Radial Striation of the S₂ Layer in Mild Compression Wood Tracheids of *Pinus radiata*

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Summary

The fine structure of the tracheid walls in *Pinus radiata* mild compression wood was examined by field emission scanning electron microscopy (FE-SEM) and transmission electron microscopy (TEM). On transverse-fractured surfaces the S₂ layer of tracheid walls exhibited distinct radial structures when observed with the FE-SEM. The TEM observations of ultrathin sections of samples from similar regions showed the S₂ layer with radial striations, corresponding in their direction to the radial structures seen with the FE-SEM. The observations are discussed in relation to the information on the structure and composition of compression wood cells.

Introduction

Wood properties to a large degree are determined by the macromolecular organisation and the chemical composition of wood cell walls in general and of the cell wall layering in particular (Fengel and Wegener 1989). Of the various morphologically and chemically distinguishable regions across the cell wall, the S₂ layer occupies the greatest area. Therefore, considerable more information is available on this layer than on other parts of the cell wall.

In softwoods the S₂ layer had been assumed to be morphologically and structurally homogenous. However, more recent observations suggest that this may be incorrect. Using a variety of techniques, including staining, chemical and biological treatments of wood cell walls, Daniel and Nilsson (1984) showed the S₂ layer in *Pinus sylvestris* tracheids to be concentrically lamellated, but with some indications of radial structures. Using high resolution field-emission scanning electron microscopy (FE-SEM) Sell and Zimmermann (1993) observed fibril agglomerates on the transverse fracture surfaces of the S₂ layer in spruce and white fir, with a radial or approximately radial orientation. Similar radial structures in the S₂ layer have also been recently observed on transverse-fracture surfaces of fiber cells in hardwoods (Zimmermann and Sell 1997). From SEM observations of southern yellow pine wood with brown rot decay or oxalic acid treatment Larsen *et al.* (1995) concluded that the tracheid walls have an inherent radial structure in the S₂ layer. Closer examinations of earlier TEM micrographs (Parameswaran and Liese 1980, 1981) and those taken more recently (Pöhler 1995; Singh and Schmitt, unpublished) also provide indications of radial striations in the S₂ layer of normal conifer tracheids and Douglas fir bark fibres, although further TEM observations

of thinner, distortion-free sections are needed to more clearly illustrate this feature.

Also, in a recent TEM study on bacterial degradation of tracheid walls in *P. radiata* mild compression wood (Singh 1996), the S₂ layer appeared radially striated. In this paper, the results of a closer examination of *P. radiata* mild compression wood cells using FE-SEM and TEM are reported, which extend the preliminary observations made earlier. The compression wood used in this study was considered mild and not severe because it had features which are characteristic of mild compression wood, such as poor development or absence of helical checks, absence or occasional presence of intercellular spaces, little or no rounding up of cells, and less lignified outer S₂ (OS₂) as compared to that in severe compression wood (Yumoto *et al.* 1983; Singh 1996).

Materials and Methods

Hand cut sections of *P. radiata* were first examined by light microscopy to select regions with mild compression wood, which were then processed for observation with TEM.

Field-emission scanning electron microscopy (FE-SEM)

Small conditioned rods (20°C/65% RH) of mild compression wood in the sapwood of *P. radiata* were loaded till fracture by static bending using a simple bending device without registration of the fracture load. SEM samples were dissected out from the tension-zone of the transverse-fracture surfaces, vacuum dried at 40°C and sputtered with platinum. Thickness of coating was approximately 15 nm. Subsequently, the samples were examined with a Jeol FE-SEM (JSM 6300 F) at 5 kV.

Transmission electron microscopy (TEM)

Small pieces of the hand cut sections were fixed in 1% w/v aqueous potassium permanganate (KMnO₄) for 1 hour at room temperature and subsequently washed with distilled water. The

Fig. 1 Transversely fractured mild compression wood tracheids. Inter-cellular cavities are lacking and tracheid walls are striated obliquely to the plane of the middle lamella. A few helical checks are present. The boxed region appears at a higher magnification in Figure 2. SEM micrograph. Bar = 10 μ m.

