tool for microfibril studies, another method was needed to non-destructively measure microfibril angles on cell walls attacked by soft rot fungi.

Confocal laser scanning microscopy (CLSM) allows optical sectioning (sectioning along the z-axis) of specimens and confocal images only contain information from the plane in focus, the focal plane. Polarisation CLSM can be used to determine the microfibril angle in the S2 layer of tracheids by optically

sectioning the cell wall and using difluorescence, *i.e.* fluorescence depending on the direction of the polarised light (Jang 1998). When the polarised laser beam is parallel to the microfibrils, maximum fluorescence occurs, *i.e.* maximum pixel intensity of the image. The method has been shown to correlate well with other S2 microfibril angle measurement techniques (Jang 1998).

Radial Norway spruce wood sections were cut according to Bergander and Salmén (2000) from the same annual rings as studied with the soft rot method (Paper III). Some sections were also prepared from soft rot attacked wood to enable measurements on the same tracheid cell walls by both methods. The sections were stained with Congo red, a fluorochrome which adsorbs to cellulose and orientates in the same orientation as the cellulose chains (Woodcock *et al.* 1995). Microfibril angles were measured by rotating the polarised laser beam 10 degrees at a time over an interval of 180°. At each 10-degree point an image was acquired and the pixel intensity of the difluorescence was analysed by image analysis software. The pixel intensities were then plotted against the angle of incident polarisation and adjusted to an equation. The procedure was repeated at three levels within the S2 layer of each tracheid, in areas of the cell wall which lacked pits. The average of these three measurements was recorded as the S2 microfibril angle of that tracheid. A statistical T-test was done in order to evaluate whether there were any differences between the soft rot method and polarisation CLSM. Average microfibril angles of the selected annual rings and measurements on individual tracheids were compared. Polarisation CLSM was also used to monitor the variability of microfibril angle in the S2 layer of annual rings and to study the correlation between microfibril angle in the S2 layer and tracheid microstructure.

Determination of the microfibril angle in the S2 layer by using X-ray diffraction

X-ray diffraction is a commonly used method for determining average microfibril angles in the S2 layer, and it was used in Paper III for comparison with the two microscopical methods. However, it was only used to generate an average microfibril angle of the selected annual rings. The microfibril angle was determined according to the method described by Sahlberg *et al.* (1997). This method measures the intensity from the (040) plane in reflection. The measured area was 5.0 x 0.4 mm in the tangential and radial directions, respectively. The average angle (θ) was determined as $\theta = 0.6T$ according to Cave (1966) and Meylan (1967). One measurement was made in the middle of each ring of the Norway spruce wood blocks later used for local microfibril angle measurements by the two microscopic methods, soft rot and polarisation CLSM.

Determination of tracheid length, width and cell wall thickness

Norway spruce tracheid microstructure (*i.e.* length, width and cell wall thickness) was studied in Paper III to investigate correlation between tracheid morphology and microfibril angle, which has been proposed earlier (Preston 1934; Wardrop 1964). The general term "fiber" was used, since we used an instrument (STFI's FiberMaster) where it is impossible to distinguish between different cell types in wood and also between broken and whole tracheids. Measurements were conducted on the same annual rings as used for microfibril angle studies. Fibre length and fibre width measurements were recorded using FiberMaster, and data on cell wall thickness was approximated by the average density obtained from X-ray microdensitometry profiles of earlywood for the annual rings studied.

Shear forces applied to wood blocks and macerated tracheids

Fracture characteristics of wood have been related to cell wall ultrastructure in several studies (Davies 1968; Côté & Hanna 1983; Donaldson 1995, 1996). Shear forces were applied to Norway spruce wood blocks and macerated tracheids in order to study the ultrastructure of the S1 layer (Paper IV). The surface of partly delignified wood blocks sheared parallel to the grain were coated with gold and studied using SEM. In addition, macerated tracheids were sheared by steel sticks. Some of the macerated tracheids were coated with gold and studied using SEM and others were embedded in resin, sectioned and observed using TEM.

Chemical treatment of wood and macerated tracheids

Chemicals acting on cell walls to produce visible changes (swelling) have been used for a long time as tools for understanding tracheid morphology (Steenberg 1947; Wardrop & Dadswell 1950; Wardrop 1954; Bucher 1957). This was used in Paper IV to study the ultrastructure of S1. Norway spruce thin (20 μm) transverse wood sections were partly delignified in hydrogen peroxide and acetic acid, and observed using light microscopy. Some partly delignified sections were also treated with ultrasound. In addition, phosphoric acid were applied onto macerated Norway spruce tracheids to study their swelling behaviour. The acid was applied directly on the tracheids with and without cover-slips, and observed using light microscopy. Untreated and partly delignified wood followed by ultrasonic treatment were also sectioned and observed in TEM.

Results and discussion

Micro- and ultrastructure of Norway spruce tracheids

The literature review (Paper I) presented information on Norway spruce tracheid micro- and ultrastructure based on research carried out during almost one hundred years. Besides presenting various results on tracheid structure, the variability in tracheid morphology within the tree was highlighted. Since tracheid morphology varies considerably, *e.g.* along the stem, from pith to bark, within and between annual rings and also along tracheids, it is very difficult to specify a typical tracheid. However, the variation can and should be used in the wood industrial processes. This may be achieved by separating different wood assortments already in the forest, at the lumber yard or within the actual process. In addition, knowledge of tracheid morphology is needed when trying to improve and change wood properties. For example, knowledge of factors which control tracheid micro- and ultrastructure variation within trees, is important in order to improve silvicultural regimes and tree breeding programs.

Paper I was based on information from studies predominantly carried out on Norway spruce, but it was not always possible to obtain ultrastructural information of this species. However, the principal architecture of the wood cell should be considered to be similar between different conifers, although the order of magnitude of the different features, such as microfibril angle in the S2 layer or thickness of the cell wall layers, may differ between species. The degree of genetic and/or environmental impact on tracheid micro- and ultrastructure was not discussed.

Paper I emphasised the importance of considering the methods and the techniques used to investigate morphological features, since different techniques can generate dissimilar results when measuring the same feature, *e.g.* cell wall thickness and lumen area using light or confocal microscopy (Donaldson & Lausberg 1998). It is also important to consider how a morphological feature is measured. For example tracheid length measurements using automated methods, *e.g.* FiberMaster or Kajaani FiberLab (Paper III, Herman *et al.* 1998). FiberMaster measures the length of objects in fibre suspensions that are longer than ca. 10 µm and have length-to-width ratios of 4 and larger (Karlsson *et al.* 1999). Since ray cells fulfil these requirements (Nyrén & Back 1960) it implies that these cells will be measured which will influence the tracheid length estimate. In addition, broken tracheids longer than 10 µm will also be measured. However, there are other, not often considered and discussed, effects of using automated methods for tracheid length measurements. One such example is the variation in ray cell frequency in different parts of the tree. It is accepted that the largest number of ray cells are formed in the early juvenile years (Larson 1994) when peripheral expansion of the cambium is greatest. In addition, wide annual rings and annual rings containing compression wood are also considered, in most reports, to consist of a larger

number of ray cells per wood section area (Timell 1986; Larson 1994). This variability in ray cell number may generate biased length estimates, *i.e.* underestimation of tracheid length in juvenile wood, wide annual rings and compression wood.

The literature review (Paper I) also emphasised the need for further studies on tracheid morphology, especially ultrastructure. In this respect, recent biotechnological advances enabling the identification and characterisation of the genes and respective enzymes involved in wood cell wall formation may lead to a general and deeper understanding of tracheid morphology.

Soft rot fungi as a tool to determine microfibril angle

The use of soft rot fungi to determine microfibril angle in wood cell walls was discussed in Paper II. It was concluded that the major advantage of the method is the detailed information, both quantitative and qualitative, that can be derived on variations in microfibril angle. The method was also shown to be reliable since it correlated well with polarisation CLSM, both when comparing measurements on the same tracheids (Table 2 and Fig. 3; Paper III) and when comparing mean microfibril angles of annual rings (Table 2 and Fig. 6 Paper III). The reliability of the method for measuring microfibril angles in the S2 layer was also shown by Anagnost *et al.* (2000). Soft rot cavities were also used to study the microfibrillar orientation of the S1 layer (Paper IV). Cavities in the S1 layer developed on both macerated tracheids and in solid wood, but cavities in the S1 layer were much less frequent than cavities in the S2 layer. This is because the S1 layer has higher lignin content (Fig. 2) which delays cavity formation and because the S1 layer is much thinner than the S2 layer. The S1 layer is approximately two (latewood) to four (earlywood) times thicker in compression wood than in normal wood (Timell 1986) and consequently, soft rot cavities in the S1 layer are more frequent in compression wood tracheids than in tracheids of normal wood (Daniel & Nilsson 1998).

