THE STRUCTURAL ORGANISATION OF THE S₁ CELL WALL LAYER OF NORWAY SPRUCE TRACHEIDS

by

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SUMMARY

The purpose of the present study was to investigate the ultrastructure of the outer layer of the secondary cell wall (S₁) of Norway spruce (*Picea abies* (L.) Karst.) tracheids with emphasis on microfibril orientation. Several methods were used to obtain complementary results. Macerated tracheids and wood blocks were exposed to degradation by soft rot fungi, different chemicals and mechanical forces. Observations were made using scanning and transmission electron microscopy and light microscopy. It was concluded that the S₁ layer is rather homogeneous with respect to microfibril orientation. The major part of the S₁ is composed of microfibrils oriented approximately perpendicular to the tracheid axis. Evidence for a crossed fibrillar structure with microfibrils in alternate S- and Z-helices was not obtained. The transition of microfibril orientation from the S₁ to the S₂ layer appeared abrupt. The transitional/intermediate lamella, where a gradual change in microfibril orientation occurs, should be considered to belong to the outer part of S₂.

Key words: Outer layer, cell wall, S₁, S₂, *Picea abies*, microfibril, ultrastructure, crossed fibrillar structure, microfibril angle.

INTRODUCTION

The outer layer of the secondary wall (i.e. S₁ layer) is c. 0.12 µm thick in earlywood and c. 0.38 µm thick in latewood cell walls of Norway spruce (*Picea abies* (L.) Karst.) tracheids and represents approximately 10% of the total cell wall thickness (i.e. 12% earlywood and 9% latewood, Fengel & Stoll 1973). The S₁ layer is much thicker in compression wood tracheids than in normal wood (Timell 1986). Since the S₁ layer is rather thin, considerable difficulties arise when trying to determine its architectural organisation. Therefore, S₁ ultrastructure has not been investigated in as much detail as the thick middle layer of the secondary wall (S₂) which represents about 80% of the total cell wall thickness (Fengel & Stoll 1973). However, the S₁ layer has a very important role for pulp fibres since it will be in direct contact with chemicals or enzymes and during beating it will be subjected to large mechanical forces. The thickness and microfibril angle of the S₁ layer has been shown to be important for the transverse elastic modulus of Norway spruce tracheids (Bergander & Salmén 2000, 2002).

Kerr and Bailey (1934) and later Bailey and Vestal (1937a) concluded that cellulose microfibrils in the S_1 layer are oriented at nearly right angles to the tracheid axis. Later, several authors claimed that the S_1 layer is composed of two striations of cellulose microfibrils with alternate helical orientations involving both left-handed S- and right-handed Z-helices (Hodge & Wardrop 1950; Emerton & Goldsmith 1956; Frei et al. 1957; Wardrop 1957; Jurbergs 1960; Dunning 1969; Crosby et al. 1972; Tang 1973). Today, this is often referred as the “crossed fibrillar structure”. Emerton and Goldsmith (1956) also claimed non-spiralling fibrils in the S_1 layer at cell wall corners but this was rejected by Wardrop (1957).

Although there are many reports of a crossed fibrillar structure in the S_1 layer, this has been questioned. Svensson (1956) studied the surface ultrastructure of Norway spruce pulp fibres and concluded that microfibrils were oriented in one helix about $50-80^\circ$ to the tracheid axis. Berenzon and Bogomolov (1972) studied ultrathin, partially delignified sections of *Pinus sylvestris* and observed c. 9 lamellae between the primary wall and the S_2 layer where microfibrils gradually changed direction from a transverse to longitudinal arrangement. Berenzon and Bogomolov (1972) claimed that the S_1 layer does not have a crossed fibrillar structure. In addition, Khalili et al. (2001) studied soft rot attacked *Pinus sylvestris* wood and suggested that the S_1 layer consists of at least one substantial lamella which is oriented almost perpendicular to the tracheid axis.