Fig. 2 High magnification view of the boxed region in Figure 1, which shows that the striations are composed of particulate wall agglomerations. SEM micrograph. Bar = 1 μ m.

samples were dehydrated in acetone and embedded in Spurr's low viscosity resin (Spurr 1969). Ultrathin sections were cut with a diamond knife on a LKB ultramicrotome, stained with 1% w/v KMnO_4 (prepared in 1% citrate buffer), and examined in a Philips 300 TEM.

Results

The FE-SEM observations of *P. radiata* mild compression wood showed the S_2 layer of tracheids to consist of formations apparently representing clusters of cellulose fibrils which were oriented radial or near-radial to the plane of the middle lamella (Fig. 1). At high magnifications, these formations appeared as aggregated wall constituents arranged as linear arrays of particles (Fig. 2).

With the TEM, the S_2 layer in these tracheids showed radial striations, which was particularly striking at lower magnifications (Fig. 3); these striations appeared to be largely confined to the less lignified inner parts of the S_2

wall (IS_2). However, high magnification views (Figs. 4, 5) revealed the striations also in the more lignified outer S_2 wall (OS_2), but not so well defined because of the high electron density of this wall, particularly in cell corner regions.

The high magnification view was instructive also in other respects. The striated walls (Fig. 3) appeared to be differentiated radially into light and dark zones (referred here as light and dark striations) (Figs. 4, 5). Although the measurements were not quantified, the light striations showed variable dimensions, the width of the smallest measuring about 30 nm. The width also varied greatly along their length with widest parts of about 300 nm. The light striations appeared to be sinuous or curvilinear (Figs. 4, 5) and some also branched (Fig. 4).

The dark striations were likewise of variable dimensions and more difficult to measure. In favourable views, the dark striations in the IS_2 wall were seen to be continuous with those of the OS_2 wall (Fig. 5). This feature was most obvious in the intercorner regions of the tracheid walls, particularly where the OS_2 wall was only slightly more lignified than the IS_2 wall (Fig. 5).

Helical checks, occurring in a few samples were of very small dimensions and corresponded closely in their orientation with the radial striations in lighter areas of the S_2 wall, i.e. light striations (Figs. 3, 5).

Discussion

The combined use of FE-SEM and TEM has provided a clear indication that in the mild compression wood of *P. radiata* the S_2 layer has also radial structures. They are identified as radial striations based primarily on the

Fig. 3 Transverse section through a corner region of mild compression wood tracheids. The middle lamella (ML) is distinct in the corner region and elsewhere. The secondary wall is differentiated into three morphologically different regions, the S_1 , the OS_2 and IS_2 . The IS_2 wall appears to be striated in a direction largely perpendicular to the plane of the middle lamella (stippled line). A helical check (arrow) traverses the IS_2 wall and penetrates half way into the OS_2 wall. TEM micrograph. Bar = 1 μ m.

Fig. 4 High magnification of an intercorner wall region where the IS₂ wall is a mosaic of light and dark areas, which gives this wall a striated appearance at low magnifications (e.g. Fig. 3). The light striations are sinuous or curvilinear and in some cases branched (arrowheads). These striations are variable in their diameter and traverse the entire thickness of the IS₂ wall. TEM micrograph. Bar = 500 nm.

Fig. 5 High magnification of an intercorner wall region where the OS₂ wall is only slightly more lignified than the IS₂ wall. The light striations are seen in both IS₂ and OS₂ walls (vertical solid lines). The dark striations are also present in both IS₂ and OS₂ walls, and in some areas they appear to be continuous across these walls (arrowheads). The stippled line indicates a region where the IS₂ wall is distinguishable from the OS₂. The arrow shows a very small crack in a light striation. TEM micrograph. ML, middle lamella; S₁, secondary wall layer. Bar = 500 nm.

appearance of this wall in low magnification TEM images. Despite some differences of their morphology, the striations display a radial or near-radial orientation and traverse the entire width of the S₂ wall in both SEM and TEM images. A rather close agreement between the diameter of these striations recorded with the TEM and that reported earlier from FE-SEM observations (Sell and Zimmermann 1993) further attests to a strong correlation between the TEM and SEM observations.