The disadvantages of using soft rot cavities for determining microfibril angle are the slow process of soft rot attack, and the need for microbiological facilities if monocultures of soft rot are used, as discussed in Paper II. In addition, when using light microscopy, it can be difficult to conclude in what plane (*i.e.* in what part of the cell wall and sometimes also in which cell) a soft rot cavity is located. This is evident when studying a double cell wall of neighbouring tracheids in a longitudinal wood section or collapsed macerated tracheid. If a confocal microscope is used, there is no problem of knowing in which cell wall a cavity is located, but the depth resolution of the CLSM is not accurate enough to determine where within the S2-layer a cavity is located. Sometimes, several cavities can appear at different angles in one specific cell wall (Fig. 2 Paper II and Khalili *et al.* 2001) but then it is impossible to state exactly where in the cell wall these cavities are located, even when using CLSM. If an ordinary light microscope is used, two opposite cell walls (of a collapsed macerated tracheid or the double cell wall of two neighbouring cells) can be separated, since cavity orientation changes from one direction to the

opposite when focusing from the closest cell wall to the cell wall behind. This is due to organisation of the microfibrils in helices.

The uncertainty of cavity location applies to longitudinal wood sections or observations on collapsed macerated tracheids, but it is not a problem in transverse wood sections. This feature was utilised in a study within this Ph.D. project but was not published. The idea was to optically section soft rot degraded wood transversely using CLSM and thereby follow cavity location in sequential images to conclude where within the S2 layer cavities were located. The purpose was to answer if cavity location moved radially within the S2 layer, if there were any differences in cavity angle (microfibril angle) between tangential and radial walls, if there were any cavities with S-helices, and if cavities run straight in cell corners as discussed by Khalili (1999). However, it was not possible to optically section the wood samples (sections of 40 µm) more than about 10-15 µm deep with satisfactory resolution, brightness and contrast which was too short in order to answer the questions. Several stains and clearing media were used, but none of them proved satisfactory. Maybe it will be possible to conduct such a study in the future using improved techniques.

Variation of S2 microfibril angle within annual rings; trends, possible causes and consequences for the living tree

Microfibril angle in the S2 layer was shown to decrease from the beginning to the end of earlywood in annual rings, using both soft rot cavity measurements and polarisation CLSM (Fig. 4 Paper III). This trend is also shown in Fig. 6, where S2 microfibril angles were measured on consecutive earlywood tracheids in three radial wood sections. The low S2 microfibril angle at the end of earlywood was also present in latewood (Fig. 4b, Paper III). The declining trend was much less pronounced in annual rings containing compression wood (Fig. 5 Paper III). Moreover, large variations in S2 microfibril angle occurred between neighbouring tracheids, especially in the beginning of earlywood where the microfibril angle could differ considerably in absolute terms. It has been shown in several studies using both microscopical methods and X-ray diffraction on various conifers that earlywood tracheids have lower microfibril angles in the S2 layer than latewood tracheids (Bailey & Vestal 1937a; Bailey 1938; Donaldson 1998; Evans 1998; Herman *et al.* 1999), but the declining trend within earlywood has not been thoroughly studied previously. There are however contradictory results reported in the literature. Studies using small-angle X-ray scattering (SAXS) have shown latewood to have much larger microfibril angles (ca. 20°) than earlywood (ca. 0°) (Jakob *et al.* 1994; Reiterer *et al.* 1998; Lichtenegger *et al.* 1999). In Paper III the high angles reported for latewood was suggested to derive from measurements on compression wood. However, the extremely low fibril angles reported for earlywood still remains unexplained. The SAXS method itself does not seem to be the problem since SAXS and X-ray diffraction have been shown to generate similar results (Lichtenegger *et al.* 1998; Andersson *et al.* 2000).

It was also concluded in Paper III that the declining trend in microfibril angle within earlywood is concealed in studies using X-ray diffraction. It should however be noted that the declining trend can be observed using X-ray diffraction on trees with very wide annual rings (Evans 1998).

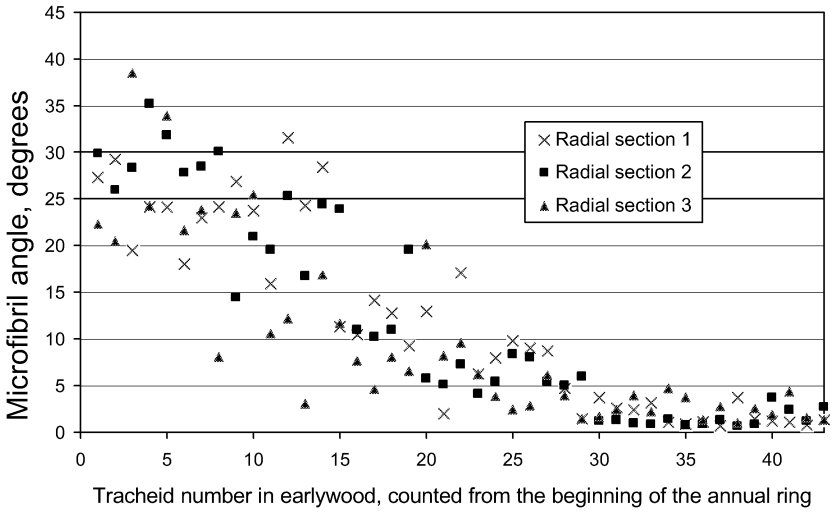


Fig. 6. Decreasing S2 microfibril angle within the earlywood of a Norway spruce annual ring. Microfibril angle was measured using the soft rot method on consecutive earlywood tracheids on three, 20 µm thick, radial wood sections. The annual ring was comprised of ca. 60 rows of cells of which ca. 43 were earlywood cells. Tracheid number (x-axis) refers to the number of the cell rows, counting from the first-produced cell in the annual ring. It was not possible to measure cavities in the latewood since that part of the annual ring was too degraded by the fungus.

It would be interesting to study the reasons behind the declining trend in microfibril angle within annual rings. It is possible that the geometrical model for cellulose deposition (Emons 1994; Emons & Kieft 1994; Emons & Mulder 2000) (see Introduction above) can explain the decrease in microfibril angle across the annual ring. According to the geometrical model, one factor affecting cellulose orientation is the size of the lumen, which is well known to decrease from early- to latewood (Jones 1958). When lumen size (*i.e.* cylinder diameter) decreases, the microfibril angle also decreases (Emons & Kieft 1994). Note that the helical angle a used in the papers of the geometrical model is 90° - the commonly defined microfibril angle. This explains why the figures in Emons and Kieft (1994) and Emons and Mulder (2000) show an increasing helical angle a with decreasing cylinder (lumen) diameter.

The large differences in S2 microfibril angle between tracheids produced in the first part of the annual ring compared to later produced cells implies that the mechanical properties of tracheids vary to a large extent within one annual ring.

Page *et al.* (1972; 1977) showed that the longitudinal strength and longitudinal elastic modulus of tracheids with S2 microfibril angles of ca. 40° was approximately one third and one fifth respectively compared to tracheids with S2 microfibril angle of ca. 5°. From a physiological point of view for the living tree, this is logical since the role of latewood tracheids is to provide mechanical support to the stem. A speculative explanation as to why earlywood tracheids have higher microfibril angles in the S2 layer than latewood tracheids is that their role, except for conducting water to the crown, may be to rapidly reset a wind-exposed tree (*i.e.* exposed to compressive stress) to its natural upright position. Bamber (2001) recently argued the role of microfibrils as helical springs in compression wood and that these helical springs would exert compressive forces on a leaning stem, "pushing" it into an upright position. Since microfibril angles in the S2 layer are similar at the beginning of earlywood (Fig. 4 Paper III and Herman *et al.* 1999) and compression wood (Fig. 5 Paper III and Sahlberg *et al.* 1997; Andersson *et al.* 2000), the effect of high microfibril angles may be the same in both first-formed earlywood and in compression wood. In addition, Bamber (2001) further stressed that the role of increased levels of lignin in compression wood, compared to normal wood, may be to bind the microfibrillar skeleton more strongly together, thus enhancing the ability to withstand the compressive forces that compression wood undergoes. Again, there is a similarity between earlywood and compression wood, since the secondary cell wall of Norway spruce earlywood is reported to have slightly, ca. 12%, higher lignin content than the secondary cell wall of latewood (Gindl & Wimmer 2000).