Observations on the ultrastructure of the S_1 layer have also been conducted on differentiating tracheids. Imamura et al. (1972) studied replicas of the inner surface of differentiating tracheids of *Pinus densiflora* using transmission electron microscopy (TEM). Cellulose microfibrils were found to be deposited with alternate helices and several transition lamellae were observed between the S_1 and the S_2 . Later, Abe et al. (1991) used field emission scanning electron microscopy (FE-SEM) to study the inner surfaces of developing tracheids of *Abies sachalinensis*. They reported the outer part of the S_1 to have an S-helix of about 45° and as more layers were added to the secondary wall the microfibril orientation changed clockwise as seen from the lumen side to c. 70° in a Z-helix. The same change in helix type and similar angles have been observed in subsequent studies (Kataoka et al. 1992; Abe et al. 1995a; Abe et al. 1997). Cortical microtubules, which are thought to direct cellulose microfibril deposition, have a similar change in orientation as the microfibrils during differentiation (Abe et al. 1995b). Kataoka et al. (1992) used TEM to study replicas of newly-formed wall surfaces of *Chamaecyparis obtusa*, *Pinus densiflora* and *Cryptomeria japonica*. They concluded that microfibril deposition occurred repeatedly with different S-angles in the S_1 layer but between the S_1 and the S_2 layers, the microfibrils were deposited unidirectionally and clockwise (as seen from the lumen side) towards the S_2 layer.

The region between the S_1 and the S_2 layers, often denoted S_{12} , has been termed the intermediate (Wardrop 1964; Tang 1973) or transition lamella (Imamura et al. 1972; Harada & Côté Jr 1985). In the S_{12} lamella microfibrils have an orientation intermediate between that of the S_1 and the S_2 , in a semi-helicoidal structure (Roland et al. 1987). The definition of S_{12} is not clear and it is not known if the lamella belongs to the S_1 or S_2 or is an independent layer. The terminology is also confused by the fact that the S_1 layer has been called the transition lamella (Meier 1955).

The crossed fibrillar structure of S₁ has been represented in several cell wall models (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). However, other cell wall models show the S₁ layer as a homogeneous layer with one microfibril helix (e.g. Dolmetsch et al. 1944; Bucher 1957; Ward et al. 1964; Côté 1967; Liese 1970). Thus, although there have been several studies on the ultrastructure of the S₁ layer, there is still considerable controversy and disagreement. In order to model the mechanical properties of the wood cell wall it is important to have increased knowledge on the microfibril orientation of the S₁ layer (Koponen 1998; Bergander & Salmén 2002). The aim of this study is therefore to investigate and discuss the structural organisation of the S₁ layer of Norway spruce tracheids with emphasis on microfibril orientation. Several methods were used in order to obtain complementary results.

MATERIALS AND METHODS

Degradation by soft rot fungi

When soft rot fungi degrade the cell walls of wood fibres they reveal the orientation of the cellulose microfibrils by the presence of cavities (Bailey & Vestal 1937b; Levi & Preston 1965; Nilsson 1974a, b; Khalili 1999; Anagnost et al. 2000; Bergander et al. 2002). The use of soft rot cavities for studies of microfibril angle in wood was reviewed by Brändström et al. (2002). Soft rot fungi were used in this study to reveal the microfibril orientation in the S₁ layer. Wood blocks were cut with a knife from a never dried, frozen Norway spruce (*Picea abies*) tree disc. Thawed wood blocks were macerated in a 1:1 solution of acetic acid and H₂O₂ (30%) at 90 °C for 6 hours and thereafter washed thoroughly in water. Glass jars were filled with moist compost and a polyester cloth laid on the surface. About 10 g of the macerated tracheids were placed on the polyester cloths which were placed between the soil and the tracheids to prevent the tracheids from being contaminated with the soil but without hindering the fungi's ability to colonize the tracheids. Glass jars were sterilized in an autoclave for 60 min at 120 °C. After cooling, the jars were inoculated with 1 ml mycelial suspension of the soft rot fungus *Humicola alopallonea* (Meyers and Moore) strain CBS 207.60 earlier used by Nilsson (1974b). Jars were stored in an environmental chamber at 26 °C and 80% relative humidity for about three months. Thereafter the tracheids were stained with aniline blue and mounted on glass slides for light microscopy. Some small wood blocks of Norway spruce were also placed in unsterile soil containing soft rot fungi for 3 months and thereafter macerated according to Khalili et al. (2001). Macerated tracheids attacked by soft rot fungi were observed using light microscopy.

