Barry Gardiner
John Barnett
Pekka Saranpää
Joseph Gril *Editors*

The Biology of Reaction Wood

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Professor Dr. Rupert Wimmer
Universität für Bodenkultur Wien (BOKU)
IFA Tulln
Institute for Natural Materials Technology
Sustainable Biomaterials Group
Konrad Lorenz Strasse 20
3430 Tulln an der Donau
Austria

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Barry Gardiner • John Barnett • Pekka Saranpää •
Joseph Gril
Editors

The Biology of Reaction Wood

Editors

Barry Gardiner
INRA - Unité EPHYSE
Villenave d'Ornon
France

John Barnett
Ashbourne, Derbyshire
United Kingdom

Pekka Saranpää
Finnish Forest Res. Inst. METLA
Vantaa
Finland

Joseph Gril
Labo. Mécanique et Génie Civil (LMGC)
Université Montpellier II CNRS
Montpellier
France

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Preface

Reaction wood is wood produced by trees in order to orientate stems and branches in response to displacement and the requirements for light. The accompanying changes in the physical and chemical properties of the wood result in its having different mechanical and physical properties compared to normal wood including differences in colour, fibre properties, workability, distortion and strength. These have important consequences for wood-based industries in the processing and serviceability of products containing reaction wood. This has resulted in increased interest among wood scientists in the factors controlling reaction wood formation, the physical and chemical properties of reaction wood cells, and the way such changes are able to generate the stresses required to reposition stems and branches.

The European COST Action program COST E50 "Cell wall macromolecules and reaction wood (CEMARE)", which ran from July 2005 to June 2009, brought together wood scientists from 19 countries. The Action covered the whole range of issues related to reaction wood from cell wall biosynthesis to forest management and wood processing. In this way it attempted to link the environmental influences on reaction wood formation to cell wall formation and cell wall structure and subsequently to the consequences for wood and fibre properties and processing. It very deliberately brought together studies on compression wood and tension wood, the normal types of reaction wood in gymnosperms and angiosperms, respectively.

The genesis of the idea for this book was the realisation amongst the scientists involved in CEMARE that there was no synthesis in one place of all the different aspects of reaction wood. In addition, the definitive work on compression wood by Tore Timell is now almost 30 years old, and no such comprehensive work on tension wood has ever been written. Therefore, it was decided to pull together in one volume the latest understanding of reaction wood and to ensure that we discussed compression wood and tension wood together in order to highlight the similarities and differences in their formation and properties. The book covers everything from reaction wood morphology, anatomy, ultrastructure and cell wall polymers to the molecular mechanisms of reaction wood induction, and the bio-mechanical action and biological functions of reaction wood. In addition the physical and mechanical properties of reaction wood at all levels are discussed,

focussing in particular on the impact of these properties on the utilisation of wood for different end products. Finally, there are chapters on detection techniques, the commercial implications of reaction wood and the influence of forest management.

The book will provide a valuable and important reference source on reaction wood for wood scientists and technologists, plant biologists and chemists, plant breeders, silviculturists, forest ecologists and anyone involved and interested in the growing of trees and the processing of wood. It is hoped that it will also provide a useful introduction to the subject for people new to this scientific area.

Villenave d'Ornon, France	Barry Gardiner
Ashbourne, Derbyshire, UK	John Barnett
Vantaa, Finland	Pekka Saranpää
Montpellier, France	Joseph Gril
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Chapter 1
Introduction

J.R. Barnett, Joseph Gril, and Pekka Saranpää

> The rings on the cross-section of the branch of a tree show the number of its years, and the greater or smaller width of these rings show which years were wetter and which drier. They also show the direction in which the branch was turned, for the part that was turned towards the north grows thicker than that turned towards the south so that the centre of the stem is nearer to the bark that faces south than to that on the north side. *Leonardo da Vinci*.

Leonardo published his observations of stem asymmetry in his notes for a treatise on painting, without any attempt at explanation. It must represent one of the earliest references to reaction wood in the literature, although there can be no doubt that carpenters and joiners had long been intuitively aware of its effects on the working and mechanical properties of timber. With the passage of time our understanding of why and how it is formed in the tree has increased, providing a scientific basis for folk knowledge, but despite extensive research, much remains to be explained.

The last major work on this topic was the *Magnum Opus* of Timell (1986), which summarised current ideas on compression wood in gymnosperms. No equivalent work has, however, been produced dealing with tension wood, its counterpart in angiosperm dicotyledonous trees. This reflects to some extent the fact that hitherto, tension wood has been of less commercial importance, although this is now changing with the breeding and development of fast-growing temperate-hardwood species. This book is intended to bring the reader up-to-date with not only the

J.R. Barnett (✉)
Birch House, Main Street, Kniveton, Ashbourne, Derbyshire DE6 1JH, UK
e-mail: j.r.barnett@reading.ac.uk

J. Gril
Laboratoire de Mécanique et Génie Civil, CNRS, Université Montpellier 2, CC048 Place
E. Bataillon, 34095 Montpellier Cedex 5, France
e-mail: joseph.gril@univ-montp2.fr

P. Saranpää
The Finnish Forest Research Institute (METLA), P.O. Box 18, 01301 Vantaa, Finland
e-mail: pekka.saranpaa@metla.fi

progress in research into reaction wood, particularly with reference to tension wood, but also the developments in compression wood research since the publication of Timell's definitive work.