The discussion above emphasises the possible role of first-formed earlywood tracheids to act as helical springs. To further stress this and try to understand the nature of compression wood development, the following line of argument may be relevant: In annual rings of both mild and fully developed compression wood, it has been observed in several studies that the first rows of tracheids often do not appear like typical compression wood tracheids. Figure 7 shows this in a fresh slightly wet Norway spruce tree disc containing compression wood.

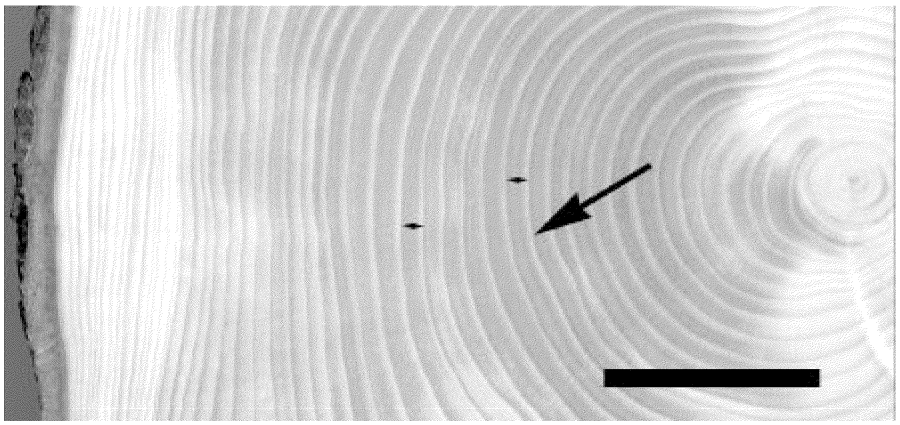


Fig. 7. Transverse section of a Norway spruce tree disc with compression wood (dark region). The light bands (arrow) at the beginning of each annual ring represent first-formed earlywood, which is followed by ordinary compression wood. Small black diamonds indicate entire annual rings. Scale bar 3 cm.

Timell (1986) reported that the first-formed earlywood tracheids appeared like normal earlywood cells, *i.e.* they had a rectangular outline, were thin-walled, lacked intercellular spaces and helical ribs were absent. Like compression wood tracheids, the S1 layer of first-formed tracheids was thicker and the S3 layer was absent. Timell (1986) discussed several hypotheses on the origin of these first-formed earlywood tracheids, but none of them provided any absolute explanation. Timell (1986) also suggested that rapid growth and lack of photosynthate early in the growing season may be responsible. However, it is suggested in this thesis that since the first-formed earlywood tracheids are "programmed" for S2 microfibrillar angles of about 30° (Paper III), there may be no need to develop fully developed compression wood tracheids. Figure 5a in Paper III represents an annual ring containing compression wood. The first 20 earlywood tracheids have S2 angles of ca. 20-30°, which is about the same as in normal wood (Fig. 4a, Paper III), and they appear like normal earlywood tracheids in transverse section (Fig. 8).

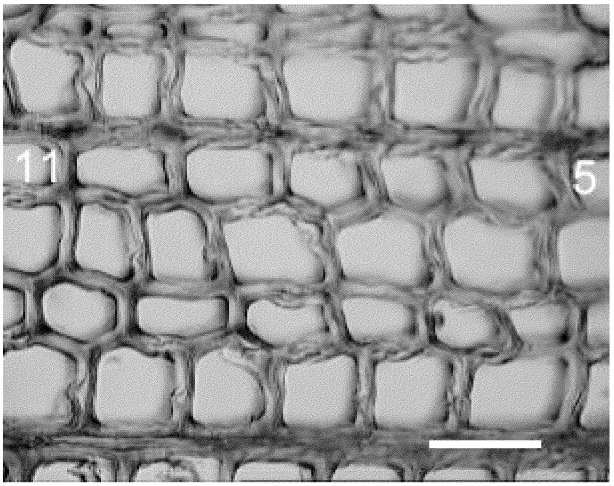


Fig. 8. First-formed earlywood tracheids in an annual ring containing compression wood. The section is generated from the same annual ring as presented in Fig. 5a Paper III. The tracheids appear like earlywood tracheids of normal wood, *i.e*. they are thin-walled, have a rectangular outline, do not have intercellular spaces and helical ribs can not be found. The numbers indicate the number of the cell-row counted from the start of the annual ring. The annual ring contained ca. 66 rows of cells. Light microscopy of transverse section. Scale bar = 50µm.

Further in the annual ring (*i.e.* from cell-row 20 and further) the high angles continue (Fig. 5a; Paper III). These tracheids appear more like normal compression wood tracheids in transverse section (Fig. 9).

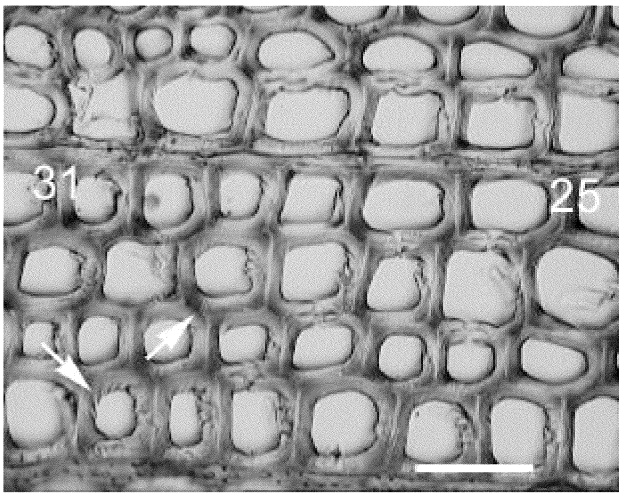


Fig. 9. First formed "normal" compression wood tracheids. The section is generated from the same annual ring as Fig. 5a Paper III presents. The tracheids have thick cell walls, a rounded outline and helical ribs can be observed (arrows). Numbers indicate the number of the cell row counted from the start of the annual ring. Light microscopy of transverse section. Scale bar = 50µm.

In addition, it was not possible to measure soft rot cavities in the whole annual ring (Fig. 5a; Paper III) because soft rot cavities were absent from tracheid row 20 and further. This is because soft rot is delayed by compression wood, as discussed in Paper II. This absence of soft rot attack from tracheid-row 20 and further also indicates that within this annual ring, ordinary compression wood tracheids are produced from about the 20th cell and further.

Variation of S2 microfibril angle within annual rings; consequences for wood fibre products

Differences in S2 microfibril angle within annual rings will also affect the strength properties of paper made from different wood assortments. For example, paper made of wood from thinnings which have high juvenile content and low latewood content, will have less strength compared to wood from sawmill reducer chips derived from the outer part of the stem (*i.e.* mature wood). Latewood content is higher in mature wood (Paper I). Thus, even if juvenile wood as a whole has a higher microfibril angle (see Paper I), the effects of the intra-annual ring variability will also cause differences. In addition, separating early- and latewood tracheids within the actual wood industrial process will of course also influence the strength properties of the paper. Besides differences in longitudinal strength and longitudinal elasticity of tracheids, there may be other less explored effects of the decreasing trend in S2 microfibril angle within annual rings. One example is the ability of tracheids to collapse during mechanical pulping processes. When producing mechanical pulps, it is important to increase the flexibility of the fibres, *e.g.* to produce collapsed fibres. Kure (1997) reported that fibres from juvenile wood collapsed less easily than fibres from the mature wood of the same cell wall thickness. This was explained as a consequence of the higher microfibril angles in the S2 layer of juvenile wood. However, the low microfibril angle at the end of earlywood which continued to the latewood (Paper III) may also indicate that these tracheids may collapse more easily despite their large cell wall thickness.