Partial delignification of wood sections

Thin wood sections (20 µm thick) were cut from never dried Norway spruce and Scots pine (*Pinus sylvestris*) blocks using a sledge microtome. Sections were partially delignified in a 1:1 solution of acetic acid and H₂O₂ (30%) at 90 °C for 2 hours followed by careful washing in distilled water. To separate cell wall layers some sections were also subjected to ultrasonic treatments in a water suspension (three 3 minute cycles). Samples were mounted on glass slides and observed using light microscopy.

Transmission electron microscopy (TEM) on untreated, partially delignified wood

Small pieces of never-dried Norway spruce wood were partially delignified and subjected to ultrasonic treatment as described above. Untreated wood pieces and the partly delignified samples were dehydrated in acetone and embedded in Spurr's low viscosity resin (Spurr 1969). Transverse ultrathin sections were cut using a diamond knife on an ultramicrotome. Sections were stained with 1% w/v KMnO_4 (prepared in 0.1% w/v sodium citrate) for 3 minutes at room temperature and later examined with a Philips 201 transmission electron microscope.

Application of shear forces to macerated tracheids and wood blocks

Macerated tracheids prepared from the soft rot study above were prepared for SEM and TEM by rapid freezing in liquid N_2 slush (i.e. at -210°C) and freeze-dried overnight using an Edwards Modulyo 4K freeze dryer. Samples were mechanically treated on glass slides by applying a shear force on the tracheids using a small steel stick. Small never-dried Norway spruce blocks (as described above) were partially delignified in a solution of acetic acid and H_2O_2 (30%) for 2 hours as described above and thereafter carefully washed in water and allowed to air dry. Shear forces were subsequently applied parallel to the grain to the partially delignified wood blocks. Sheared wood blocks and macerated tracheids to be observed by SEM were thereafter mounted on stubs bearing double-sided cell-tape and coated with gold using a Polaron E5000 sputter device and observed in a Philips XL 30 ESEM in conventional mode. Macerated tracheids to be observed by TEM were dehydrated in ethanol followed by acetone and thereafter embedded in Spurr's low viscosity resin (Spurr 1969). Ultrathin transverse sections were cut using a diamond knife and stained with 4% uranyl acetate in 50% ethanolic uranyl acetate. Sections were examined with a Philips CM12 transmission electron microscope.

Phosphoric acid treatment of macerated tracheids

Wood blocks were macerated as described above, but for four hours. Tracheids were transferred onto glass slides and excess water on the tracheid surfaces removed using a Kleenex. Phosphoric acid (85%) was applied to the tracheids under cover-slips and directly to tracheids without cover-slips and observed using light microscopy.

RESULTS

Soft rot cavities in the S_1 -layer

Soft rot cavities produced on macerated tracheids were oriented approximately at 70° (Fig. 1a) to the tracheid axis while cavities produced in wood blocks were oriented approximately perpendicular to the tracheid axis (Fig. 1b).

Observations on partially delignified wood sections

The S_1 layer appeared homogeneous and it retained a rectangular outline with clearly pronounced cell corners, as is characteristic of tracheid cell walls of untreated wood observed in transverse section (Fig. 2a, b). The S_2 and S_3 layers split from the S_1 and

buckled into the cell lumen (Fig. 2a, b). Ultrasonic treatment showed the S₂ layer to separate from the S₁ (Fig. 2b, c). This was particularly evident for Scots pine tracheids (Fig. 2c).

Fig. 1. Light micrographs of soft rot cavities (arrows), oriented approximately perpendicular to the tracheid axis, in the S₁ layer of Norway spruce tracheids. — a: Cavities produced on macerated tracheids; b: cavities produced in wood blocks which were later macerated. — Scale bars for a = 20 μ m, for b = 30 μ m.

Fig. 2. Light micrographs of a partially delignified wood sections of Norway spruce (a & b) and Scots pine (c) tracheids, without (a) and with (b & c) ultrasonic treatment. The S₁ layer (upper arrows) has retained its shape after delignification. In a and b the S₂ and S₃ layers (lower arrows) have swollen and buckled into the cell lumen. In c the S₂ and S₃ have separated totally from S₁. — Scale bars = 10 μ m.

TEM studies on untreated and partially delignified wood

The S₁ layer also appeared homogeneous in TEM observations (Fig. 3). The interface between S₁ and S₂ appeared abrupt but in the outer part of the S₂ a sinusoidal appearance of lignin/cellulose matrix was apparent (Fig. 3a). The S₂ layer separated from the S₁ layer in partially delignified samples (Fig. 3b).