1.1   What Is Reaction Wood?

Reaction wood has been defined by the Committee on Nomenclature of the International Association of Wood Anatomists (IAWA 1964) as "Wood with more or less distinctive anatomical characters, formed typically in parts of leaning or crooked stems and in branches and tending to restore the original position, if this has been disturbed. It is divided into two types: tension wood in dicotyledons and compression wood in conifers". The Committee further defines compression wood as "Reaction wood formed typically on the lower sides of branches and leaning or crooked stems of coniferous trees and characterized anatomically by heavily lignified tracheids that are rounded in transverse section and bear spiral wall checks; zones of compression wood are typically denser and darker than the surrounding tissue". Tension wood is: "Reaction wood formed typically on the upper sides of branches and leaning or crooked stems of dicotyledonous trees and characterized anatomically by lack of cell wall lignification and often by the presence of an internal gelatinous layer in the fibres".

As might be expected, and as will become clear in this book, there are many examples of variations in detail from these necessarily succinct definitions. For example, in the case of so-called mild compression wood, cell walls may lack spiral wall checks and not necessarily be rounded, while the gelatinous layer is not present in tension wood of many species. The Oxford English Dictionary provides several definitions of the word "reaction" some of which encompass the nature and function of the term when used in conjunction with wood. Perhaps the two most appropriate are: "The response made by a system or an organ to an external stimulus" and "A movement towards a reversal of an existing tendency or state of things . . . or desire to return, to a previous condition of affairs". The first definition is appropriate to the formation of reaction wood, while the second is appropriate to its function in the tree.

Briefly, reaction wood is formed in response to mechanical stress experienced by a tree. Its formation can work to restore vertical growth (gravitropy) in main stems, providing the stem is not already too thick to make this possible. It can be used also to incline stems in order to move the canopy in towards light (heliotropy). In the case of a branch, reaction wood formation is carefully controlled to balance its continuously increasing weight, either as a buttress in the case of compression wood in gymnosperms, or as a cantilever, in the case of tension wood in angiosperm dicotyledons, thereby maintaining the branches pre-ordained orientation and the architecture of the tree. It is noteworthy that reaction wood in a branch does not tend to force the branch into a vertical alignment unless the dominance of the apical shoot is lost. However, reaction wood is required to change the orientation of a

lateral branch to the vertical in the event of damage to or loss of the leading shoot. Compression wood and tension wood sectors in the stem are always associated with local growth stresses which are very different from the normal tensile stress state common to gymnosperms and angiosperms: compressive stress in the case of compression wood, very high tensile stress in the case of tension wood.

There are, however, as will become apparent, variations on the theme. For example, compression wood may form around the entire growth ring in straight vertical fast-growing conifer stems. This may be a result of almost continual movement in the wind of the long, recently formed apical internodes, which are highly flexible owing to the high microfibril angle in the S_2 layer in the juvenile tracheids. Of all existing types of compression wood the so-called spiral compression wood is most peculiar. A band of compression wood that spirals from the pith towards the cambium may last for decades (Fig. 1.1). The reason for formation of spiral compression wood is unknown. Also, gelatinous fibres of the type normally associated with tension wood are sometimes found distributed randomly in vertical stems of fast-growing hybrid aspen. These phenomena might be explained by the existence of extraordinary growth stresses in fast growing trees or by some variation in the level of growth regulators.

Some workers have observed cases in which the "normal" pattern of reaction wood formation was not found. For instance, Höster and Liese (1966) described compression wood in angiosperm species whose main axial elements were tracheids, observations confirmed by Yoshizawa et al. (1993) and Baillères et al. (1997). In contrast, Jacquiot and Trenard (1974) described gelatinous fibres in coniferous wood.

1.2 Historical Background

The reason why branches and many tree stems are elliptical in cross-section, with growth rings having different widths on opposite sides, and pith located to the side of the narrower growth rings, was already a subject of investigation in the nineteenth century. It was noted that in conifers growing on mountain slopes more growth occurred on the side of the stem facing the slope. Attempts were made to explain this in terms of nutrient distribution to the cambium, in that nutrients moved preferentially to areas to stimulate growth. Büsgen and Münch (1929) pointed out that in fact the opposite was the case, as suggested by Cotta (1806), in that growth stimulates the movement of nutrients to where they are required. They suggested that this process was set in motion by stimuli which at that time were unknown. They also noted that in Germany, where south-west and west winds predominate, conifer stems take on an elliptical form with the long axis of the ellipse parallel to the wind direction and greatest growth on the leeward side of the stem. Similarly they noted that in leaning conifer stems, greatest growth occurred on the lower side. Thus the tree presents its least flexible profile to the prevailing stress. It was also noted that those roots aligned with the direction of the stress, whether wind or

Fig. 1.1 Spiral compression wood in a Scots pine (*Pinus sylvestris* L.) stem from a first thinning site in southern Finland. The *disk* shows a band of compression wood that spirals four times clockwise from the pith towards the cambium. The formation of compression wood began when the tree was 5–6 years old and continued for several decades

gravitational pull, also developed an elliptical profile. They proposed that this helped to prevent the stem from falling over.

Hartig (1901) with spruce and Rasdorsky (1925) with *Helianthus* induced elliptical stem form by rocking the experimental plant from side to side. Büsgen and Münch (1929) interpreted this to mean that eccentric growth in branches and leaning stems was caused by mechanical stimulation. The fact that this response was also found by Hartig (1901) in a fallen spruce stem supported by the ground and therefore not under any bending stress led to the view that the force of gravity played the most important part in the eccentricity of branches.

The facts that in conifers, reaction wood is produced on the underside of leaning stems and branches under compressive stress, and that it has a reddish hue, led to its being referred to in the German literature as Druckholz (pressure wood) or Rotholz (red wood). These terms were supplanted by the name compression wood as it was believed to be formed as a result of the tissue being under a compressive load. In contrast, reaction wood produced in angiosperm dicots, which is formed in tissues under tensile stress, and which is light in colour was referred to as tension wood or Weissholz (white wood). As Dadswell and Wardrop (1949) pointed out, the latter name is confusing as it was also used to describe wood formed in conifers on the opposite side of the stem to Rotholz. The terms compression wood and tension wood eventually acquired universal acceptance as reflecting the stress conditions under which they are usually formed.

