

Chapter 2

Morphology, Anatomy and Ultrastructure of Reaction Wood

Julien Ruelle

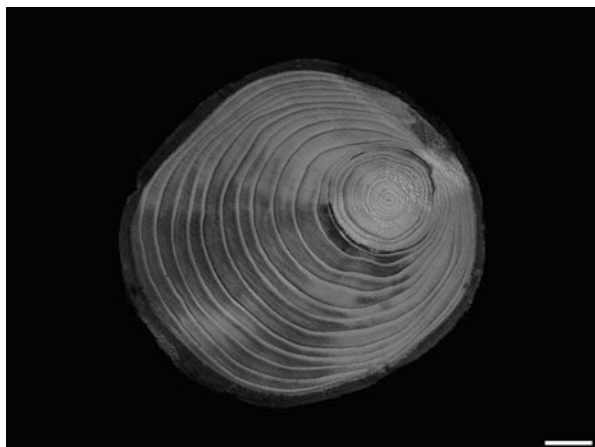
Abstract Whatever the species considered, trees reorient their axis by a very active mechanical action driven by variations of cambial activity. These variations of cambial activity will lead to variations in anatomy and ultrastructure of the xylem to achieve this biomechanical function, forming a type of wood called reaction wood, i.e. tension wood in angiosperms and compression wood in gymnosperms. This chapter focuses on the structure of reaction wood from the macroscopic level to the ultrastructural scale via the macro-, meso- and microscopic scales. It focuses in particular on differences between areas of reaction wood and other areas of wood around the circumference of the tree in terms of variation in appearance and structural organization. Therefore, the chapter starts with a description of the macroscopic appearance, followed by a description of the impact of reaction wood formation on the various tissues of the wood structure (vessels elements, fibres and parenchyma) leading to the variation occurring in the fibre cell wall and in the organization of the macromolecules inside the wall. Some methods or key features are described, for each scale, in order to highlight the occurrence of reaction wood. In addition, the limits of the described methods are discussed.

Whatever the species considered, trees reorient their axis by a very active mechanical action driven by variations of cambial activity (Sinnott 1952). Those variations of cambial activity will lead to variations in anatomy and ultrastructure of the xylem to achieve this biomechanical function, forming a type of wood called reaction wood. As it was discussed in the previous chapter the term normal wood is often used to describe any wood that is not reaction wood. However, because

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Fig. 2.1 Observation of compression wood in a stem of *Picea abies*. Scale bar = 5 cm



wood from the opposite¹ side of reaction wood can also show some variations in terms of anatomy or properties we decided to name the wood from the lateral and opposite zones relative to any reaction wood using the term “non-reaction wood” wherever possible.

During this chapter we will focus on each different scale of reaction wood one by one, especially on the differences between the reaction wood sector and other sectors around the circumference of the tree. One very important point to remember is that the process of axis reorientation in trees is always based on circumferential heterogeneity in cambial region activity occurring at various distinct structural levels.

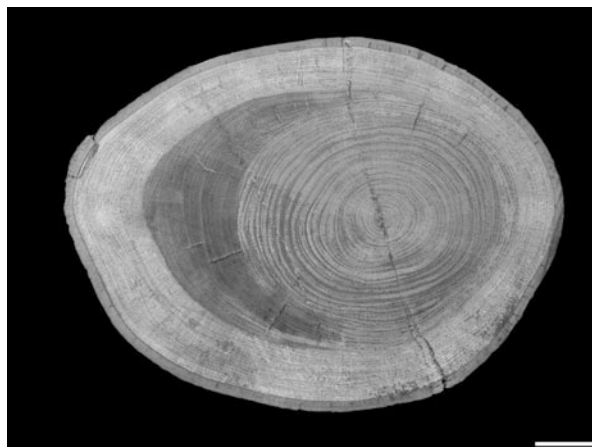
2.1 Macroscopic Appearance

The macroscopic appearance of compression wood is often described as darker in colour, varying in different species from brown to dark reddish brown. Its occurrence is associated with eccentricity of the stem, the pith being further away from the side containing compression wood (Fig. 2.1). The growth ring is therefore normally wider on the lower side of stems and branches and in species with distinct growth rings the latewood in the compression wood is wider and more marked (Dadswell and Wardrop 1949).

Tension wood in angiosperms is not always as conspicuous as compression wood in gymnosperms. It is normally also associated with eccentricity of the stem or branch with the wider rings normally being on the upper side of the stem or branches (Fig. 2.2), i.e. on the tension wood side. However, some authors

¹ In this book “opposite wood” is used to describe the wood directly across the pith from any reaction wood.

Fig. 2.2 Observation of a strong eccentricity related to the occurrence of tension wood in a stem of *Eperua falcata*. Scale bar = 5 cm



demonstrated a lack of eccentricity with tension wood occurrence or eccentricity opposite to the tension wood (Chanson 1989).

It seems that tension wood is preferentially observed in the earlywood of temperate species, but it can also be observed in latewood. Its distribution does not seem to be proportional to ring thickness as tension wood fibres can be observed both in large or thin wood rings (Jourez 1997a, b).

Tension wood can be made more visible by brushing the surface of a disk with various solutions, such as phloroglucinol in hydrochloric acid or zinc chloro-iodide solution also known as Herzberg's reagent (Jourez 1997a, b). Chlorine destroys hydrogen bonds between macro-polymers of cellulose and thus promotes the accumulation of iodine molecules. This last method seems to be more efficient (Grzeskowiak et al. 1996) and colours tension wood light purple to violet, and non-reaction wood, yellow. However, since iodine is degraded by light, the colour is transient and lasts for only around 10 min. Even in a "natural state" definite bands of tension wood have been observed in a number of species, these bands are much darker in colour than the other sectors on a disk (Dadswell and Wardrop 1949). Another example of tension wood macroscopic observation is the tension wood of poplar (*Populus* spp.) that has a shiny appearance on freshly sawn disks; some authors using this property to quantify tension wood macroscopically (Badia et al. 2005).

2.2 Tissue Level

The structure of reaction wood generally differs from non-reaction wood. If we look at the tissue organisation we see that in compression wood it is largely the tracheids that display a different anatomy, whereas the other tissues of the wood structure appear to be less affected. The transition from earlywood to latewood is very

Fig. 2.3 Traumatic vertical resin canals in a cross section of compression wood (Lee and Eom 1988). Scale bar = 200 μm

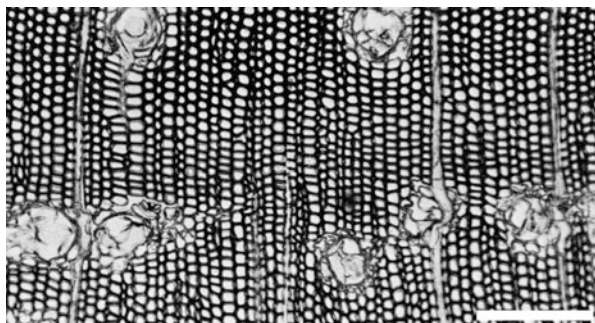
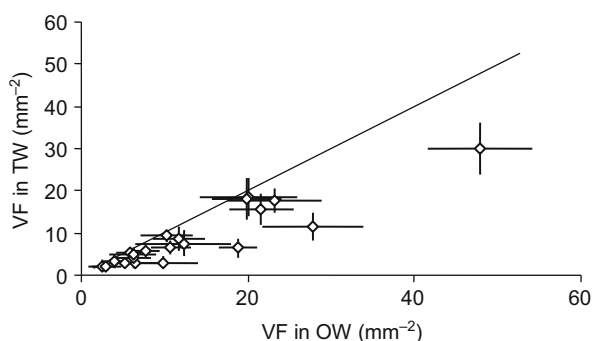