Shear forces applied to macerated tracheids and wood blocks

The S₁ layer loosened from the underlying S₂ layer as shear forces were applied to the macerated tracheids (Fig. 4a & 5a, b) and wood blocks (Fig. 4b). The angle between

Fig. 3. Transmission electron micrographs of transverse sections of Norway spruce wood. – a: Untreated wood. The S₁ layer appears as a homogeneous layer lying adpressed to the middle lamella region. The zone of gradually changing lignin/cellulose distribution belongs to the outer part of the S₂ (running arrows). – b: Partly delignified wood cell followed by ultrasonic treatment. The S₂ layer has separated from the S₁ layer abruptly in the S₁/S₂ interface (arrows). In the cell corner (below white arrow) a small part of the outermost part of S₂ remained on the S₁ layer. – CCML = cell corner middle lamella; ML = middle lamella. — Scale bars = 0.25 μ m.

Fig. 4. Results of shear forces applied to macerated tracheids (a) and wood blocks (b). – a: SEM micrograph of a Norway spruce tracheid. The S₁ layer has split from the underlying S₂ layer and has cracked in several regions; the cracks are oriented approximately at right angles to the tracheid axis, consistent with the microfibrillar alignment. – b: SEM micrograph of the radial face of the bordered pit region of a Norway spruce tracheid from a partly delignified wood block, sheared parallel to grain. The S₁ layer has loosened from the S₂ and cracks (arrows) are apparent. The angle of the cracks are approximately perpendicular to the tracheid axis below the bordered pit but close to the pit border (upper left arrow) the angle of the cracks changes.

the cracks in the loosened S_1 and the tracheid axes was about 90° except for cracks close to pit borders (Fig. 4a, b). The separation of the S_1 and S_2 in response to shear forces apparently is due to the abrupt change in microfibril orientation between these layers (Fig. 5a, b). A similar separation was seen between the S_2 and S_3 (not shown). In addition, concentrically oriented slits were also observed within the S_1 and S_2 layers (Fig. 5a, b).

Fig. 5a & b. Transverse sections of sheared macerated earlywood Norway spruce tracheids observed using TEM. The S_1 layer separated from the S_2 layer and concentrically oriented slits (CS) occurred within the S_2 layers. — L = lumen; PW = primary wall; S = slit between S_1 and S_2 . — Scale bars for a & b = $1.0\ \mu\text{m}$.

Tracheids exposed to phosphoric acid

Delignified tracheids treated with phosphoric acid swelled and developed ‘balloons’ and spirals. As the tracheids swelled, they twisted along their axes and their length decreased rapidly. Some tracheids were strongly affected while others were less affected. Spirals were observed as bands upon balloons (Fig. 6). A double spiral with opposite directions, i. e. both S- and Z-helices, was never observed.

Fig. 6. Macerated Norway spruce tracheids exposed to phosphoric acid. Spirals (black arrows) which are thought to belong to the S₁ appear on the swollen S₂ (white arrows). — Scale bar = 25 μ m.

DISCUSSION

It was possible to produce soft rot cavities in the S₁ layer, in both macerated tracheids (Fig. 1a) and in wood blocks (Fig. 1b). The angle between the cavities and the tracheid axis was between c. 70° and 90° which is consistent with reports on other softwood species (Aaron & Wilson 1955; Khalili et al. 2001). The cavities produced in macerated tracheids (Fig. 1a) are most likely caused by erosion since the fine hypha (proboscis) of *Humicola alopallanella* is thicker (i. e. 0.4 μ m, Hale & Eaton 1985) than the thickness of the S₁ layer in most tracheids and also the surface of the S₁ is directly exposed to the fungal hyphae. However, the cavities produced in unsterile soil (Fig. 1b) were caused by soft rot penetrating the cell wall since these cavities were produced in wood blocks and not macerated tracheids where the S₁ was not directly exposed. In unsterile soil, various soft rot fungi can degrade the cell wall and since the fine hyphae of some soft rot fungi can be as thin as 0.2 μ m (Hale & Eaton 1985; Bardage & Daniel 1997) they can grow within the S₁ layer. The fact that soft rot cavities were present in the S₁ layer indicates that the S₁ is likely to be rather homogeneous in microfibril orientation but the existence of cavities does not imply that the microfibril angle can not change to some extent between neighbouring lamellae.