However, there are circumstances in which tension wood can form in tissues under compressive stress and vice versa. Experiments by Ewart and Mason-Jones (1906) in which they bent conifer twigs into vertical loops (Fig. 1.2) demonstrated that compression wood formed on the lower side of the twigs at both the top of the loop (where the developing wood was under pressure) and the bottom (where it was

Fig. 1.2 Diagram after Jaccard (1938). Diagrammatic representations of (**a**) Loop made in a conifer stem. Compression wood is shown as a *thicker line* on the lower sides of the upper and lower parts of the loop. (**b**) Loop made in a woody dicotyledon stem. Tension wood is shown as a *thicker line* on the upper sides of the upper and lower parts of the loop. (**c**) and (**d**) The effect of cutting the loops is similar in each case suggesting compression wood acts by pushing, while tension wood by pulling against the normal wood
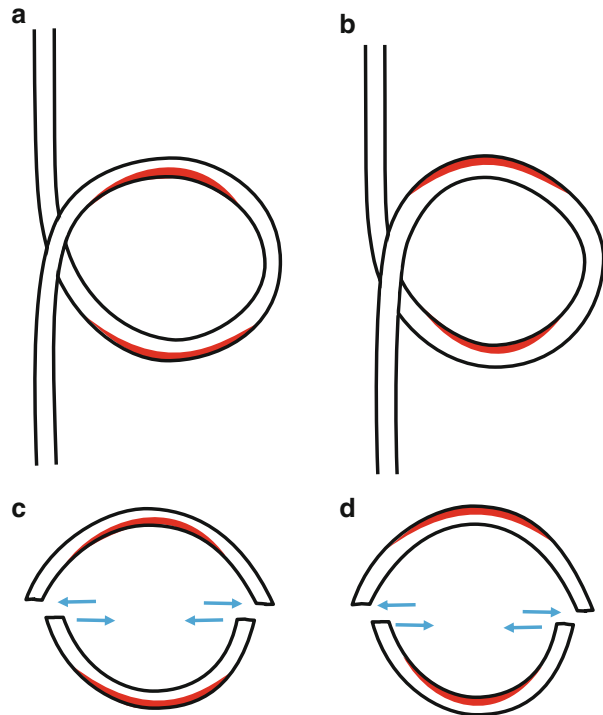
under tension). Jaccard (1938) repeated the experiment and found that in angiosperm saplings tension wood always formed on the upper side of the top and bottom of the loop. This, coupled with the discovery of auxin and its effects as a growth regulator which moves basipetally in tissues under the influence of gravity, led to the proposition that auxin accumulation on the lower side of conifer branches and leaning stems stimulated compression wood formation, while depletion of auxin from the upper side in angiosperms led to tension wood formation. The work of Wershing and Bailey (1942), who found that external applications of auxin induced compression wood formation, lent support to this view. Conversely, Nečesaný (1958) found that the application of auxin to the upper side of an angiosperm branch inhibited tension wood formation, while Lachaud (1987) applied tritiated auxin to loops made in the manner of Jaccard (1938) and found that it moved to the lower side of the loop while tension wood formed on the upper side. This effect was most pronounced when the loop was still attached to the plant, no movement of auxin taking place in a detached loop.

In essence this theory was accepted until questioned by Boyd (1977), who felt that reaction wood formation was stimulated by stress, rather than auxin concentration changes. His view was supported by Wilson et al. (1989) following measurement of auxin levels in bent branches of Douglas fir made using gas chromatography–mass spectroscopy. It was found that auxin levels were higher

on the upper side of these branches even though compression wood was formed on the lower side. Sundberg et al. (1994) measured auxin concentrations with a relatively high degree of resolution in the cambium of tilted pine stems and also found no difference in auxin distribution between tilted and control stems.

The work performed by the French consortium ASMA (Fournier et al. 1991a, b; Thibaut et al. 2001) demonstrated that reaction wood is not a consequence of stresses acting on a zone in the tree but rather, its role is to generate specific levels of stress when and where they are needed. The fact that while the main stem of the tree produces reaction wood in an apparent attempt to maintain as vertical an alignment as possible, while branches produce it to maintain a particular orientation has led to the suggestion that the stress imposed by gravitational force does not of itself stimulate reaction wood formation. Rather it is the tree's perception of the effect of the gravitational force in displacing the stem or branch from its pre-ordained alignment that provokes the response.

Recent developments in this area are described in Chaps. 4 and 5.

1.3 Structure of Reaction Wood

The anatomical and structural features of reaction wood are usually described by comparison with the so-called normal wood. The latter term actually refers to wood which has those properties most desired by the timber industry, for example, straight grain, high density, high bending strength and uniform shrinkage without distortion on drying. Interestingly there appears to be no definition of normal wood and it is noteworthy that the IAWA glossary in its definitions of reaction wood, tension wood and compression wood avoids the use of comparatives.

Those features usually associated with what authors refer to as normal wood and which are responsible for the desirable characteristics of good timber are determined by the anatomical structure of the wood and the structure of fibre (hardwoods) and tracheid (softwood) walls. In particular, tracheids and fibres have the classical three-layered secondary wall (Fig. 1.3) with the microfibril angle in the S_2 layer being small, resulting in a high modulus of elasticity. In reacting to gravity-induced displacement of the stem or branch, the tree produces fibres or tracheids whose structure differs to a greater or lesser extent from this "ideal". These variations and other differences in reaction wood will be discussed in more detail in Chap. 2.