Fig. 2.4 Distribution of the 21 trees belonging to 21 tropical species in the comparison of tension wood (TW) and opposite wood (OW) for VF = vessel frequency (Ruelle et al. 2006)



gradual in compression wood so that the demarcation between earlywood and latewood is more difficult (Timell 1986; Lee and Eom 1988). Lee and Eom (1988) did observe traumatic vertical resin canals in the compression wood of *Pinus koraiensis* (Fig. 2.3), but this feature does not seem to be a consistent feature of compression wood.

In hardwood species tension wood structure shows variation for vessel frequency and proportion and fibre proportion for numerous species (Wicker 1979; Jourez 1997a, b; Ruelle et al. 2006). Even if vessel parietal structure in tension wood tissue seems to be unchanged most authors report a decrease in their diameter and frequency (Figs. 2.4 and 2.5) in tension wood tissue in comparison with non-tension wood (Jourez et al. 2001; Ruelle et al. 2006). This feature was also observed in species that do not show a peculiar unusual structure in their tension wood, such as *Magnolia* species (Yoshizawa et al. 2000) or other tropical species (Ruelle et al. 2006) and in some hardwood species from Japan (Sultana et al. 2010).

Jourez et al. (2001) did extensive work on poplar tension wood and found that not only vessel frequency but also the area of vessel lumen is lower in tension wood and consequently the proportion of vessel lumen is lowest in tension wood. Little information is available about rays and axial parenchyma in tension wood. Tsai et al. (2006) found that axial parenchyma is less abundant in tension wood of *Swietenia macrophylla* and Jourez et al. (2001) found that the number of rays is highest in the tension wood of poplar. They also found that fibres length was longer

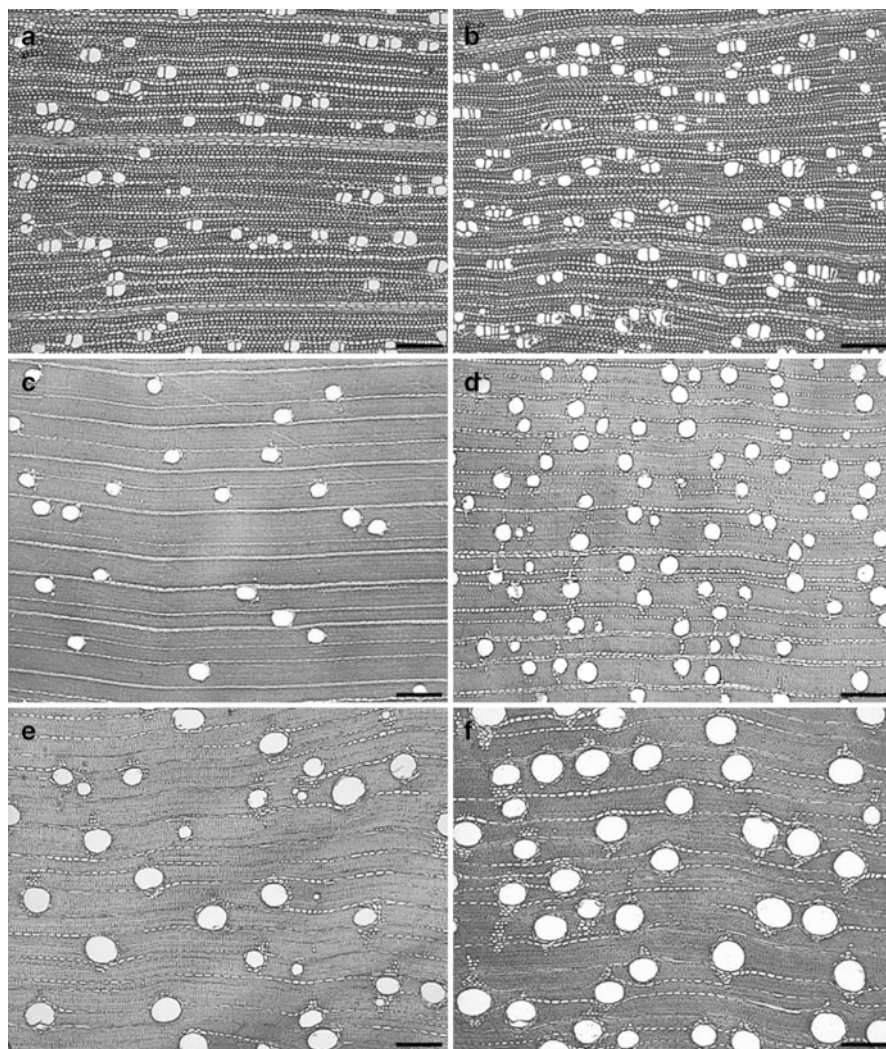


Fig. 2.5 Comparison of transverse sections of tension wood (on the left) and opposite wood (on the right) of *Casearia javitensis* (a and b), *Cassipourea guianensis* (c and d) and *Hebeptalum humiriifolium* (e and f). Scale bars = 200 μ m (Ruelle et al. 2006)

in tension wood, a result that is partially in accordance with the literature. Tension wood fibres have been described as being longer, having equivalent length or being shorter compared to non-tension wood fibres (Chow 1946; Dadswell and Wardrop 1949; Onaka 1949) and these differences appear to be strongly related to the particular species studied. Measurements of fibres transversal dimensions in several studies gave conflicting observations, with tension wood fibres narrower or wider than non-tension wood fibres. Jourez et al. (2001) found out that the diameter of

radial fibres is lower in the tension wood of poplar. During an extensive work on numerous tropical species, we found that fibre diameter or cell wall thickness did not reveal any general trend in variation between tension and non-tension wood (Ruelle et al. 2006). These results suggest that the stem eccentricity often observed with the formation of tension wood results from a larger number of cell divisions and not from larger diameters of fibres. It appears to demonstrate that the cell division rate, i.e. cambial activity, is higher in tension wood tissue.

The increase of fibre proportions observed in tension wood structure raises for several authors the concept of a “priority” being given to supporting elements during the synthesis of tension wood. If we take a further look at fibres in tension wood, we see that some authors have taken particular interest in the way that the unusual fibres synthesised in some species, called gelatinous fibres (G-fibres), are distributed in the entire cross section, for example in arcs or in a diffuse manner so they are very rare and isolated (Clair et al. 2006). Furthermore the proportion of those G-fibres is closely related to the “intensity” of tensile stress (Clair et al. 2003; Abe and Yamamoto 2007; Fang et al. 2008). Other criteria have been considered in the classification of tension wood at the cell wall level and we will consider this aspect in the next part of the chapter.