The S₁ layer appeared as a homogeneous layer when studied by light microscopy of partially delignified wood sections (Fig. 2) and by TEM of untreated (Fig. 3a) and

partly delignified wood pieces (Fig. 3b). The partially delignified sections observed using light microscopy (Fig. 2) also emphasised the enclosing feature of S_1 , since the S_2 and S_3 layers buckled into the cell lumen. The S_1 layer also appeared rigid since it remained rather intact at the cell corners (Fig. 2a, b). The sinusoidal appearance of lignin/cellulose in the outer part of S_2 (Fig. 3) is probably due to a changing microfibrillar angle. Thus, the previously observed region of gradual change of microfibrillar angle from S_1 to S_2 also called the transition or intermediate lamella, belongs to the S_2 layer.

Several studies have shown that the region between the S_1 and S_2 layers represents a zone of weakness when wood is fractured (Davies 1968; Côté & Hanna 1983; Donaldson 1995, 1996). The shear forces applied in this study confirm that the S_1 may be easily separated from the S_2 layer (Fig. 4 & 5). The characteristic cracks, which appeared in the S_1 layer, were all almost perpendicular to the tracheid axis indicating that the angle between the tracheid axis and the microfibrils was also oriented in a similar way (Fig. 4). Similar results have been presented by Donaldson (1995). In pit border regions, the angle of the cracks changed (Fig. 4b) because of changing microfibril angle in pit border regions (Bailey & Vestal 1937a). The results suggest that the bulk of the S_1 is comprised of cellulose microfibrils oriented almost perpendicular to the tracheid axis and that the transition from the S_1 to the S_2 layer is abrupt. Earlier high-resolution micrographs of the surface ultrastructure of S_1 confirm the perpendicular orientation of microfibrils in S_1 (Svensson 1956; Liese 1970; Duchesne 2001; Duchesne et al. 2001).

Macerated tracheids exposed to phosphoric acid twisted along their axes, swelled and developed 'balloons' and spirals (Fig. 6). These phenomena are similar to those reported by Steenberg (1947) for pulp fibres. It was later proposed that the balloons are derived from the swelling of the S_2 layer and that the constrictions between the balloons represent thin bands derived from the S_1 layer (Wardrop & Dadswell 1950; Wardrop 1954). The spirals observed in Figure 6 are most probably parts of the S_1 layer. The similarities to Steenberg's (1947) results, the high angle of the pitch and the thin nature of the spirals and the fact that they appear localised upon the balloons (S_2) (Fig. 6) are all consistent with this suggestion. In addition, the fact that double spirals with simultaneous S- and Z-helices were never observed, which agrees with other swelling experiments (Steenberg 1947; Wardrop & Dadswell 1950; Wardrop 1954; Meier 1957), indicates that the S_1 is unlikely to be composed of a crossed fibrillar structure.

There may be several reasons for the reported crossed fibrillar structure. The S_1 layer is very thin, therefore it is very difficult during electron microscopic studies of replicas to examine the S_1 layer alone, without some overlapping of the S_2 or S_{12} . This overlapping may give the effect of a crossed fibrillar structure. Another possibility may be that fragments from adjacent tracheid cell walls are retained on the studied cell wall. In addition, since it has been shown that microfibrils are deposited repeatedly in two adjacent directions (Kataoka et al. 1992) this may generate both S- and Z-helices if microfibrils are deposited close to 90° . Other reported evidence for a crossed fibrillar structure was based on the fact that opposite cell walls of the same tracheid were similarly birefringent in sections cut at different angles to the transverse axis (Crosby

et al. 1972; Tang 1973). However, the similarity in birefringence can be explained by the fact that the microfibril angle changes from the outermost part of S₁ to the outer part of S₂ (Abe et al. 1991; Kataoka et al. 1992), and by the fact that the resolution of light microscopy is not sufficient enough to distinguish exactly from where in the S₁/S₂ region birefringence occurs. As a matter of fact, the diagrams of Crosby et al. (1972) and Tang (1973) show that maximum birefringence occurs between 70 to 90° to the tracheid axis indicating that the majority of microfibrils are oriented close to these angles, which is consistent with this study.