In brief, compression wood is a darker colour than normal wood, the tracheids are more rounded in cross-section with the result that intercellular spaces are formed. They are also shorter than normal wood tracheids in the same tree. Cell walls of compression wood tracheids are normally thicker than those of normal wood tracheids in the same part of the tree. This coupled with the greater proportion of lignin in the cell wall makes the wood denser, more impermeable and stronger in compression. The microfibril angle in the S_2 layer is larger, which reduces the tensile strength and modulus of elasticity and increases the brittleness of the wood,
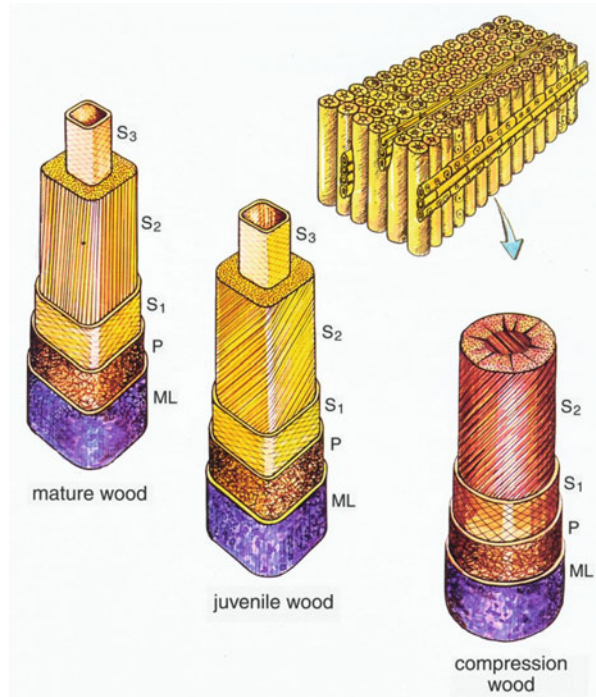
making it unsuitable for uses in which it is likely to experience high stresses. The
larger microfibril angle also means that the wood has a higher longitudinal shrink-
age on drying, but a lower transverse shrinkage. This explains the distortion on
drying of pieces of wood containing both normal and compression wood. In severe
cases the S_3 wall layer is absent and the S_2 layer contains splits which lie parallel to
the microfibril angle.

The structure of tension wood fibres is less clear-cut than that of compression
wood tracheids. Tension wood fibres are longer than those in normal wood and have
been found to contain a lower proportion of lignin than normal wood, giving it a
whiter appearance. They are most commonly described as lacking an S_3 layer and
having variable amounts of the S_2 remaining, inside which is a gelatinous layer
composed mainly of hydrated cellulose microfibrils (Norberg and Meier 1966)
oriented almost parallel to the long axis of the fibre. This gives the wood a
glistening gelatinous appearance when wet. Variations on this theme have been
reported, however. For example, Faruya et al. (1970) reported the presence of a
gelatinous layer in fibres of *Populus euroamericana* which had retained their S_3
layer, while Côté et al. (1969) reported an S_3 layer inside a gelatinous layer. More
and more variations on the structure of tension wood fibres are being reported, with
numerous species apparently lacking a gelatinous layer in their fibre walls.

These structural variations, which are adapted to the mechanical role the fibres
and tracheids must fulfil, are of course, a normal response by the tree to enhance its

chances of survival, either by helping to maintain the upward movement of the crown by the shortest possible route, by restoring verticality of trees partially uprooted by wind, or by maintaining the optimum branch architecture for efficient light capture. As such, for the tree, making reaction wood is as normal as making the normal wood beloved of the timber merchant, engineer, carpenter or joiner. The use of the term normal for the latter carries the implication that reaction wood is abnormal, and by extension that it is of no value. However, trees as we know them could not have evolved without reaction wood, a fact which needs to be borne in mind by those working to improve wood quality.

The formation of reaction wood demonstrates that the tree is capable of fine-tuning the structure of its fibre or tracheid walls to generate growth stress. It does this by adjusting the proportions and arrangement of its major wall components, cellulose, hemicellulose and lignin. The structure and composition of the cell wall in reaction wood forms the subject of Chap. 3.

1.4   How Reaction Wood Works

It is now well known that reaction wood effects stem reorientation by generating a long-lasting flexure momentum (Almeras et al. 2005). This is linked to asymmetry between wood production (new ring) on the two sides of the wooden axis: asymmetry of generated growth stress and often asymmetry of ring width. But it is not yet well understood how growth stress level is tuned during cell differentiation. Various theories have been put forward, but none has so far proved satisfactory. Brodski (1972) proposed that the water in the developing S_2 layer of the compression wood tracheid wall was replaced during maturation by a compound, laricinan, and that this insertion provided the force needed to push the stem upright. Boyd (1978) published a convincing argument against such a role for laricinan. The tensional effect of the gelatinous layer of tension wood fibres is also difficult to explain. When gelatinous fibres are severed, the gelatinous layer may be observed to detach itself from the other wall layers and contract. This is despite the fact that its major component is cellulose microfibrils with their major axis parallel to the direction of shrinkage. As the microfibrils are highly crystalline they would not be expected to shrink in this way. Current thinking on these topics is covered in Chap. 5.

1.5   Why Is Reaction Wood so Important?

It is precisely those properties which enable reaction wood to carry out its function in the tree that render it a problem for the timber industry. When Jaccard (1938) cut loops in which reaction wood had formed, the curvature immediately changed as internal stresses were released (Fig. 1.2). In the case of the gymnosperm loop, in which compression wood had formed on the convex side of the lower curve, and the
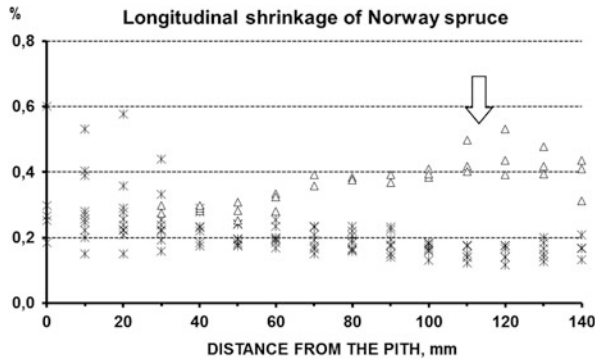
Fig. 1.4 Variation of longitudinal shrinkage of Norway spruce [*Picea abies* (L.) Karst.] from the pith to the bark. Juvenile wood has typically larger longitudinal shrinkage due to higher microfibril angle of the tracheid cell wall. Samples containing compression wood (*triangle*) especially in the sapwood (*arrow*) show significantly larger longitudinal shrinkage than defect-free wood. Large longitudinal shrinkage causes warping and bending of boards upon drying