2.3 Cell Wall Level

In cross section compression wood tracheids are typically rounded in appearance and many intercellular spaces can be seen between individual cells; this appearance contrasts markedly with the more rectangular to hexagonal cross section of non-reaction wood tracheids and the complete lack of intercellular spaces (Fig. 2.6). The thick and heavily lignified wall of compression wood tracheids also often show cracks. These features can be used for compression wood classification, because they are more or less pronounced in mild, moderate and severe compression wood. Donaldson and Turner (2001) observed the absence of an S_3 layer in the compression wood of *Pinus radiata*. This last feature seems to be particularly related to severe forms of compression wood, because the absence of the S_3 layer is variable in the mildest forms (Singh and Donaldson 1999). The occurrence of a highly lignified outer S_2 layer that is continuous around the perimeter of the cell is also related to severe compression wood. It seems that the presence of cavities in cell corners may be common to both mild and severe compression wood.

In longitudinal sections of compression wood the most striking feature is the presence of spiral markings or spiral checks in the cell walls; they may be associated with the bordered pits, in which case they appear to extend from the pit apertures (Fig. 2.7). These structures give a definite indication of the cell wall organisation, as it has been shown that they follow the microfibril orientation in the S_2 layer of the secondary wall, which varies considerably depending on the severity

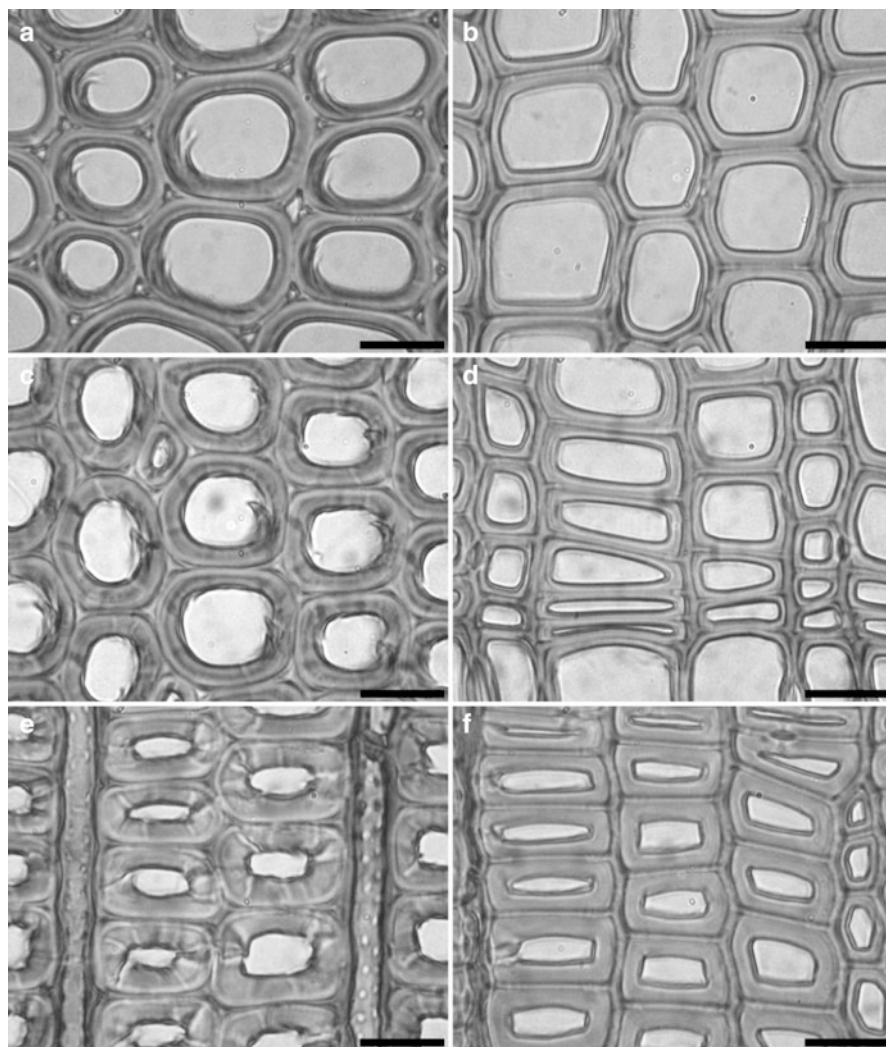


Fig. 2.6 Comparison of transverse sections of tension wood (on the left) and opposite wood (on the right) of *Picea abies* (a and b), *Pinus pinaster* (c and d), *Pinus sylvestris* (e and f). Scale bars = 20 μm

of the compression wood. However, even in very mild compression wood the extension of the pit apertures with spiral markings in the cell wall is quite evident.

In the majority of the references it is clearly stated that compression wood tracheids are shorter than those of non-reaction wood from the same tree (Dadswell and Wardrop 1949; Lee and Eom 1988). Occasionally distorted tracheid tips occur in compression wood (Fig. 2.8), this tracheid distortion was observed by several authors and was considered as a feature of compression wood (Onaka 1949; Lee

Fig. 2.7 Spiral striations in compression wood of tracheids situated in the centre of annual rings. The *arrow* shows a bordered pit with a partly hidden aperture (Mayr et al. 2006). Scale bar = 5 μm

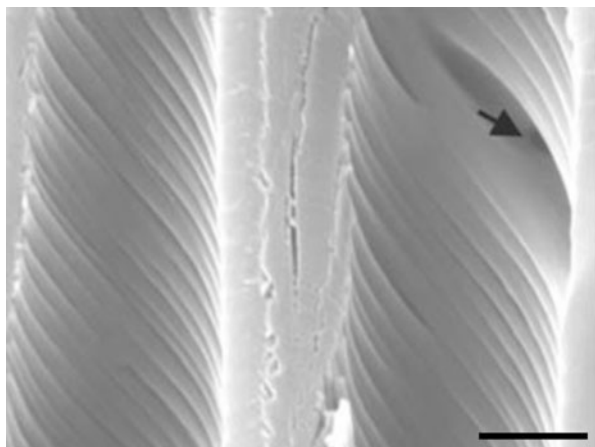
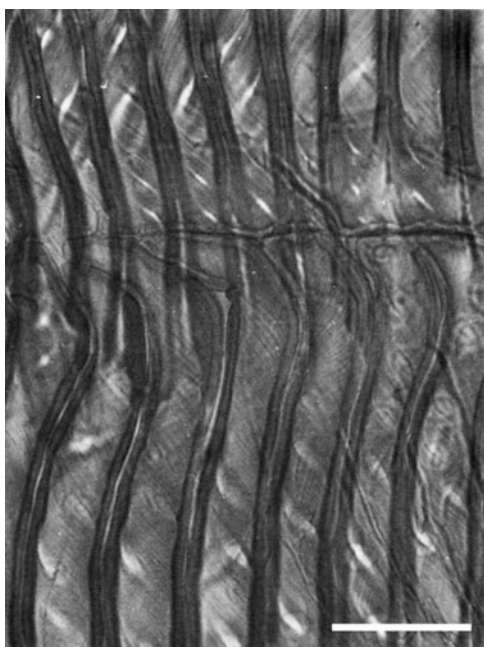


Fig. 2.8 Distorted tips of tracheids in radial section of compression wood (Lee and Eom 1988). Scale bar = 1 μm



and Eom 1988). It seems that flattened and L-shaped tips of tracheids increase in number with the development of compression wood (Yoshizawa et al. 1987).