Relationship between tracheid microstructure and microfibril angle in the S2 layer

Preston (1934) proposed a correlation between tracheid length and S2 microfibril angle (*i.e.* short tracheids = large microfibril angle), a feature later confirmed by other authors (Kantola & Seitsonen 1969; Marton *et al.* 1972), but this has been questioned (Hirakawa & Fujisawa 1995; Sahlberg *et al.* 1997). No correlation was found between tracheid microstructure (*i.e.* length, width, cell wall thickness) and S2 microfibril angle (Paper III). However, there may be some relationship between tracheid length and microfibril angle, since these parameters behave in the same way from pith to bark, and also between wide and narrow annual rings (discussed in Paper I). It should be noted that the microstructural measurements (Paper III) are imprecise. One reason for the uncertainty is that the data on tracheid length and tracheid width were derived from FiberMaster. The FiberMaster measures all types of cells in a wood sample, as discussed above, and also broken tracheids. Another

reason for the uncertainty is that the length and width measurements were done on the whole annual rings, thus not only on earlywood which we were focused on. In addition, the data on cell wall thickness was derived from density measurements, but density is not only correlated with cell wall thickness. Density is also correlated with cell and lumen width (Yasue *et al.* 2000; Hannrup *et al.* 2001).

Microfibril orientation in the outer layer (S1) of the secondary wall

It has been assumed that the outer layer of the secondary wall (S1) is composed of lamellae with alternate S- and Z microfibril helices, the so-called "crossed fibrillar structure" (Hodge & Wardrop 1950; Wardrop 1957; Roelofsen 1959; Tang 1973) (Fig. 5b). This is also shown in several cell wall models, (*e.g.* Fengel & Wegener 1984; Harada & Côté Jr 1985; Niklas 1992; Dinwoodie 2000). Despite the fact that there are early TEM studies on S1 which do not consider the crossed fibrillar hypothesis (Svensson 1956; Liese 1970; Berenzon & Bogomolov 1972) and recent studies on tracheid differentiation using high resolution techniques (Abe *et al.* 1991; Kataoka *et al.* 1992) which do not show alternate S and Z microfibril helices, the crossed fibrillar structure is still considered to be valid by many. Considering these recent findings on cellulose deposition and by the use of various methods for revealing the ultrastructure of S1, it was concluded in Paper IV that the bulk of S1 is composed of microfibrils oriented at approximate right angles to the tracheid axes. Soft rot cavities in the S1 layer (Fig. 1 Paper IV) and studies on mechanically (Figs. 10 and 11 and Figs. 4 and 5 Paper IV) and chemically (Fig. 6, Paper IV) treated wood blocks and macerated tracheids supported this conclusion.

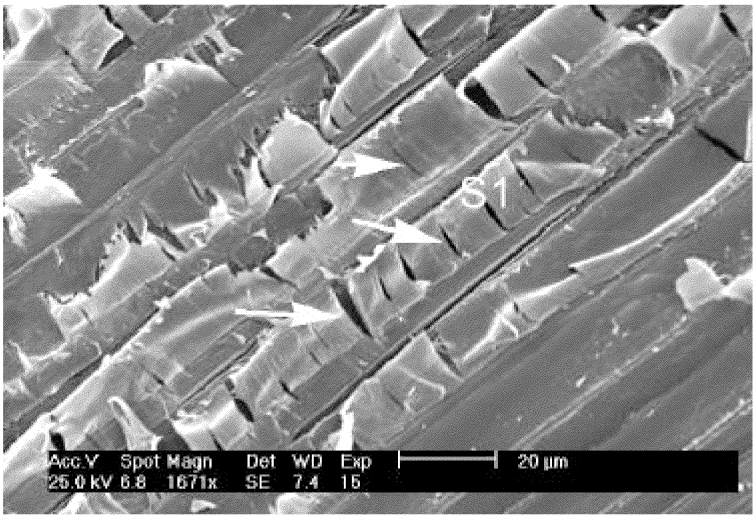


Fig. 10. SEM micrograph of a partly delignified wood block of Norway spruce sheared parallel to the grain. The S1 layer has separated from the underlying S2 and developed cracks (arrows) oriented perpendicular to the tracheid axis.

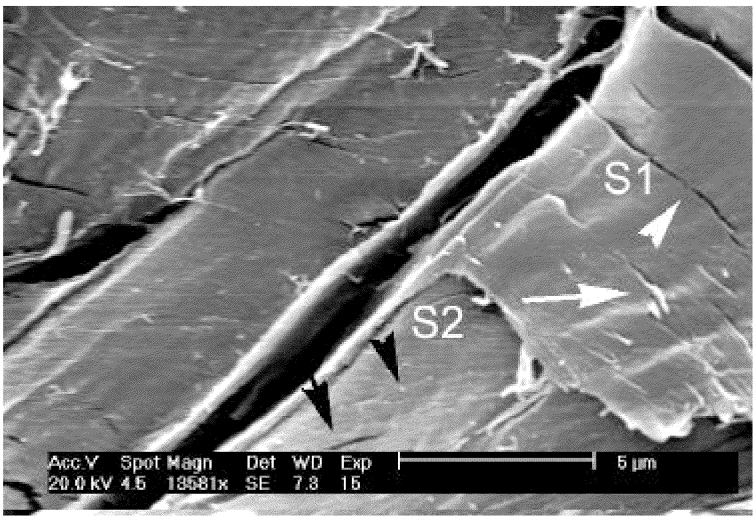


Fig. 11. The S1 layer has separated from the underlying S2. Cracks in S1 (white arrows) are oriented perpendicular to the tracheid axis while the underlying S2 has a crack (black arrows) oriented at approximately 30° degrees. The tracheid axis runs from lower left to upper right in the image. Similar treatment as for Fig. 10.

There may be microfibrils that cross each other, but these are not deposited in alternate helices but rather within a helix, as shown by Kataoka *et al.* (1992). The

transition of microfibril orientation from the S1 to the S2 layer appeared abrupt. It was further suggested that the transitional/intermediate lamella (Wardrop 1964; Imamura *et al.* 1972; Tang 1973; Harada & Côté Jr 1985), where a gradual change in microfibril angle occurs, should be considered to belong to the outer part of S2. Micrographs also highlighted the rigidity and homogeneity of the S1 layer (Figs. 2 and 3 Paper IV).

The general concept of helicoidal plant cell wall organisation reviewed by Roland *et al.* (1987) also supports the idea that the S1 is composed of microfibrils oriented in one predominant direction (Fig. 4). In addition, when considering the nature of cellulose deposition (discussed above) it seems unlikely that microfibrils have alternate S and Z helices within the S1. If the microtubular hypothesis is considered, it has been shown that microtubules follow the gradual change in orientation (Abe *et al.* 1995b; Funada *et al.* 2000) similar to that of microfibrils. The other major hypothesis, the geometrical model, does not necessarily eliminate the possibility of a crossed cell wall but in order to change the microfibril angle according to the geometrical model, at least one of the three prerequisites upon which it is based (see above) has to be changed. Due to the fact that each lamella is so thin, it seems unlikely that any one of the other two prerequisites (*i.e.* density of active cellulose synthases in the plasma membrane and the distance between individual microfibrils) would cause a shift in the helix between lamellae.

Conclusions

The soft rot method was found to be a reliable technique for determining microfibril angle in wood cell walls. Comparison with polarisation CLSM showed good correlation. The soft rot method enables detailed information at the cell wall level, and it provides a useful tool in wood ultrastructural research.

Both the soft rot and polarisation CLSM methods showed the same trend of high fibril angles in the first part of the earlywood, followed by a decrease towards the end of earlywood. The declining trend was less pronounced in annual rings containing compression wood. No correlation was found between fibre microstructure (*i.e.* length, width, and cell wall thickness) and microfibril angle.

It was concluded that the S1 layer is rather homogenous with respect to microfibrillar orientation, *i.e.* the bulk of its structure is composed of microfibrils predominantly oriented ca. 70-90° to the tracheid axis in a single helix. Evidence for the crossed fibrillar structure with alternate S and Z helices was not obtained. The gradual transition of microfibrils, also called S12, from S1 to S2 should be designated as a part of the S2 layer, *i.e.* the transitional/intermediate lamella (S12) is situated in the S2 layer.

Considering information presented in the literature review (Paper I) and from papers III and IV, three new cell wall models are presented (Fig. 12). They are intended to diagrammatically summarise current opinion on the cell wall ultrastructure of Norway spruce tracheids with respect to microfibril orientation. The models are in many aspects similar to some of the earlier presented models (*cf.* Fig. 1) but they are based on recent research findings, critical reading of older literature and results generated within this study. The models (Fig. 12) also show some of the variability between early- and latewood and juvenile- and mature wood.