concave side of the upper curve, the effect of cutting the loop was to increase the radius of curvature of the lower section and decrease the radius of curvature of the upper section. Thus the compression wood appeared to have been exerting a positive pressure prior to release. In the case of the angiosperm loop, where tension wood formed on the upper sides of the loop, the effect was the opposite, with release prompting a shortening of the tension wood side. These movements illustrate the phenomena associated with growth stress release during wood processing, further amplified by drying-induced deformations (Fig. 1.4). The commercial significance of such effects is enormous, since timber containing reaction wood cannot be used where dimensional stability is required (see Chaps. 8 and 9).

Other problems associated with compression wood include the difficulty of working the hard timber. Büsgen and Münch (1929) commented that it is very difficult to drive a nail into it. It is also brittle and prone to brash compression failure. The higher levels of lignin also increase costs for the pulp and paper industry since lignin is expensive to remove and was hitherto difficult to dispose of. However, increasing pressure on non-renewable natural resources mean that research is now looking into utilisation of this abundant waste product.

Tension wood suffers from similar problems of dimensional stability and is also difficult to work. The gelatinous fibres tear rather than cut, giving a wood surface containing tension wood a woolly appearance. On the other hand, the lower levels of lignin might be considered advantageous for the pulp industry. The problems created by reaction wood for industry are discussed in Chaps. 6, 8 and 9.

If reaction wood were present in wood in only small amounts, these problems might be ameliorated. However, the fact that tree growth takes place subject to environmental pressures means that trees constantly have to make some reaction wood to brace themselves against the wind, to correct for windthrow and to support branches at the optimum angle. Even those trees which are perfect in form (from the

forester's and timber merchant's point of view), with straight and vertical stems, may contain significant amounts of reaction wood. Vertical growth may only be achieved by constant corrections of tendencies to lean under the influence of wind, whose direction may change from day to day. For this reason it is essential that we have the tools for identification of reaction wood. It is not always easy to do this, and methods for doing so are reviewed in Chap. 7.

The evolution of reaction wood was an essential step in the evolution of trees. Without it, tall trees could not exist and there would be no timber of any size for industry to use. It is certain therefore that the wood-based industries will have to live with reaction wood and allow for its behaviour. However, it is possible to reduce the levels of reaction wood by careful forest management and it may be possible to reduce the levels to the minimum required for successful tree growth through focused tree breeding. These and other commercial issues are discussed in Chap. 9.

In a world where pressure on forests is increasing as demand for wood for traditional purposes is added to by demand from new uses such as biofuel, it is essential that we grow trees with high quality wood while optimising biomass production. This means that we need to find ways of keeping the amount of reaction wood in timber to a minimum compatible with the safety of the tree. In addition we should be actively seeking ways of using reaction wood and ensuring that wastage is kept to a minimum. This book provides the best scientific understanding of the formation, function and behaviour of reaction wood, which will help achieve these goals.

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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

J. Ruelle (✉)
INRA, UMR 1092, Laboratoire d'Etude des Ressources Forêt-Bois (LERFoB), Centre INRA de Nancy-Lorraine, 54280 Champenoux, France
e-mail: jruelle@nancy.inra.fr

Fig. 2.1 Observation of compression wood in a stem of *Picea abies*. Scale bar = 5 cm
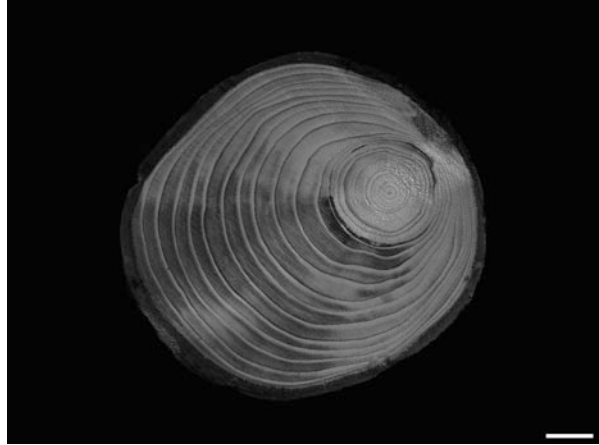
wood from the opposite[1] side of reaction wood can also show some variations in terms of anatomy or properties we decided to name the wood from the lateral and opposite zones relative to any reaction wood using the term "non-reaction wood" wherever possible.

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

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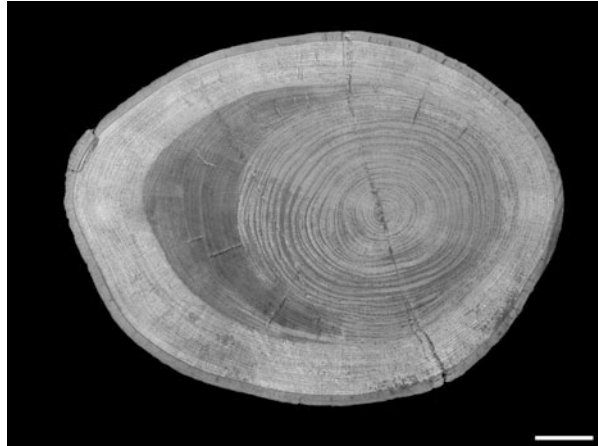
demonstrated a lack of eccentricity with tension wood occurrence or eccentricity opposite to the tension wood (Chanson 1989).