In angiosperms, for many commonly studied species such as beech (*Fagus* spp.), poplar (*Populus* spp.), oak (*Quercus* spp.) or chestnut (*Castanea* spp.), tension wood is characterised by the occurrence of fibres with a particular morphology and chemical composition due to the development of the so-called gelatinous layer (G-layer). This layer was discovered by Th. Hartig at the end of the nineteenth century and is named the cellulosic layer, mucilaginous layer, cartilaginous layer,

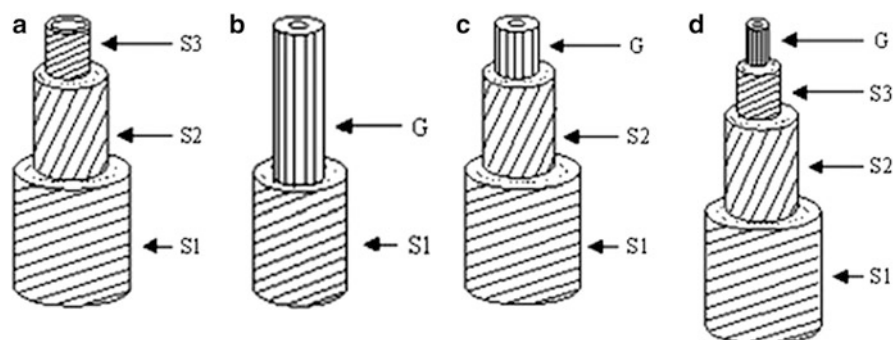


Fig. 2.9 Schematic models for the cell wall structures of fibres in normal wood (a) and tension wood (b–d), redrawn from Wardrop and Dadswell (1955). Solid lines indicate cellulose microfibril orientation. (a) Normal fibres do not develop a G-layer. (b) G-layer where S₂ and S₃ layers develop normally. (c) S₃ layer replaced with G-layer. (d) G-layer forms as the innermost layer next to the S₃ layer (Kwon 2008)

or gelatinous layer because of its cellulose content, and translucent and jelly-like appearance. Although gelatinous fibres can often be detected on unstained sections it is preferable to use a staining method to highlight the occurrence of G-layer such as fast-green/safranin (Chow 1946), safranin/astra blue (Jourez et al. 2001) or Azur II© (Clair et al. 2003).

The cell wall organization of gelatinous fibres can show some variation, both in the same species and in different species (Fig. 2.9). Actually the literature abounds in sometimes conflicting observations on gelatinous fibre morphology, linked, for example, to the species in question, the area sampled in the tree or in the ring, or the presence of axis eccentricity (Jourez et al. 2001). In the same way that ordinary fibres show a three-layered structure in their secondary wall with the S₁, S₂ and S₃ layers, gelatinous fibres can show various patterns, i.e. S₁+G, S₁+S₂+G, S₁+S₂+S₃+G. Onaka (1949) referred to three types of gelatinous layer which may correspond to the ones cited above. However, he has indicated that each type is to be found in certain genera or families, whereas the present observations have demonstrated the occurrence of more than one type in the same tree or particular specimen (Wardrop and Dadswell 1955; Araki et al. 1983).

Besides the above variations in structure that can appear in any specimen containing gelatinous fibres, a variation in the intensity of the development of the gelatinous layer exists inside the same tree and expressed through the thickness of the gelatinous layer. However, the border effect observed by Clair et al. (2005b) brings doubt to this point (Fig. 2.10). Their study shows that during cross sectioning, some major changes occur in the G-layer thickness and the transverse shape near the surface. Further results by Clair et al. (2005a) clearly demonstrate that the use of transverse cross sections for anatomical observations of tension wood containing a G-layer can be misleading. Most standard methods for sectioning wood samples do not include embedding, but perform sectioning on softened

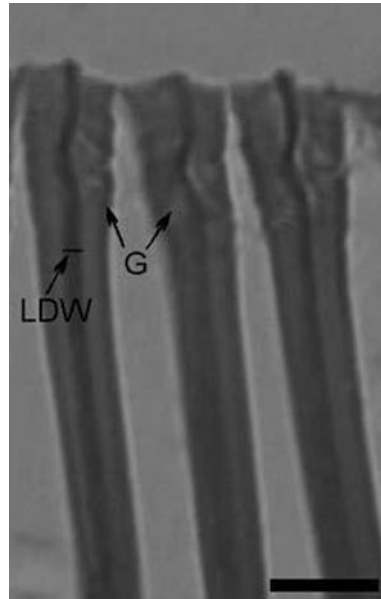


Fig. 2.10 Longitudinal section of poplar tension wood fibres showing an increase in the G-layer thickness near the cutting surface. LDW = lignified double wall (S_2+S_1+P +intercellular layer+ $P+S_1+S_2$), G = G-layer. Scale bar = 10 μm (Clair et al. 2005a)

samples after boiling in water. Thus, on a 10–20 μm thick section, a G-layer is always observed in the transversally swollen condition. However, because the distance to the border of embedded samples is generally not taken into account while sectioning with a microtome, measurements of the G-layer thickness in this condition will over-estimate the G-layer thickness of the cell wall compared to the *in vivo* state. Furthermore the G-layer has always been described as loosely attached to the rest of the secondary wall (Wardrop and Dadswell 1955; Côté and Day 1964), but this appears to be an effect produced by cutting in the transversal direction. This phenomenon is something that only affects the first 100 μm from the cutting plane (Fig. 2.11). These observations lead to the conclusion that the G-layer is always adhered to the S_2 layer in tension wood (Clair et al. 2005b).

In species where tension wood exhibits a G-layer, its occurrence is always correlated with tensile growth stresses with the proportion of G fibres directly correlated with the magnitude of the growth stresses (Fang et al. 2008). When all fibres contain a G-layer, the G-layer proportion within each cell wall then appears to directly affect the magnitude of the growth stress so that the thicker the G-layer the larger the growth stresses (Fig. 2.12).