CONCLUSION

The generally accepted terminology proposed by Kerr and Bailey (1934) states that a cell wall layer is characterised by a rather homogeneous orientation of microfibrils. In this respect, we conclude from the present study of Norway spruce tracheids and the literature, that the bulk of the S₁ layer of Norway spruce is composed of microfibrils predominantly oriented c. 70–90 degrees to the tracheid axis in a single helix. Evidence for a crossed fibrillar structure with microfibrils in alternate S- and Z-helices was not found. It is only when the repetitive deposition of microfibrils (Kataoka et al. 1992) occurs in angles of about 90° that alternate S- and Z-helices may be present. It is further emphasised that the S₁ layer is rather homogeneous and also rigid since it separates from the underlying S₂. This suggests that the microfibrillar orientation undergoes an abrupt change between S₁ and S₂ and that the transitional/intermediate lamella should be considered as belonging to the outer part of the S₂ layer.

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REFERENCES

- Aaron, J.R. & K. Wilson. 1955. Soft rotting fungi in timber. *Wood* 20: 186–189.
- 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. *Ann. Bot.* 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.
- Abe, H., J. Ohtani & K. Fukazawa. 1991. FE-SEM observations on the microfibrillar orientation in the secondary wall of tracheids. *IAWA Bull.* n. s. 12: 431–438.
- 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 Fiber Sci.* 32: 81–87.

- Bailey, I.W. & M.G. Vestal. 1937a. The orientation of cellulose in the secondary wall of tracheary cells. *J Arnold Arbor*. 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. *J Arnold Arbor*. 18: 196–205.
- Bardage, S.L. & G. Daniel. 1997. The ability of fungi to penetrate micropores: implications for wood surface coatings. *Mater Organismen* 31: 233–245.
- Berenzon, M.F. & B.D. Bogomolov. 1972. Electron-microscope study of the structure of the cell-walls of tracheids of *Pinus sylvestris*. *Lesnoi Zhurnal* 15: 126–132 [In Russian].
- Bergander, A., J. Brändström, G. Daniel & L. Salmén. 2002. Fibril angle variability in earlywood of Norway spruce using soft rot cavities and polarization confocal microscopy. *J. Wood Sci.* 48: 255–263.
- Bergander, A. & L. Salmén. 2000. Variations in transverse fibre wall properties: Relations between elastic properties and structure. *Holzforschung* 54: 654–660.
- Bergander, A. & L. Salmén. 2002. Cell wall properties and their effects on the mechanical properties of fibres. *J. Materials Sci.* 37: 151–156.
- Brändström, J., G. Daniel & T. Nilsson. 2002. Use of soft rot cavities to determine microfibril angles in wood; advantages, disadvantages and possibilities. *Holzforschung* 56: 468–472.
- Bucher, H. 1957. Die Struktur der Tertiärwand von Holzfasern. *Holzforschung* 11: 97–102.
- Côté, W.A. 1967. Wood ultrastructure. University of Washington Press, Seattle.
- Côté, W.A. & R.B. Hanna. 1983. Ultrastructural characteristics of wood fracture surfaces. *Wood Fiber Sci.* 15: 135–163.
- Crosby, C.M., C. de Zeeuw & R. Marton. 1972. Fibrillar angle variation in Red pine determined by senarmon compensation. *Wood Sci. Technol.* 6: 185–195.
- Davies, G.W. 1968. Microscopic observations of wood fracture. *Holzforschung* 22: 177–180.
- Dinwoodie, J.M. 2000. Timber: its nature and behaviour (2nd Ed.). E & FN Spon, London.
- Dolmetsch, H., E. Franz & E. Correns. 1944. Zusammenhänge zwischen morphologischer Struktur und Kettenlänge bei nativen Zellulosefasern. *Kolloid-Zeitschrift* Bd 106, Heft 3: 175.
- Donaldson, L.A. 1995. Cell wall fracture properties in relation to lignin distribution and cell dimensions among three genetic groups of radiata pine. *Wood Sci Technol.* 29: 51–63.
- Donaldson, L.A. 1996. Clonal variation in the fracture properties of radiata pine wood. In: L. A. Donaldson, A.P. Singh & B.G. Butterfield (eds.), *Recent advances in wood anatomy*: 283–291. New Zealand Forest Research Institute Ltd, Rotorua, New Zealand.
- Duchesne, I. 2001. Electron microscopic and spectroscopic studies on the surface ultrastructure of kraft pulp fibres. Doctoral thesis, Department of Wood Science, Swedish University of Agricultural Sciences, Uppsala.
- Duchesne, I., E. Hult, U. Molin, G. Daniel, T. Iversen & H. Lennholm. 2001. The influence of hemicellulose on fibril aggregation of kraft pulp fibres as revealed by FE-SEM and CP/MAS C-13-NMR. *Cellulose* 8: 103–111.
- Dunning, C.E. 1969. The structure of longleaf pine latewood I. Cell-wall morphology and the effect of alkaline extraction. *Tappi J.* 52: 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.
- 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.
- 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. *J. Exp. Bot.* 8: 139–146.