The shape of the models is derived from a three-dimensional reconstruction of a hydrated Norway spruce tracheid (Bardage 2001). The models have been created by Computer Aided Design (CAD) and a 3D visualisation program (Rhinoceros 1.1). No attempt was made to reflect the relative thickness of the different layers. They are intended to be representative for cell walls without pits and they should not be regarded as representative of tracheid tips. However, ultrastructural studies and visualisation of pit regions and tracheid tips are important areas of future research.

The models (Fig. 12) do not indicate any preferred lamellation of the S2 layer (*i.e.* concentric, radial or random). This was discussed in Paper I but has not been considered in Fig. 12 since there is still a lot of disagreement on type of lamellation. Donaldson (2001) recently argued that concentric lamellations observed in TEM micrographs do not necessarily reflect the underlying

ultrastructure of the cell wall, since the helical organisation of the cell wall can generate tangential textures irrespectively of microfibril arrangement. Donaldson (2001) also suggested that the most probable organisation of the cell wall is a random arrangement of microfibrils with varying amounts of concentric and radial organisation.

The models (Fig. 12) are built up of the following parts, described from the primary wall (P) towards the lumen.

Primary wall (P)

- This part of the cell wall was discussed in Paper I. The microfibrils in the primary wall are organised in a loose, irregular interwoven texture but there is some organisation. The outer part of P is composed of microfibrils oriented approximately axially and the inner part is composed of microfibrils oriented approximately transversely as shown by Abe *et al.* (1995b) studying *Abies sachaliensis*. The primary wall is considered to be one layer, despite the fact that the orientation changes within P, since P is not composed of organised lamellae.

Secondary wall

S1 layer

- The S1 layer of Norway spruce tracheids was studied in Paper IV. The S1 layer is considered as one layer composed of microfibrils oriented approximately perpendicular to the tracheid axis according to Paper IV. In many other cell wall models the crossed fibrillar structure of S1 has been represented (*e.g.* Emerton & Goldsmith 1956; Roelofsen 1959; Forgacs 1963; Wardrop 1964; Dunning 1969; Meylan & Butterfield 1972; Fengel & Wegener 1984; Harada & Côté Jr 1985; Niklas 1992; Dinwoodie 2000) but evidence for a crossed fibrillar structure was not found in Paper IV.

S2 layer

- Inside the S1 layer, two thin lamellae are shown. These should be considered as part of S2 (Paper IV). The reason for showing them as individual lamellae is because it is very difficult to visualise the gradual transition of microfibrils from S1 to S2 in any other way. The microfibril angles and the direction of the gradual transition of microfibrils between S1 and S2 are based on studies of *Chamaecyparis obtusa*, *Pinus densiflora* and *Cryptomeria japonica* by Kataoka *et al.* (1992).

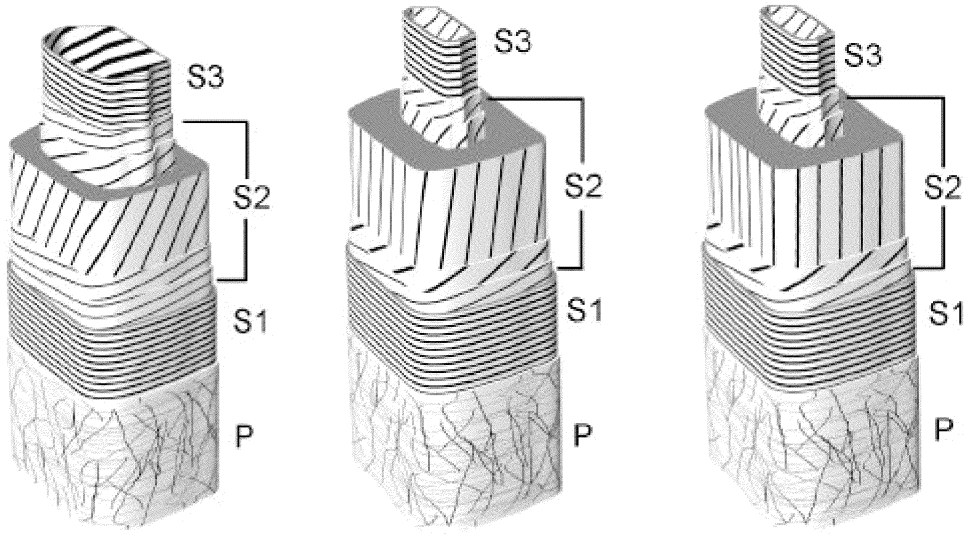
- Fig. 12a. Earlywood tracheid of juvenile- and mature wood. Microfibrillar angles in the S2 layer of Norway spruce earlywood tracheids have been reported to be ca. 28° in juvenile wood (Saranpää *et al.* 1998) and ca. 30° in first-formed earlywood tracheids of Norway spruce mature wood (Paper III).

- Fig. 12b. Latewood tracheid of juvenile wood. Sahlberg *et al.* (1997) reported microfibrillar angles of approximately 10° in the S2 layer of Norway spruce tracheids.
- Fig. 12c. Latewood tracheid of mature wood. Microfibril angle in the S2 layer of Norway spruce mature wood was reported to be ca. 2-5° (Paper III). This figure may also be representative of last-formed earlywood tracheids of mature wood, since the microfibril angle in the S2 layer was similar in last-formed earlywood and latewood (Paper III).

- Inside the S2 layer, two thin lamellae are shown in Fig. 12. It was not investigated in this thesis if these layers belong to S2 or S3. Assuming that this transition is similar to the transition between S1 and S2, they should be considered as part of S2. The reason for showing them as individual lamellae is because it is very difficult to visualise the gradual transition of microfibrils from S2 to S3 in any other way. The microfibril angles and the direction of the gradual transition of microfibrils between S2 and S3 are based on studies of *Chamaecyparis obtusa*, *Pinus densiflora* and *Cryptomeria japonica* by Kataoka *et al.* (1992).

S3 layer

- This layer was discussed in Paper I. According to Abe *et al.* (1992), both S and Z helices appeared on the innermost surfaces of Norway spruce tracheids but S helices of ca. 40-50° were predominant. When the last deposited microfibrils have an S-helix, the layer beneath often had a flatter S-helix (Abe *et al.* 1992). This is why Fig. 12 shows the outer part of S3 layer as being composed of microfibrils oriented approximately perpendicular to the tracheid axis, and the inner part with microfibrils oriented ca. 50° in an S-helix. Note that in Fig. 12 the innermost S3 is shown from the lumen, which is why the helix is Z. There is still a large amount of information missing on the ultrastructural organisation of S3 and thus it would be interesting to study this in the future.



a) b) c)

Fig. 12. Cell wall models of Norway spruce tracheids. a) earlywood tracheid, b) latewood tracheid from the juvenile wood, and c) latewood tracheid from the mature wood. The layers of the models are explained in the text above.

References

Abe, H., J. Ohtani & K. Fukazawa. 1991. FE-SEM observations on the microfibrillar orientation in the secondary wall of tracheids. *IAWA Bulletin 12*(4), 431-438.

Abe, H., J. Ohtani & K. Fukazawa. 1992. Microfibrillar orientation of the innermost surface of conifer tracheid walls. *IAWA Bulletin 13*(4), 411-417.

Abe, H., R. Funada, H. Imaizumi, J. Ohtani & K. Fukazawa. 1995a. Dynamic changes in the arrangement of cortical microtubules in conifer tracheids during differentiation. *Planta 197*, 418-421.

Abe, H., R. Funada, J. Ohtani & K. Fukazawa. 1995b. Changes in the arrangement of microtubules and microfibrils in differentiating conifer tracheids during the expansion of cells. *Annals of Botany 75*, 305-310.

Abe, H., R. Funada, J. Ohtani & K. Fukazawa. 1997. Changes in the arrangement of cellulose microfibrils associated with the cessation of cell expansion in tracheids. *Trees 11*, 328-332.

Anagnost, S.E., R.E. Mark & R.B. Hanna. 2000. Utilization of soft-rot cavity orientation for the determination of microfibril angle. Part I. *Wood and Fiber Science 32*(1), 81-87.

Andersson, S., R. Serimaa, M. Torkkeli, T. Paakkari, P. Saranpää & E. Pesonen. 2000. Microfibril angle of Norway spruce *Picea abies* (L.) Karst. compression wood: comparison of measuring techniques. *Journal of Wood Science 46*(5), 343-349.

Bailey, I.W. & M.G. Vestal. 1937a. The orientation of cellulose in the secondary wall of tracheary cells. *Journal of the Arnold Arboretum 18*, 185-195.