The G-layer has long been thought to be composed of nearly pure cellulose (Norberg and Meier 1966). However, a slight deposition of lignin has been controversially discussed in the past (Scurfield and Wardrop 1963; Yoshida et al. 2002; Joseleau et al. 2004; Pilate et al. 2004; Gierlinger and Schwanninger 2006). First

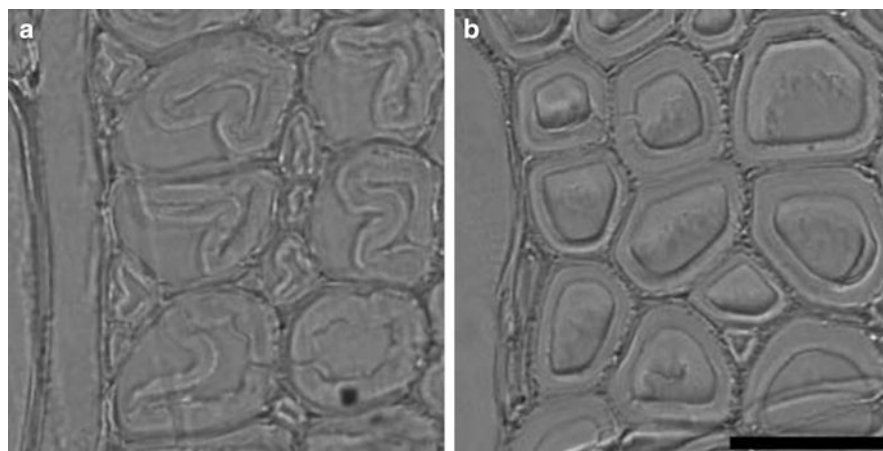


Fig. 2.11 Transverse sections of never-dried poplar tension wood. Observation of detachment of the G-layer from S_2 layer versus distance (D) to the reference face (*cutting surface*). (a) 10 μm , (b) 150 μm . Bar 20 μm (Clair et al. 2005b)

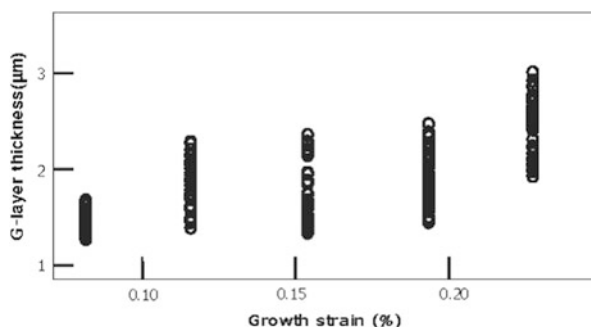


Fig. 2.12 Relation between G-layer thickness (μm) and growth strain (%) (Fang et al. 2008)

evidence that the G-layer may consist of more than pure cellulose was given by Casperson (1967), by means of electron microscopy investigations on tension wood tissue of *Quercus robur*. Concentric and diffuse incrustation of dark contrasting substances in the G-layer was detected and was interpreted by the author as evidence for lignin deposition. Evidence of deposition of aromatic compounds in and attached to the G-layer of tension fibres of *Acer* spp., *Fagus sylvatica* and *Q. robur* was shown (Fig. 2.13) after staining with potassium permanganate and viewed by transmission electron microscopy (Lehringer et al. 2009).

Furthermore the layer may contain polysaccharides including pectin and hemicellulose in addition to cellulose. Evidence of xyloglucan and xyloglucan-synthesising proteins in the G-layer has also been reported and recent works highlighted the occurrence of rhamnogalacturonan I, arabinogalactan and arabinogalactan proteins (Bowling and Vaughn 2008). For more details, please see Chap. 3.

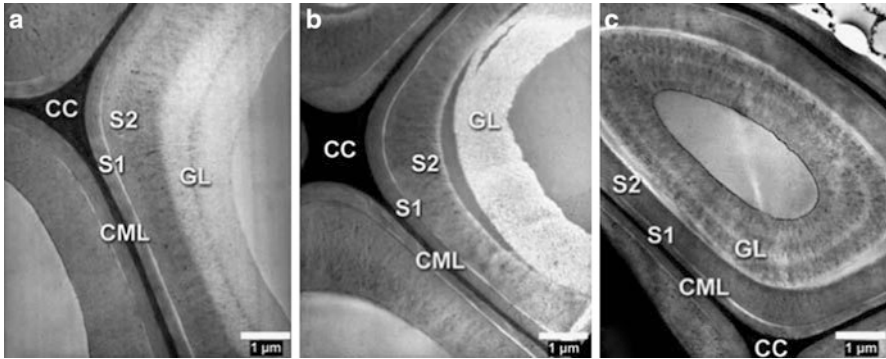


Fig. 2.13 TEM cross sections of tension wood in *Acer* spp. (a), *F. sylvatica* (b) and *Q. robur* (c). Note the concentric contrast in the G-layers of maple and oak indicating a slight deposition of aromatic compounds (CC cell corner; CML compound middle lamella, S₁, S₂ secondary wall, GL gelatinous layer) (Lehringer et al. 2009)

In the past literature tension wood was almost always defined by the occurrence of the so-called gelatinous fibres. Actually the G-layer was considered for a long time as the indicator of tension wood occurrence, but this is only true for species synthesising it. Several studies have shown that the formation of the supplementary G-layer is not constant in tension wood fibres. Out of the 346 species cited by Onaka (1949), fibres with a G-layer were observed in only 136 (39 %). Fisher and Stevenson (1981), working on tension wood in the branches of 122 species demonstrated the G-layer in only 46 % of them. However, these studies were based on the assumption that the upper parts of leaning stems would be made of tension wood, i.e. should be in very high tensile stress state compared to non-reaction and opposite wood, but growth stresses were not in fact measured. Only in a few studies has the G-layer been absent in a given species when there was measurable mechanical tensile stress in the tension wood (Yoshida et al. 2000, 2002; Clair et al. 2006; Ruelle et al. 2006, 2007a; Chang et al. 2009). In a study of 21 naturally tilted trees from 18 families of tropical angiosperms we found that only 7 trees among 7 distinct families showed a well-differentiated G-layer associated with high tensile stress values (Clair et al. 2006). During this study we found an unusual structure in the tension wood of *Casearia javitensis* from the Flacourtiaceae family (Fig. 2.5). Later we found the same kind of polylaminate secondary wall in the tension wood of *Laetia procera* (Fig. 2.14), another Flacourtiaceae (Ruelle et al. 2007b). In *L. procera* this structure consists of alternating thick and thin layers, with an average of five to six thin layers with thick layers between them (the thick layers are approximately ten times larger than thin ones). Observations on longitudinal sections also show a lignified layer inside the lumen of tension wood fibres. After delignification treatment this layer showed a large microfibril angle (MFA), a feature that is typical of the S₃ layer commonly observed in non-tension wood fibres. This kind of structure was also observed by Daniel and Nilsson (1996) in

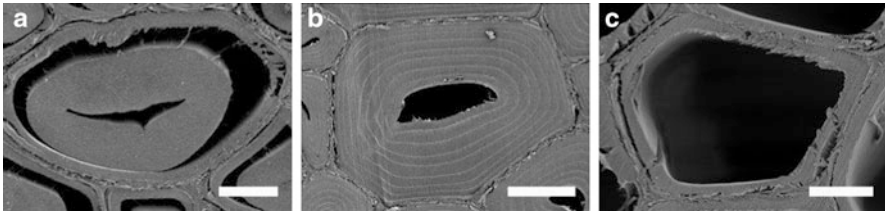


Fig. 2.14 Cross sections of tension wood (a) *Eperua falcata* showing a well-developed G-layer (b) *Laetia procera* showing a polylaminate organisation, and (c) *Simarouba amara* showing no difference with non-reaction wood fibres. Bars, 5 μm (from Ruelle et al. 2007a)

another species from the Flacoutiaceae family, *Homalium foetidum*, but in their study its occurrence was not identified as a tension wood feature. Observations of this peculiar structure in tension wood fibres emphasise the difficulty of classifying tension wood structures (Clair et al. 2006).