- Hale, M. D. & R. A. Eaton. 1985. The ultrastructure of soft rot fungi. I. Fine hyphae in wood cell walls. *Mycologia* 77: 447–463.
- Harada, H. & W. A. Côté Jr. 1985. Structure of wood. In: T. Higuchi (ed.), *Biosynthesis and biodegradation of wood components*: 1–42. Academic Press, Orlando.
- 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.
- 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. *Bull. Kyoto Univ. Forests* No 44: 183–193.
- Jurbergs, K. A. 1960. Morphological properties of cotton and wood fibres. II. Slash Pine. *Tappi J.* 43: 561–568.
- Kataoka, Y., H. Saiki & M. Fujita. 1992. Arrangement and superimposition of cellulose microfibrils in the secondary walls of coniferous tracheids. *Mokuzai Gakkaishi* 38: 327–335.
- Kerr, T. & I. W. Bailey. 1934. The cambium and its derivative tissues. X. Structure, optical properties and chemical composition of the so-called middle lamella. *J. Arnold Arbor.* 15: 327–349.
- Khalili, S. 1999. Microscopical studies on plant fibre structure, PhD 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 Roh Werkst* 58: 439–447.
- Koponen, S. 1998. Effect of wood micro-structure on mechanical and moisture physical properties. In: B. G. Butterfield (ed.), *Microfibril angle in wood*: 348–366. IAWA, and IUFRO, Christchurch, New Zealand.
- 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: 183–190.
- Liese, W. 1970. Elektronenmikroskopie des Holzes. In: H. Freund (ed.), *Handbuch der Mikroskopie in der Technik*, Bd V. Teil 1: Mikroskopie des Rohholzes und der Rinden: 109–170. Umschau Verlag, Frankfurt am Main.
- Meier, H. 1955. Über den Zellwandabbau durch Holzvermorschungspilze und die submikroskopische Struktur von Fichtentracheiden und Birkenholzfäsern. *Holz Roh Werkst* 13: 323–338.
- Meier, H. 1957. Discussion of the cell wall organization of tracheids and fibres. *Holzforschung* 11: 41–46.
- Meylan, B. A. & B. G. Butterfield. 1972. Three-dimensional structure of wood : a scanning electron microscope study. Chapman & Hall, London.
- Niklas, K. J. 1992. *Plant biomechanics: an engineering approach to plant form and function*. University of Chicago Press, Chicago.
- 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 alopallonia* Meyers and More. *Studia Forestalia Suecica* 112: 1–30.
- Roelofs, P. A. 1959. *The Plant Cell-Wall*, Encyclopedia of plant anatomy. Gebr. Borntraeger, Berlin.
- 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.
- Spurr, A. R. 1969. A low viscosity embedding medium for electron microscopy. *J. Ultrastruct. Res.* 26: 31–43.

- 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: 239–250.
- Tang, R. C. 1973. The microfibrillar orientation in cell-wall layers of Virginia Pine tracheids. *Wood Fiber Sci* 5: 181–186.
- Timell, T. E. 1986. The structure of compression wood. Springer-Verlag, Berlin, Heidelberg New York, Tokyo 1: 705.
- Ward, R. J., W. A. Côté Jr. & A. C. Day. 1964. The wood substrate-coating interface. Repr. from Official Digest, Federation of Societies for Paint Technology, Philadelphia 36: 1091–1098.
- 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: M. H. Zimmermann (ed.), *The formation of wood in forest trees*: 87–134. Academic Press, New York.
- 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.