Bailey, I.W. & M.G. Vestal. 1937b. The significance of certain wood-destroying fungi in the study of the enzymatic hydrolysis of cellulose. *Journal of the Arnold Arboretum 18*, 196-205.

Bailey, I.W. 1938. Cell wall structure of higher plants. *Industrial and Engineering Chemistry 30*, 40-47.

Bamber, R.K. 2001. A general theory for the origin of growth stresses in reaction wood: How trees stay upright. *IAWA Journal 22*(3), 205-212.

Bardage, S.L. 2001. Three-dimensional modeling and visualization of whole Norway spruce latewood tracheids. *Wood and Fiber Science 33*(4), 627-638.

Barnett, J.R. 1981. Secondary xylem cell development. *In: Xylem cell development*. Ed. J. R. Barnett. Castle House Publications Ltd., Tunbridge Wells, Kent, UK. pp. 47-95.

Barrett, J.D., A.P. Schniewind & R.L. Taylor. 1972. Theoretical shrinkage model for wood cell walls. *Wood Science 4*(3), 178-192.

Baskin, T.I. 2001. On the alignment of cellulose microfibrils by cortical microtubules: a review and a model. *Protoplasma 215*, 150-171.

Batchelor, W.J., A.B. Conn & I.H. Parker. 1997. Measuring the fibril angle of fibres using confocal microscopy. *Appita Journal 50*(5), 377-380.

Batchelor, W.J., A.B. Conn & I.H. Parker. 2000. Comparison of techniques to measure the fibril angle. *Appita Journal 53*(6), 432-437.

Berenzon, M.F. & B.D. Bogomolov. 1972. Electron-microscope study of the structure of the cell-walls of tracheids of *Pinus sylvestris* (In Russian). *Lesnoi Zhurnal 15*(5), 126-132.

Bergander, A. & L. Salmén. 2000. Variations in transverse fibre wall properties: Relations between elastic properties and structure. *Holzforschung 54*(6), 654-660.

Bergander, A. & L. Salmén. 2002. Cell wall properties and their effects on the mechanical properties of fibres. *Journal of Materials Science 37*(1), 151-156.

Berglund, A., H. Brelid, A. Rindby & P. Engström. 1999. Spatial distribution of metal ions in spruce wood by synchrotron radiation microbeam X-ray fluorescence analysis. *Holzforschung 53*(5), 474-480.

Bogomolov, B.D. & M.F. Berenzon. 1971. The fibrillar structure of the S layer of the cell-wall of wood. *Khimiya Drevesiny 7*, 171-174.
Brown, R.M. 1996. The biosynthesis of cellulose. *Journal of Macromolecular Science-Pure and Applied Chemistry A33*(10), 1345-1373.
Brown, R.M. 1999. Cellulose structure and biosynthesis. *Pure and Applied Chemistry 71*(5), 767-775.
Bucher, H. 1957. Die Struktur der Tertiarwand von Holzfasern. *Holzforschung 11*, 97-102.
Cave, I.D. 1966. Theory of X-ray measurement of microfibril angle in wood.
Cave, I.D. 1968. The anisotropic elasticity of the plant cell wall. *Wood Science and Technology 2*(4), 268-278.
Chaffey, N., J.R. Barnett & P. Barlow. 1999. A cytoskeletal basis for wood formation in angiosperm trees: the involvement of cortical microtubules. *Planta 201*(1), 19-30.
Côté, W.A. & R.B. Hanna. 1983. Ultrastructural characteristics of wood fracture surfaces. *Wood and Fiber Science 15*(2), 135-163.
Daniel, G. & T. Nilsson. 1998. Developements in the study of soft rot and bacterial decay. *In: Forest Products Biotechnology*. Eds. A. Bruce and J. W. Palfreyman. Taylor and Francis, London. pp. 37-62.
Davies, G.W. 1968. Microscopic observations of wood fracture. *Holzforschung 22*(6), 177-180.
Denne, M.P. 1974. Effects of light intensity on tracheid dimensions in *Picea sitchensis*. *Annals of Botany 38*, 337-345.
Dinwoodie, J.M. 2000. *Timber: Its nature and behaviour (Second Edition)*. E & FN Spon, London. pp. 257.
Dodd, R.S. & P. Fox. 1990. Kinetics of tracheid differentiation in Douglas-fir. *Annals of Botany 65*, 649-657.
Dolmetsch, H., E. Franz & E. Correns. 1944. Zusammenhänge zwischen morphologischer Struktur und Kettenlänge bei nativen Zellulosefasern. *Kolloid-Zeitschrift Band 106, Heft 3*, 175.
Donaldson, L.A. 1991. The use of pit apertures as windows to measure microfibril angle in chemical pulp fibers. *Wood and Fiber Science 23*(2), 290-295.
Donaldson, L.A. 1995. Cell wall fracture properties in relation to lignin distribution and cell dimensions among three genetic groups of radiata pine. *Wood Science and Technology 29*(1), 51-63.
Donaldson, L.A. 1996. Clonal variation in the fracture properties of radiata pine wood. *In: Recent advances in wood anatomy*. Eds. L. A. Donaldson, A. P. Singh and B. G. Butterfield. New Zealand Forest Research Institute Ltd, Rotorua, New Zealand. pp. 283-291.
Donaldson, L.A. 1998. Between-tracheid variability of microfibril angles in radiata pine. *In: Microfibril Angle in Wood*. Ed. B. G. Butterfield. IAWA, and IUFRO, Christchurch, New Zealand. pp. 206-224.
Donaldson, L.A. & M.J.F. Lausberg. 1998. Comparison of conventional transmitted light and confocal microscopy for measuring wood cell dimensions by image analysis. *IAWA J. 19*, 321-326.
Donaldson, L.A. 2001. A three-dimensional computer model of the tracheid cell wall as a tool for interpretation of wood cell wall ultrastructure. *IAWA Journal 22*(3), 213-233.
Dunning, C.E. 1969. The structure of longleaf pine latewood I. Cell-wall morphology and the effect of alkaline extraction. *Tappi 52*(7), 1326-1335.
Emerton, H.W. & V. Goldsmith. 1956. The structure of the outer secondary wall of pine tracheids from kraft pulps. *Holzforschung 10*, 108-115.
Emons, A.M.C. 1994. Winding threads around plant cells: a geometrical model for microfibril deposition. *Plant, Cell Environment 17*, 3-14.
Emons, A.M.C. & H. Kieft. 1994. Winding threads around plant cells: Applications of the geometrical model for microfibril deposition. *Protoplasma 180*(1-2), 59-69.