If we do not consider the variations occurring in G-layer structure, then tension wood shows at least three anatomical variations (Onaka 1949; Fisher and Stevenson 1981; Clair et al. 2006; Ruelle et al. 2007b) (Fig. 2.14):

- Tension wood fibres with a G-layer,
- Tension wood fibres with polylaminate secondary wall structure, and
- Tension wood fibres not differing from non-reaction wood fibres.

A first general observation based on combined anatomical observations and mechanical measurements (Clair et al. 2006; Ruelle et al. 2007a) is that the presence of an unusual structure such as a G-layer or polylaminate organization is not a prerequisite for the production of high tensile stressed wood. It is clear that various cell wall structures can occur in tension wood; so the question still remains as to whether there are ultrastructural features that are characteristic of tension wood independent of the occurrence of the G-layer.

This variability of tension wood structure, from species showing no difference between tension and opposite wood, species with thicker or multi-layered cell wall in tension wood, and species having a G-layer, means that the reaction wood of angiosperms is not easy to define.

2.4 Ultrastructural Level

In this section of the chapter only the morphological aspect of macromolecules in the cell wall is considered, in particular the structure and organization of cellulose in the cell wall of reaction wood. The biochemical aspect of macromolecules in reaction wood will be treated in Chap. 3.

2.4.1 *Artefacts or True Observations?*

The studies on gelatinous layers in the 1960s provided two hypotheses about its ultrastructure. The first view was that the gelatinous layer had a honeycomb structure, visible when the layer became swollen. A different view that the gelatinous layer had a distinctly lamellar structure, like the three layers in the secondary wall, has been advocated by a number of investigators. The main reason for these two different views on the structure of the G-layer is due to the fact that artefacts appear when tension wood specimen are cut, dehydrated and embedded for preparation of thin or ultra-thin sections. Actually the observation of the honeycomb structure was made after strong and rapid swelling and it is clear that highly swollen cell wall organization does not reflect the organization of the native fibre. However, the honeycomb aspect after swelling suggested that microfibrils must be less firmly bound together than normal owing to the lack of lignin in the matrix that surrounds them (Cote et al. 1969). Even when the gelatinous layer shows some fibrillar structure it never has an ordered fibrillar aspect as observed in normal S_1 or S_2 wall layers. One of the characteristics of tension wood is the variability which occurs in the stratification of poly-lamellate walls. This is particularly clear in the wall of fibres in which the angle between the microfibril orientation of the S_1 and S_2 layers varies from fibre to fibre.

More recently Clair et al. (2005a) showed that the wavy outline of the G-layer, supposed to be characteristic of this layer, is an artefact (Fig. 2.10). Both an increase in thickness and wavy structure indicate that a change has occurred in the G-layer organisation. Cellulose molecules should be less ordered in the swollen condition than in the native state with an increase of the inter-microfibrillar space allowing a loss of the perfectly parallel arrangement of microfibrils. Sections of 30 μm thick prepared by Norberg and Meier (1966) using conventional techniques were followed by an ultrasonic treatment to extract G-layer tubes from the sections. They reported that the estimated birefringence of cellulose in the G-layer tube was slightly smaller than that of ramie fibres. This could indicate that the ultrastructure of cellulose, particularly the cellulose orientation, was somehow disordered close to the cut surface by the sectioning procedure. To avoid the end effect due to cutting, the use of classical microtomy has to be avoided. Sectioning after embedding, taking into account the distance of the sectioning area from the cut surface provides a good solution. Use of confocal microscopy, which permits optical sectioning at monitored depths below the cutting edge, provides another. Sections to be examined must be cut at least 30 μm from the end surface to ensure that artefacts are avoided.

2.4.2 *Gel Structure*

Recently, Clair et al. (2008), using nitrogen adsorption–desorption isotherms of supercritically dried tension wood and non-reaction wood, demonstrated that the

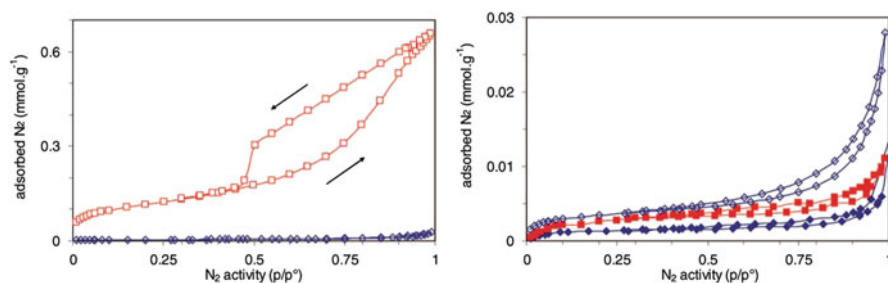


Fig. 2.15 N₂ adsorption–desorption isotherms: (*left*) aerogel of tension wood (TW) and normal wood (NW); (*right*) NW and TW xerogel compared to NW aerogel. Key: *square*, TW; *diamond*, NW; *void shapes*, aerogel; *filled shapes*, xerogel

G-layer is really constituted like a gel (Fig. 2.15). The isotherms showed that the tension wood fibre cell wall of *Castanea sativa* Mill. has a hydrogel structure characterised by the occurrence of mesopores (pores size between 2 and 50 nm), with a pore surface more than 30 times greater than that in non-reaction wood. As normal wood samples showed very little porosity, the authors suggested that the observed results in tension wood have to be attributed to the G-layer, the component which differentiates tension wood from normal wood in the studied species. These results will have great significance for the way the behaviour and the properties of the G-layer are analysed in future. A study using this technique has been conducted on six tropical species, showing a range of tension wood fibre anatomy, i.e. one species with thick G-layer, three with thin ones and two with a lack of G-layer (Chang et al. 2009). In species without a G-layer, mesoporosity was low and at the same level in normal and tension wood. The species with a thick G-layer showed porosity parameters similar to what was described for *C. sativa*. Other species, with a thin G-layer, present an extremely low mesopore volume.

2.4.3 Variation of Cellulose Structure in Cell Walls

In all types of reaction wood, MFA shows a variation from that in non-reaction wood. It is smaller in tension wood and larger (up to 45°) in compression wood with respect to the fibre axis. The MFA in the gelatinous layer is almost parallel to the fibre axis, but even in tension wood without a G-layer a decrease in the MFA of the main layer of the secondary wall is observed (Okuyama et al. 1994; Yoshizawa et al. 2000; Ruelle et al. 2007a, b).