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
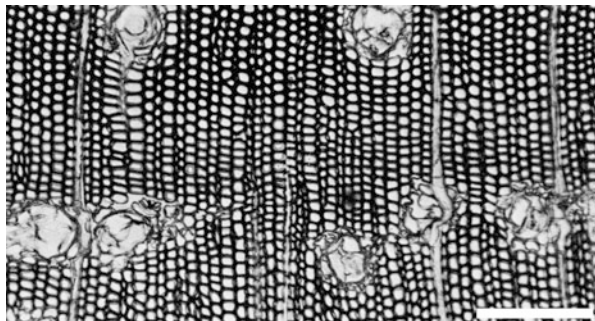
Fig. 2.4 Distribution of the 21 trees belonging to 21 tropical species in the comparison of tension wood (TW) and opposite wood (OW) for VF = vessel frequency (Ruelle et al. 2006)
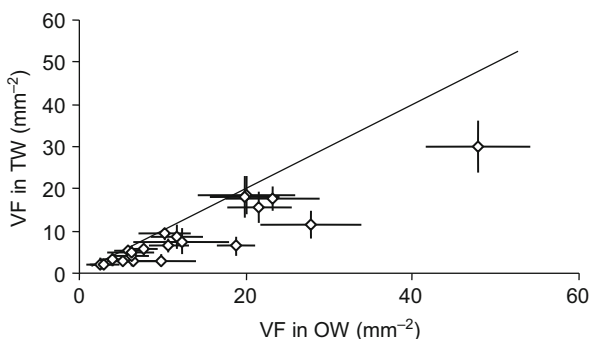
gradual in compression wood so that the demarcation between earlywood and latewood is more difficult (Timell 1986; Lee and Eom 1988). Lee and Eom (1988) did observe traumatic vertical resin canals in the compression wood of *Pinus koraiensis* (Fig. 2.3), but this feature does not seem to be a consistent 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 *Hebepetalum humiriifolium* (**e** and **f**). Scale bars = 200 μm (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
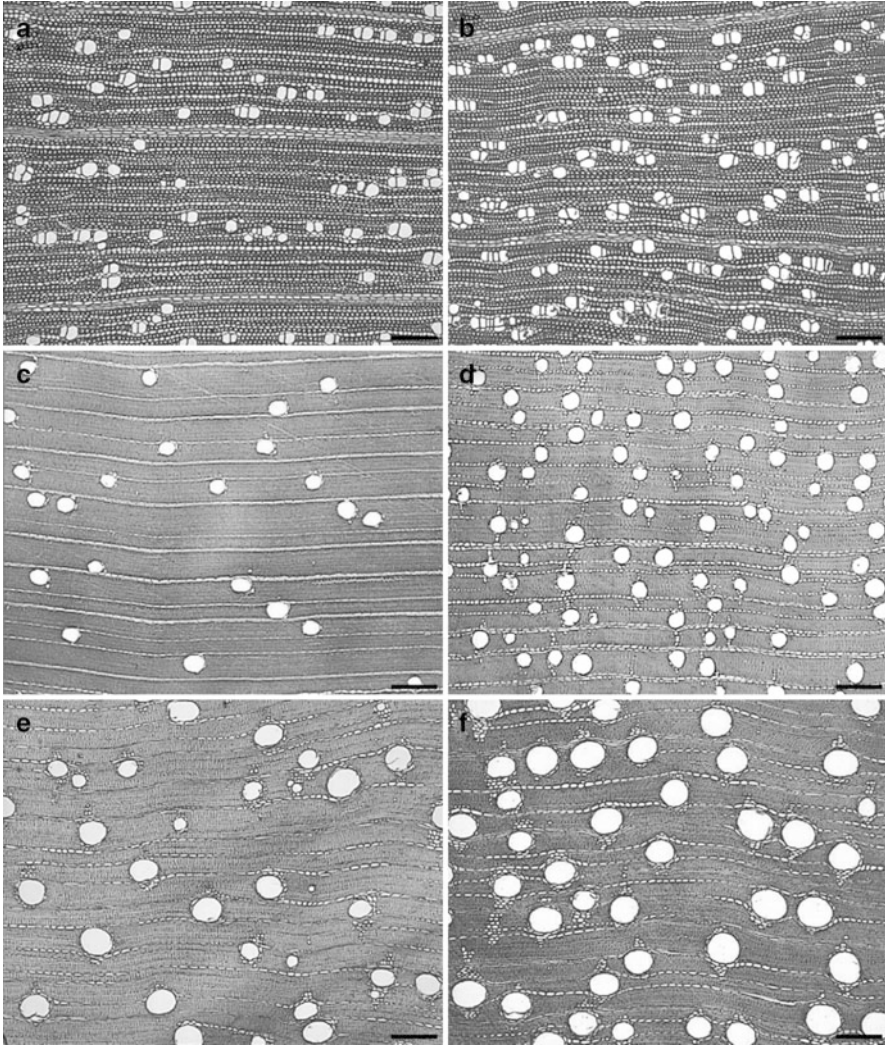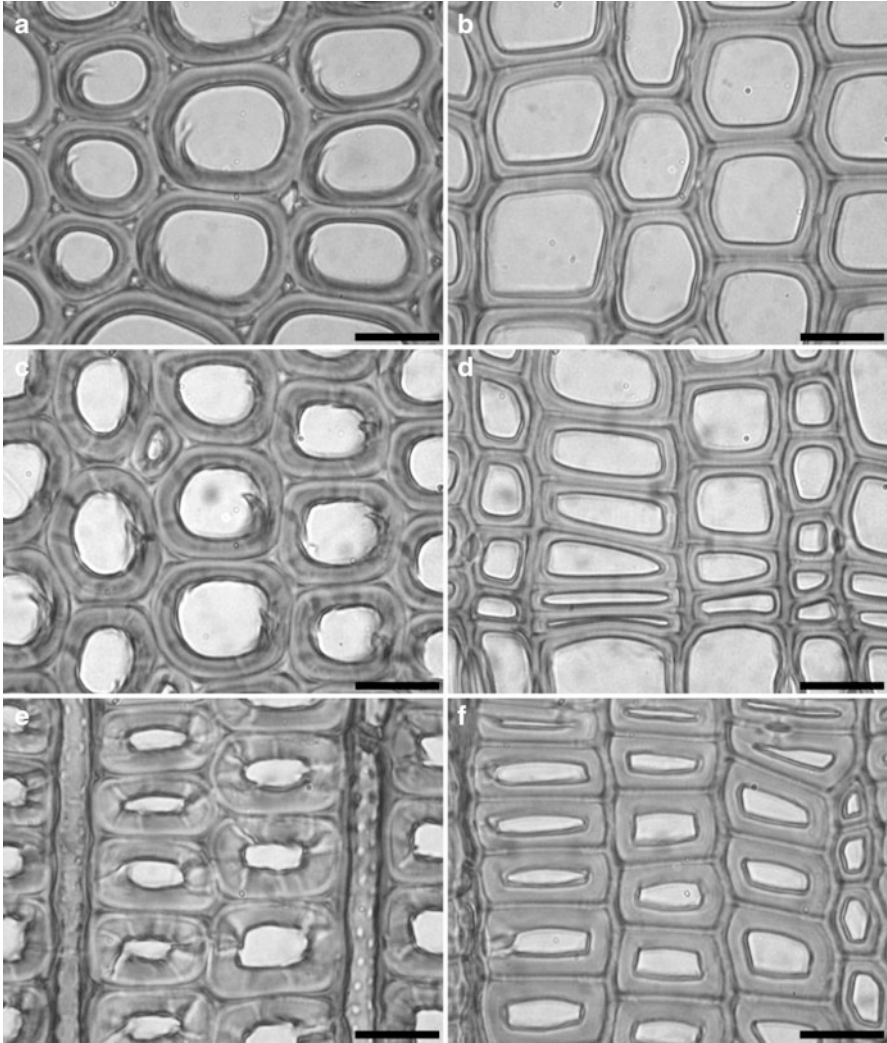
Fig. 2.6 Comparison of transverse sections of tension wood (on the *left*) and opposite wood (on the *right*) of *Picea abies* (**a** and **b**), *Pinus pinaster* (**c** and **d**), *Pinus sylvestris* (**e** and **f**). Scale bars = 20 μm