The TEM observations provided additional information. The better visibility of the striations in the IS₂ wall as compared to the OS₂ wall may be because the OS₂ wall is more highly lignified and thus stains more intensely with KMnO₄. The differentiation of striations in this region of the wall is therefore masked. The TEM views of intercorner regions of

the tracheids, where the OS₂ wall is only marginally more lignified than the IS₂ wall, confirmed the striations in both regions of the S₂ wall.

Despite some reservations to the specificity of KMnO₄ for lignin (Bland *et al.* 1971; Hoffmann and Parameswaran 1976), this stain has been widely used to contrast lignin in plant and wood cell walls (Hepler *et al.* 1970; Maurer and Fengel 1990; Diaz-Vaz *et al.* 1991; Blanchette *et al.* 1994; Donaldson 1995; Singh 1996). Recognising this, we assume for the *P. radiata* mild compression wood that lighter areas (light striations) are less lignified than darker areas (dark striations) within the mosaic of variable densities in the S₂ wall. According to Larsen *et al.* (1995), radial striation may also indicate alternating areas of higher and lower concentrations of a hemicellulose or, alternatively, a hemicellulose-lignin matrix.

Among the factors which may be related to the identification of wall striations, the quality of ultrathin sections is very important, with thinner sections yielding a better differentiation between light and dark areas. It is therefore not surprising that the presence of radial striations in the walls of mild compression wood tracheids was recorded only recently (Fig. 3a in Donaldson 1995; Singh 1996), and that evidence of this inhomogeneity of the S₂ wall in radial direction in normal wood cells has been presented only in a few studies (Daniel and Nilsson 1984; Sell and Zimmermann 1993; Larsen *et al.* 1995). Such a radial inhomogeneity in the S₂ wall was revealed only under certain conditions or with special technical approaches, e.g. examination of transverse fracture faces by FE-SEM (Sell and Zimmermann 1993), of extremely thin, distortion-free sections by TEM after KMnO₄ (prepared in citrate buffer) staining (Schmitt and Singh, unpublished), of biologically modified wood by TEM (Daniel and Nilsson 1984) or SEM (Larsen *et al.* 1995), and of mildly delignified wood by TEM (Pöhler 1995; Singh and Schmitt, unpublished).

The correspondence observed in the direction of orientation of helical checks and that of radial striations as well as the presence of very fine checks within light striations (e.g. Fig. 5) suggest that the checks which form after the lignification of tracheid walls, as revealed for spruce (Timell 1986), may largely develop in the less lignified areas of the S₂ wall. Although no evidence has been presented here that the IS₂ wall in the mild compression wood tracheids in *P. radiata* may also be radially inhomogeneous in its polysaccharide composition, our observations support the hypothesis of Fujita *et al.* (1973) that the helical checks in the compression wood cells of *Cryptomeria japonica* are formed by irregular deposition of cell wall substances.

The observation of radial striations in the S₂ wall of mild compression wood tracheids in *P. radiata* extends our understanding on the ultrastructure of compression wood cell walls. Further studies will be needed to determine the chemical basis for the striated appearance of the S₂ wall. Developmental studies (Takabe *et al.* 1992) would be required to understand the relationship of the ultrastructural and chemical organisation of compression wood cell walls

to the formation of helical checks. In this regard, mild compression wood cells may prove to be a more suitable system than severe compression wood cells. The importance of understanding the formation and structure of mild compression wood is growing particularly in those parts of the world such as New Zealand or Chile where fast growth of *P. radiata* and other conifer trees is likely to result in cambium becoming more prone to the stresses of adverse environmental factors.

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