The process by which cellulose microfibril orientation during deposition in fibre walls is controlled has been extensively investigated, in particular the relationship between cortical microtubule orientation at the time of cellulose deposition and MFA. The orientation of cellulose microfibrils (MFs) and cortical microtubules (MTs), in developing tension wood fibres of artificially inclined *Fraxinus*

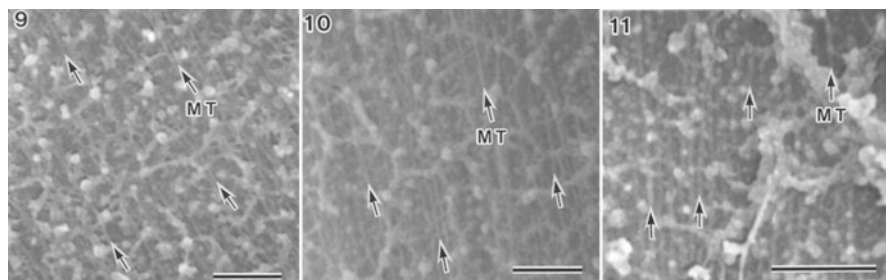


Fig. 2.16 Micrographs (FE-SEM) showing the progressive changes in orientation of MTs, with clockwise rotation (viewed from the lumen side), during formation of the G-layer in tension wood fibres of *Fraxinus mandshurica*. (9) The MTs (arrows) are oriented at an angle of about 35–40° to the fibre axis in a Z-helix at the beginning of formation of the G-layer. Note the high degree of parallelism among the MTs. MT, microtubule. Bar = 0.5 μm . (10) The MTs (arrows) are oriented at an angle of about 10° to the fibre axis in a steep Z-helix. MT, microtubule. Bar = 0.5 μm . (11) The MTs (arrows) are oriented parallel to the fibre axis. Note that the MTs are closely spaced with a high degree of parallelism. MT, microtubule. Bar = 0.5 μm (Funada et al. 1996)

mandshurica trees, was investigated by electron microscopy and immunofluorescence microscopy (Funada et al. 1996). The secondary wall of tension wood fibres was identified as the S_1+G type. The MFs were deposited at an angle of about 45–50° to the longitudinal fibre axis in a flat S-helical orientation at the initiation of secondary wall thickening and the orientation changed progressively in a clockwise direction, as seen from the lumen side, eventually becoming parallel to the longitudinal axis of the fibre. The orientation then remained fixed resulting in the formation of a thick G-layer. A further counter-clockwise rotation of MFs was observed in some of the tension wood fibres at a late stage of G-layer deposition. The MFs showed a high degree of parallelism at all stages of deposition during G-layer formation. On the basis of these results, a model of the orientation and deposition of MFs in the secondary wall of tension wood fibres could be developed.

The orientation of MTs also changed progressively in a clockwise direction, as seen from the lumen side, from an angle of about 35–40° in a steep Z-helix to parallel to the fibre axis during G-layer formation (Fig. 2.16). Parallelism in the orientation between MTs and newly deposited MFs was evident. These results indicated that the MTs play a role in controlling the orientation of MFs in the developing tension wood fibres (Funada et al. 1996).

Work by Prodhon et al. (1995) showed that the change in the orientation of the microfibrils in mature cells is progressive, from the layer adjacent to the G-layer and from the inner to the outer part of the G-layer. However, variations occur between fibres. Field-emission scanning electron micrographs showed that the orientation of microfibrils on the innermost surface (G-layer) of tension wood fibres varied from fibre to fibre, ranging from 0° to 25° relative to the fibre axis. Most of the microfibrils observed in the G-layer were found in the range from 5° to 10°. A more recent study in which MFA was directly observed using scanning electron microscopy of three tropical species with various types of tension wood fibre

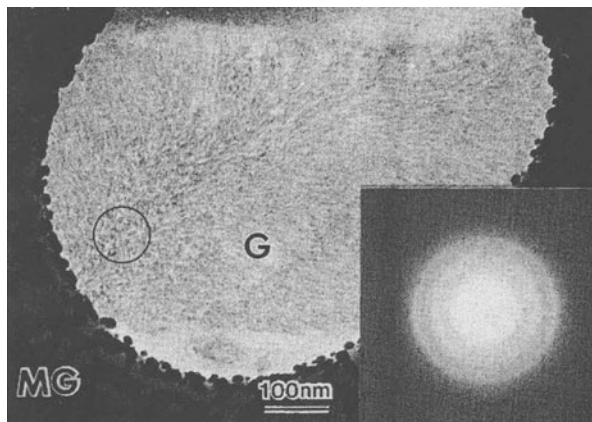
showed that tension wood fibres always had a lower MFA than non-tension wood. *Eperua falcata* a species which synthesises a G-layer had MFAs ranging from 1° to 26° but mainly between 1° and 16°. *L. procera* showed an MFA ranging from 3° to 12° in the thick layers of the polylaminate structure of tension wood fibres, and the species showing no difference between tension and non-tension fibres had an MFA in the S₂ layer ranging from 4° to 17.5°. Muller et al. (2006) using X-ray diffraction on a single tension wood fibre showed that the variation in MFA inside the same fibre was very low. The authors also revealed that MFA of the non-G-layers (S₁ and S₂) of the tension wood fibre was larger than that of non-tension wood. These results lead to the conclusion that the presence of a G-layer is not the only morphological change occurring during the formation of tension wood in species which synthesise a G-layer.

Other structural parameters of cellulose, such as cellulose crystallinity, cellulose-matrix aggregates, also called microfibrils, and cellulose crystallites show variation in tension wood. Even though the impact of these variations on tension wood properties remains unclear, some of them, such as cellulose crystallite size, are used to indicate the occurrence of reaction wood in living trees (Washusen and Evans 2001). Differences in apparent crystallite width have been found between reaction wood (both tension and compression wood) and non-reaction wood. Using X-ray diffraction, apparent crystallite width was always found to be larger in tension wood than in non-tension wood in the same species (Blaho et al. 1994; Washusen and Evans 2001; Ruelle et al. 2007a). The mean values determined by X-ray diffraction for crystallite size in tension and non-tension wood were reported by Washusen and Evans (2001) to be 3.6 and 3.2 nm, respectively, for *Eucalyptus globulus*. A study on three tropical angiosperm species showing various anatomical features of tension wood fibres (Ruelle et al. 2007a) showed mean values of 3.6 and 2.5 nm, respectively, in tension and opposite wood of *E. falcata* whose tension wood exhibits a G-layer, 3.6 and 2.6 nm for *L. procera*, whose tension exhibits a polylaminate structure, and 2.8 and 2.4 nm for *Simarouba amara* that does not show any variation from non-tension wood in its tension wood fibres. Actually the use of X-ray diffraction to estimate cellulose crystallite size has to be questioned because the method is affected by factors other than just the crystal size, including the degree of order of cellulose within the cell wall. But the estimation of crystallite size is still useful in highlighting the occurrence of tension wood in trees, as tension wood also shows variation in the degree of order of the cellulose. But actual variation of crystallite size needs to be determined using direct observation or other independent methods.

Goto et al. (1975) found that crystallite width observed by electron microscopy was in the range of 2.0–4.0 nm in the tension wood of poplar. The diameter of microfibrils in the G-layer of poplar, as measured by Sugiyama et al. (1986), is about 4–6 nm. These were observed with bright field imaging (Fig. 2.17). Electron diffraction patterns also showed that cellulose crystallites were non-preferentially oriented.