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
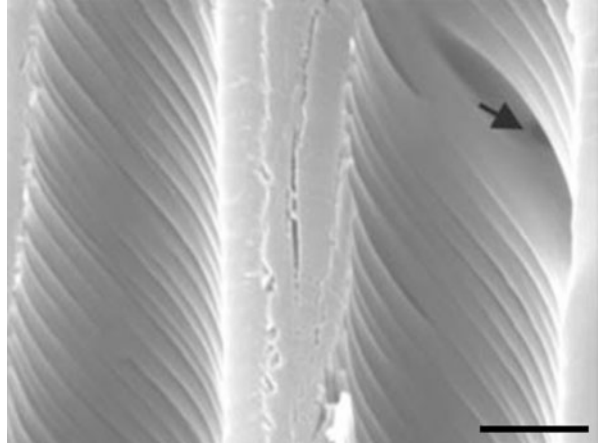
Fig. 2.8 Distorted tips of tracheids in radial section of compression wood (Lee and Eom 1988). Scale bar = 1 μm
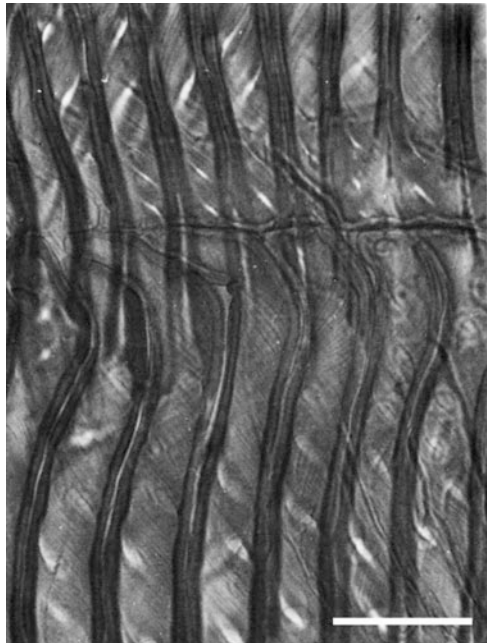
and Eom 1988). It seems that flattened and L-shaped tips of tracheids increase in number with the development of compression wood (Yoshizawa et al. 1987).