Emons, A.M.C. & B.M. Mulder. 1998. The making of the architecture of the plant cell wall: How cells exploit geometry. *Proceedings of the National Academy of Sciences of the United States of America 95*(12), 7215-7219.
Emons, A.M.C. & B.M. Mulder. 2000. How the deposition of cellulose microfibrils builds cell wall architecture. *Trends in Plant Science 5*(1), 35-40.
Evans, R. 1998. Rapid scanning of microfibril angle in increment cores by x-ray diffractometry. *In: Microfibril angle in wood*. Ed. B. G. Butterfield. IAWA, and IUFRO, Christchurch, New Zealand. pp. 116-139.
Fengel, D. & M. Stoll. 1973. Variation in cell cross-sectional area, cell-wall thickness and wall layers of spruce tracheids within an annual ring. *Holzforschung 27*, 1-7.
Fengel, D. & G. Wegener. 1984. *Wood: Chemistry, Ultrastructure, Reactions*. Walter de Gruyter, Berlin. pp. 613.
Forgacs, O.L. 1963. The characterization of mechanical pulp. *Pulp Paper Mag. Can. 64*, T89-T118.
Frei, E., R.D. Preston & G.W. Ripley. 1957. The fine structure of the walls of conifer tracheids. VI. Electron microscope investigations of sections. *Journal of Experimental Botany 8*, 139-146.
Funada, R., O. Furusawa, M. Shibagaki, H. Miura, T. Miura, H. Abe & J. Ohtani. 2000. The role of cytoskeleton in secondary xylem differentation in conifers. *In: Cell and Molecular Biology of Wood Formation*. Eds. R. Savidge, J. R. Barnett and R. Napier. BIOS Scientific Publishers Limited, Oxford. pp. 255-264.
Gindl, W. & R. Wimmer (2000). Relationship between lignin content and tracheid morphology in spruce. *In:* Proceedings of 3rd Plant Biomechanics Conference, Aug 27-Sept 2, Freiburg, Germany, pp. 163-168.
Gullichsen, J. 2000. Chemical pulping. *In: Chemical pulping*. Eds. J. Gullichsen and C.-J. Fogelholm. Finnish Paper Engineers' Association and TAPPI, Jyväskylä. pp. A19-A243.
Hannrup, B., O. Danell, I. Ekberg & M. Moell. 2001. Relationships between wood density and tracheid dimensions in Pinus sylvestris L. *Wood and Fiber Science 33*(2), 173-181.
Harada, H. & W.A. Côté Jr. 1985. Structure of wood. *In: Biosynthesis and biodegradation of wood components*. Ed. T. Higuchi. Academic Press, Orlando. pp. 1-42.
Haygreen, J.G. & J.L. Bowyer. 1989. *Forest products and Wood Science. An introduction. Second edition*. Iowa State Univ. Press, Ames, Iowa, US.
Herman, M., P. Dutilleul & T. Avella-Shaw. 1998. Intra-ring and inter-ring variations of tracheid length in fast-grown versus slow-grown Norway spruce (*Picea abies*). *IAWA J. 19*, 3-23.
Herman, M., P. Dutilleul & T. Avella-Shaw. 1999. Growth rate effects on intra-ring and inter-ring trajectories of microfibril angle in Norway spruce (*Picea abies*). *IAWA Journal 20*(1), 3-21.
Hiller, C.H. 1964. Correlation of fibril angle with wall thickness of tracheids in summerwood of slash and loblolly Pine. *Tappi Journal 47*(2), 125-128).
Hirakawa, Y. & Y. Fujisawa. 1995. The relationships between microfibril angles of the S2 layer and latewood tracheid lengths in elite Sugi tree (*Cryptomeria japonica*) clones. *Mokuzai Gakkaishi 41*(2), 123-131.
Hodge, A.J. & A.B. Wardrop. 1950. An electron microscopic investigation of the cell wall organization of conifer tracheids and conifer cambium. *Aust. J. Sci. Res. 3*, 265-269.
Huang, C., N.P. Kutcha, G.L. Leaf & R.A. Megraw. 1998. Comparison of microfibril angle measurement techniques. *In: Microfibril Angle in Wood*. Ed. B. G. Butterfield. IAWA, and IUFRO, Christchurch, New Zealand. pp. 177-205.
Huang, C.-L. 1995. Revealing fibril angle in wood sections by ultrasonic treatment. *Wood and Fiber Science 27*(1), 49-54.
Imamura, Y., H. Harada & H. Saiki. 1972. Electron microscopic study on the formation and organization of the cell wall in coniferous tracheids: crisscrossed and transition

structures in the secondary wall. *Bulletin of the Kyoto University Forests No 44*, 183-193.
Jakob, H.F., P. Fratzl & S.E. Tschegg. 1994. Size and arrangement of elementary cellulose fibrils in wood cells: A small-angle x-ray scattering study of *Picea abies*. *J. Struct. Biol. 113*, 13-22.
Jang, H.F. 1998. Measurement of fibril angle in wood fibres with polarization confocal microscopy. *Journal of Pulp and Paper Science 24*(7), 224-230.
Jones, G.B. 1958. Variations in cell size and cell wall thickness in Norway Spruce, *Picea abies* Karst. *Journal Oxford University Forestry Society 6*, 35-43.
Kantola, M. & H. Kähkönen. 1963. Small-angle X-ray investigation of the orientation of crystallites in Finnish coniferous and deciduous wood fibres. *Ann Acad Sci Fenn A VI 137*, 1-14.
Kantola, M. & S. Seitsonen. 1969. On the relation between tracheid length and microfibrillar orientation measured by X-ray diffraction in conifer wood. *Ann. Acad. Sci. Fenn. A. 300*, 2-10.
Karlsson, H., P.-I. Fransson & U.-B. Mohlin (1999). STFI Fibermaster. *In:* 6th International Conference on New Available Technologies., Stockholm, Sweden, pp. 367-374.
Kataoka, Y., H. Saiki & M. Fujita. 1992. Arrangement and superimposition of cellulose microfibrils in the secondary walls of coniferous tracheids. *Mokuzai Gakkaishi 38*(4), 327-335.
Kerr, T. & I.W. Bailey. 1934. The cambium and its derivative tissues. No X. Structure, optical properties and chemical composition of the so-called middle lamella. *Journal Arnold Arboretum 15*, 327-349.
Khalili, S. 1999. Microscopical studies on plant fibre structure, Ph.D. Thesis, Swedish University of Agricultural Sciences; Uppsala; Sweden.
Khalili, S., T. Nilsson & G. Daniel. 2001. The use of soft rot fungi for determining the microfibrillar orientation in the S2 layer of pine tracheids. *Holz als Roh- und Werkstoff 58*(6), 439-447.
Kure, K.A. (1997). The alteration of the wood fibres in refining. *In:* International mechanical pulping conference, 9-13 June 1997, Stockholm, Sweden pp. 137-150.
Larson, L.R. 1994. *The vascular cambium: development and structure*. Springer Verlag, Berlin. pp. 725.
Ledbetter, M.C. & K.R. Porter. 1963. A "microtubule" in plant cell fine structure. *Journal of Cell Biolology 19*, 239-250.
Leney, L. 1981. A technique for measuring fibril angle using polarized light. *Wood Fiber 13*(1), 13-16.
Levi, M.P. & R.D. Preston. 1965. A chemical and microscopic examination of the action of the soft-rot fungus *Chaetomium globosum* on Beechwood (*Fagus sylvatica*). *Holzforschung 19*(6), 183-190.
Lichtenegger, H., A. Reiterer, S.E. Tschegg & P. Fratzl. 1998. Determination of spiral angles of elementary fibrils in the wood cell wall: comparison of small-angle X-ray scattering and wide-angle X-ray diffraction. *In: Microfibril angle in wood*. Ed. B. G. Butterfield. IAWA & IUFRO, Christchurch, New Zealand. pp. 140-156.
Lichtenegger, H., A. Reiterer, T.S.E. Stanzl & P. Fratzl. 1999. Variation of cellulose microfibril angles in softwoods and hardwoods: A possible strategy of mechanical optimization. *Journal of Structural Biology 128*(3), 257-269.
Liese, W. 1970. Elektronenmikroskopie des Holzes. *In: Handbuch der Mikroskopie in der Technik, Bd V. Teil 1: Mikroskopie des Rohholzes un der Rinden*. Ed. H. Freund. Umschau Verlag, Frankfurt am Main. pp. 109-170.
Long, J.M., A.B. Conn, W.J. Batchelor & R. Evans. 2000. Comparison of methods to measure fibril angle in wood fibres. *Appita Journal 53*(3), 206-209.
Lüdtke, M. 1931. Untersuchungen über Aufbau und Bildung der pflanzlichen Zellmembran und ihrer stofflichen Komponenten (In german). *Biochemische Zeitschrift 233*, 1-57.