In a study by Muller et al. (2006) on an isolated single cell, a mean value of 6.49 nm was estimated for crystallite size in the G-layer of poplar although the

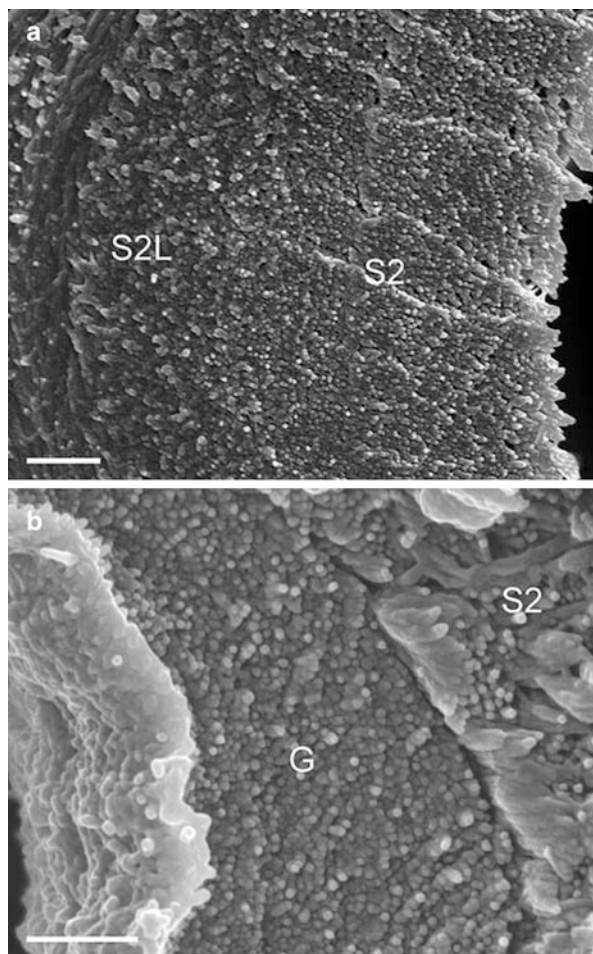
Fig. 2.17 Typical bright field images using diffraction contrast obtained from transverse ultrathin sections of the G-layer of poplar. Inserted is the electron diffraction diagram taken from the corresponding image. MG: microgrid, G: gelatinous layer (Sugiyama et al. 1986)



value for the S_2 layer of the same tension wood cell was 3.14 nm. Such a large value for G-layer crystallites and the difference from that found in the S_2 layer could be explained by two scenarios given by the authors: either cellulose biosynthesis is considerably different between the G-layer and the S_2 layer or the cellulose microfibrils aggregate to larger crystalline regions upon drying, a process facilitated by the very low content of hemicelluloses and lignin in the G-layer. Bamber (1979) suggested that this difference may be explained by an increase in the extent of cellulose crystallization in tension wood after cell elongation has been completed. Compression wood shows smaller apparent crystallite width than non-compression wood (Tanaka et al. 1981).

The organization of wood cell wall components involves aggregates of cellulose microfibrils and matrix known as macrofibrils. Donaldson (2007) attempted to determine the relationships between macrofibrils, microfibrils and matrices and how these components vary among cell wall types, including normal and reaction wood of radiata pine (*P. radiata*) and poplar as examples of a typical softwood and hardwood, respectively. Macrofibrils in tension wood were slightly smaller than in normal wood of poplar (Fig. 2.18), while compression wood in radiata pine had larger macrofibrils compared to normal wood with some variation in their organisation (Fig. 2.18). The inner S_2 region of radiata pine compression wood contained macrofibrils with a tendency towards radial alignment, while in some cells there was a distinct inner region adjacent to the lumen where they were randomly arranged and noticeably smaller (not shown). In the outer S_2 region, macrofibrils were distinctly larger and more randomly arranged and the cell wall was less porous (Fig. 2.18). The microfibril orientation in the G-layer could often be determined by observation of the macrofibril structure and appeared to be more or less parallel to the fibre axis, in contrast to the main part of the secondary wall, which had a larger MFA. Microfibril orientation did not seem to affect the appearance of macrofibrils or their arrangement but this requires more detailed investigation. Values for the smallest macrofibrils were 14 nm in diameter found in the G-layer of poplar. Normal poplar wood fibres had an average macrofibril diameter of 16 nm compared

Fig. 2.18 Field-emission scanning electron micrographs of macrofibrils in reaction wood: (a) S₂ and S₂L regions of *P. radiata* compression wood tracheid. Samples were coated with 12 nm of chromium. Scale bar = 0.5 μ m; (b) G-layer of *P. deltoides* tension wood fibre. Scale bar 0.25 μ m (Donaldson 2007)



to radiata pine tracheids with an average of 19 nm. The S₂ region of compression wood tracheids had the largest macrofibrils at 22 nm diameter. The above trend suggests a relationship with the degree of lignification among these cell wall types. The G-layer of poplar tension wood is thought to be unlignified (Pilate et al. 2004) and compression wood is known to be more lignified than the normal wood of hardwoods. Macrofibril diameter appears to show an approximately linear relationship with lignin concentration, ranging from 14 nm in the non-lignified G-layer of poplar tension wood, to 22 nm in the highly lignified outer S₂ layer of compression wood tracheids in radiata pine. While it is possible to show a relationship between lignin content and macrofibril size, other cell wall components such as hemicelluloses, are also known to vary in content and type among cell wall regions. Unfortunately such variations are rather poorly understood in comparison with the extensive literature on lignin topochemistry.

In a recent study, Lehringer et al. (2009) measured macrofibrils in tension and opposite wood of *Acer* spp., *F. sylvatica* and *Q. robur*. They did not find any differences in macrofibrils diameter between the G-layer, the S₂ layer from tension wood and the S₂ layer from opposite wood. For all species the macrofibrils of the G-layers and the S₂ layer showed a diameter between 9 and 22 nm. A concentric alignment of macrofibrils in the G-layer of *Acer* spp. and *Q. robur* was also observed. The macrofibrils showed a strict and regular order and, although partly delaminated during sample sectioning, showed concentric layering. Corresponding observations were made by Daniel et al. (2006) on tension wood fibres of *Populus tremula* and *Betula verrucosa*. In a study on poplar G-layer cellulose aggregates, macrofibrils occurred predominantly in a random arrangement (Donaldson 2007).

Of course variations in reaction wood ultrastructure concern not only the cellulose but also the other macromolecules, lignin and hemicelluloses. Those points will be covered in the next chapter.

2.5 Conclusions

As was underlined in the brief introduction to this chapter, the process of axis reorientation, related to the formation of reaction wood, is based on the heterogeneity of cambial region activity. This heterogeneity occurs at the macroscopic, mesoscopic, microscopic and ultrastructural level. More precisely the mechanism allowing reorientation of the axis originates in structural modifications at the cell wall level. Indeed, these micro-structural modifications induce a variation in the behaviour of reaction wood, leading to variations in its properties. Some of these variations can complicate saw-milling and using timber from trees that have had an active phase of vertical restoration (see Chaps. 6, 8 and 9 for further details on the physical properties of reaction wood and the implications for industrial processing). However, the peculiar structure of reaction wood allows us to highlight the strong influence of microscopic and ultrastructural parameters on wood properties and to study their impact on trees biomechanics.

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