Marton, R., P. Rushton, J.S. Sacco & K. Sumiya. 1972. Dimensions and ultrastructure in growing fibers. *Tappi Journal 55*, 1499-1504.
Meier, H. 1955. Uber den Zellwandabbau durch Holzvermorschungspilze und die submikroskopische Struktur von Fichtentracheiden und Birkenholzfasern. *Holz als Roh- und Werkstoff 13*, 323-338.
Meylan, B.A. 1967. Measurement of microfibril angle by X-ray diffraction.
Meylan, B.A. & B.G. Butterfield. 1972. *Three-dimensional structure of wood : a scanning electron microscope study*. Chapman and Hall, London. pp. 80.
National Board of Forestry. 2001. Skogsstatistisk årsbok 2001. Statistical Yearbook of Forestry 2001. National Board of Forestry, Jönköping, Sweden.
Niklas, K.J. 1992. *Plant biomechanics: an engineering approach to plant form and function*. University of Chicago Press, Chicago. pp. 607.
Nilsson, T. 1974a. Microscopic studies on the degradation of cellophane and various cellulosic fibres by wood-attacking microfungi. *Studia Forestalia Suecica 117*, 1-32.
Nilsson, T. 1974b. Formation of soft rot cavities in various cellulose fibres by *Humicola alopallonella* Meyers and More. *Studia Forestalia Suecica 112*, 1-30.
Nyrén, V. & E. Back. 1960. The dimensions of tracheidal and parenchymatous ray cells of *Picea abies* (Karst.) pulpwood. The resin in parenchymatous cells and resin canals of conifers. VII. *Svensk Papperstidning 63*(18), 619-624.
Page, D.H. 1969. A method for determining the fibrillar angle in wood tracheids. *Journal of Microscopy 90*, 137-143.
Page, D.H., F. El Hosseiny, K. Winkler & R. Bain. 1972. The mechanical properties of single wood-pulp fibres. Part I: A new approach. *Pulp and Paper Magazine of Canada 73*(8), 72-77.
Page, D.H., F. El Hosseiny, K. Winkler & A.P.S. Lancaster. 1977. Elastic modulus of single wood pulp fibers. *Tappi 60*(4), 114-117.
Panshin, A.J. & C. de Zeeuw. 1980. *Textbook of Wood Technology, 4th Ed.* McGraw-Hill Book Company, New York. pp. 722.
Persson, K. 2000. Micromechanical modelling of wood and fibre properties, Doctoral thesis, Lund University, Lund.
Petrić, B. & V. Šćukanec. 1973. Volume percentage of tissues in wood of conifers grown in Yugoslavia. *IAWA Bulletin 2*, 3-7.
Preston, R.D. 1934. The organization of the cell wall of the conifer tracheid. *Phil. Trans. R. Soc. Lond. Biol. 224*, 131-173.
Reiterer, A., H.F. Jakob, S.E. Stanzl Tschegg & P. Fratzl. 1998. Spiral angle of elementary cellulose fibrils in cell walls of *Picea abies* determined by small-angle X-ray scattering. *Wood Science and Technology 32*(5), 335-345.
Roelofsen, P.A. 1959. *The Plant Cell-Wall, Encyclopedia of plant anatomy*. Gebrüder Borntraeger, Berlin. pp. 335.
Roland, J.C., D. Reis, B. Vian, B. Satiat-Jeunemaitre & M. Mosiniak. 1987. Morphogenesis of cell walls at the supramolecular level: internal geometry and versatility of helicoidal expression. *Protoplasma 140*, 75-97.
Sahlberg, U., L. Salmen & A. Oscarsson. 1997. The fibrillar orientation in the S2-layer of wood fibres as determined by X-ray diffraction analysis. *Wood Science and Technology 31*, 77-86.
Salmén, L. & A. de Ruvo. 1985. A model for the prediction of fiber elasticity. *Wood and Fiber Science 17*(3), 336-350.
Saranpää, P., R. Serimaa, S. Andersson, E. Pesonen, T. Suni & T. Paakkari. 1998. Variation of microfibril angle of Norway spruce (*Picea abies* (L.) Karst.) and Scots pine (*Pinus sylvestris* L.) -comparing x-ray diffraction and optical methods. *In: Microfibril angle in wood*. Ed. B. G. Butterfield. IAWA & IUFRO, Christchurch, New Zealand. pp. 240-252.

Sell, J. & T. Zimmermann. 1993. Radial fibril agglomerations of the S2 on transverse-fracture surfaces of tracheids of tension-loaded spruce and white fir. *Holz als Roh- und Werkstoff 51*, 384.
Senft, J.F. & B.A. Bendtsen. 1985. Measuring microfibrillar angles using light microscopy. *Wood and Fiber Science 17*(4), 564-567.
Skene, D.S. 1969. The period of time taken by cambial derivatives to grow and differentiate into tracheids in *Pinus radiata* D. Don. *Annals of Botany 33*(253-262).
Skene, D.S. 1972. The kinetics of tracheid development in *Tsuga canadensis* Carr. and its relation to tree vigour. *Annals of Botany 36*, 170-187.
Steenberg, B. 1947. Beating process studied by fibre swelling. *Svensk Papperstidning 11B*, 155-163.
Svensson, A.A. 1956. The surface fine structure of conifer cell walls. *Arkiv för Kemi 10*(3), 239-50.
Tang, R.C. 1973. The microfibrillar orientation in cell-wall layers of Virginia Pine tracheids. *Wood Science 5*(3), 181-186.
Timell, T.E. 1986. The structure of compression wood. *Springer-Verlag, Berlin, Heidelberg New York Tokyo 1*, 705.
Wang, H.H., J.G. Drummond, S.M. Reath & P.A. Watson. 2001. An improved fibril angle measurement method for wood fibres. *Wood Science and Technology 34*, 493-503.
Wardrop, A.B. & H.E. Dadswell. 1950. The swelling behaviour of conifer tracheids and the concept of a skin substance. *Proc. Aust. Pulp Pap. Ind. tech. Ass. 4*, 198-226.
Wardrop, A.B. 1954. The fine structure of the conifer tracheid. *Holzforschung 8*, 12-29.
Wardrop, A.B. 1957. The organization and properties of the outer layer of the secondary wall in conifer tracheids. *Holzforschung 11*, 102-110.
Wardrop, A.B. 1964. The structure and formation of the cell wall in xylem. *In: The formation of wood in forest trees*. Ed. M. H. Zimmermann. Academic Press, New York. pp. 87-134.
Varhimo, A. & O. Tuovinen. 1999. Raw materials. *In: Mechanical pulping*. Ed. J. Sundholm. Finnish Paper Engineers' Association and TAPPI, Jyväskylä. pp. 67-104.
Westermark, U., H.L. Hardell & T. Iversen. 1986. The content of protein and pectin in the lignified middle lamella primary wall from spruce fibers. *Holzforschung 40*(2), 65-68.
Wilson, B.F. & R.A. Howard. 1968. A computer model for cambial activity. *Forest Science 14*(1), 77-90.
Wodzicki, T.J. 1971. Mechanism of xylem differentiation in Pinus sylvestris L. *Journal of Experimental Botany 22*(72), 670-687.
Woodcock, S., B. Henrissat & J. Sugiyama. 1995. Docking of Congo Red to the surface of crystalline cellulose using molecular mechanics. *Biopolymers 36*(2), 201-210.
Wymer, C. & C. Lloyd. 1996. Dynamic microtubules: implications for cell wall patterns. *Trends in Plant Science 7*, 222-228.
Yasue, K., R. Funada, O. Kobayashi & J. Ohtani. 2000. The effects of tracheid dimensions on variations in maximum density of *Picea glehnii* and relationships to climatic factors. *Trees: Structure and Function 14*(4), 223-229.
Ye, C. & O. Sundström. 1997. Determination of S2-fibril-angle and fiber-wall thickness by microscopic transmission ellipsometry. *Tappi 80*(6), 181-190.

Acknowledgements

This work was carried out at the Wood Ultrastructure Research Centre (WURC), Department of Wood Science, Swedish University of Agricultural Sciences. WURC is gratefully acknowledged for financial support of this project.

I am very grateful to my supervisors Professor Thomas Nilsson and Professor Geoffrey Daniel for guiding me through the fascinating world of wood ultrastructure and microscopy and for all advice, support and encouragement during my Ph.D. studies. Stig Bardage is gratefully acknowledged for fruitful discussions, critical reading of the thesis, and for helping me with 3D-modeling. Nasko Terziev is gratefully acknowledged for careful and critical reading of the this thesis and a special thanks to Lloyd Donaldson, Forest Research Institute, New Zealand for giving valuable comments on the thesis

A very warm thank to all my colleagues at the Department of Forest Products and later Department of Wood Science, especially Isabelle Duchesne, Jonas Hafrén, Ann-Sofie Hansén, Björn Henningsson, Lars Hildén, Eva Myrsell Nilsson, Erik Persson, Karolina Nyholm and Miyuki Takeuchi and to all my WURC colleagues at universities, institutes and companies. My co-authors, Anna Bergander and Lennart Salmén at STFI are gratefully acknowledged for fruitful co-operation.

I had the pleasure to broaden my knowledge of wood fibres from the industrial perspective during my stay in Piteå at AssiDomän. Ove Rehnberg (today Kappa Kraftliner) is gratefully acknowledged for making that time so great. Gunilla Söderstam StoraEnso and Inger Eriksson, SCA are also gratefully acknowledged for giving me an industrial perspective of wood and tracheid morphology.

Finally I would like to thank my family, my parents and my sisters for their love and for always supporting and believing in me and, above all, my beloved wife Karin, for all her support and unconditional love and to our children Oskar and Elsa for being the essence of joy and happiness in life.

This thesis is dedicated to my parents, my mother Christina and my late father Sven.