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,
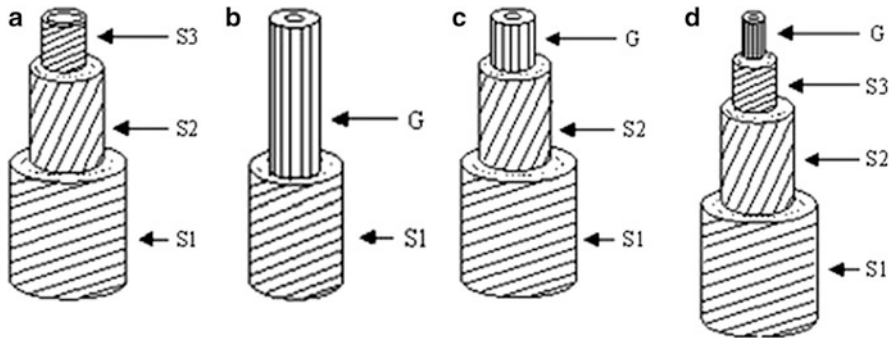
Fig. 2.9 Schematic models for the cell wall structures of fibres in normal wood (**a**) and tension wood (**b–d**), redrawn from Wardrop and Dadswell (1955). *Solid lines* indicate cellulose microfibril orientation. (**a**) Normal fibres do not develop a G-layer. (**b**) G-layer where S_2 and S_3 layers develop normally. (**c**) S_3 layer replaced with G-layer. (**d**) G-layer forms as the innermost layer next to the S_3 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 S_1, S_2 and S_3 layers, gelatinous fibres can show various patterns, i.e. S_1+G, S_1+S_2+G, $S_1+S_2+S_3+G$. 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
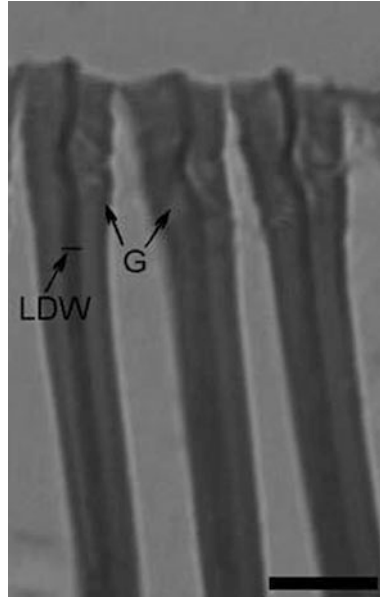
Fig. 2.10 Longitudinal section of poplar tension wood fibres showing an increase in the G-layer thickness near the cutting surface. LDW = lignified double wall (S_2+S_1+P+intercellular layer$+P+S_1+S_2$), G = G-layer. Scale bar = 10 μm (Clair et al. 2005a)

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
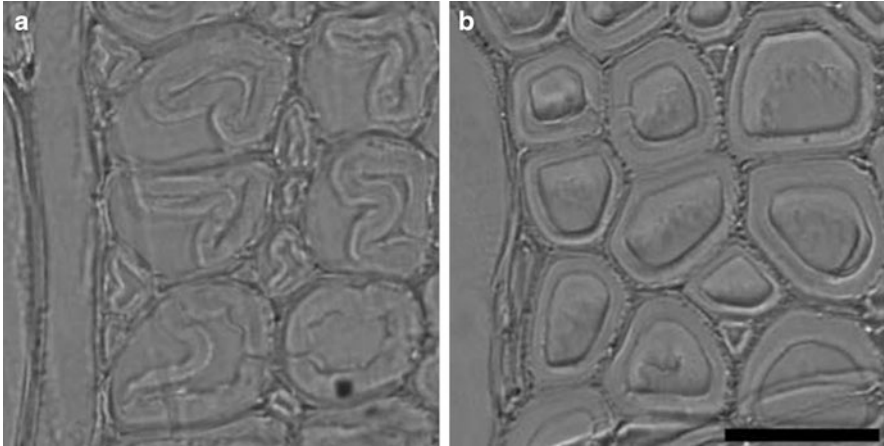
Fig. 2.11 Transverse sections of never-dried poplar tension wood. Observation of detachment of the G-layer from S$_2$ layer versus distance (*D*) to the reference face (*cutting surface*). (**a**) 10 μm, (**b**) 150 μm. Bar 20 μm (Clair et al. 2005b)
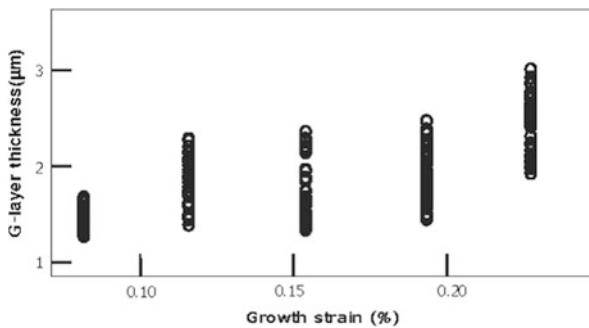
Fig. 2.12 Relation between G-layer thickness (μm) and growth strain (%) (Fang et al. 2008)

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.
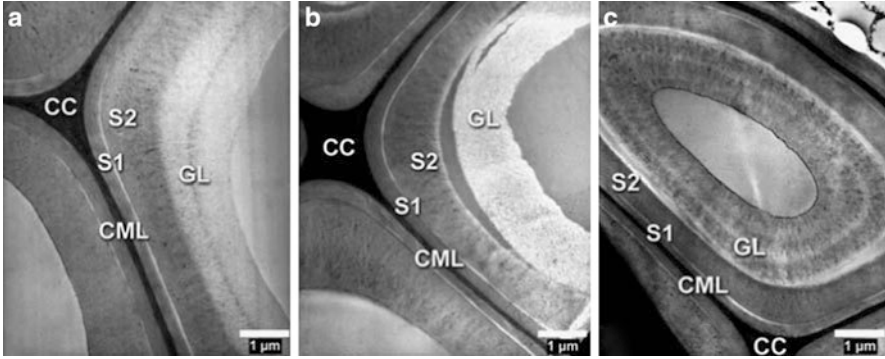
Fig. 2.13 TEM cross sections of tension wood in *Acer* spp. (**a**), *F. sylvatica* (**b**) and *Q. robur* (**c**). Note the concentric contrast in the G-layers of maple and oak indicating a slight deposition of aromatic compounds (CC cell corner; CML compound middle lamella, S_1, S_2 secondary wall, 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 S_3 layer commonly observed in non-tension wood fibres. This kind of structure was also observed by Daniel and Nilsson (1996) in
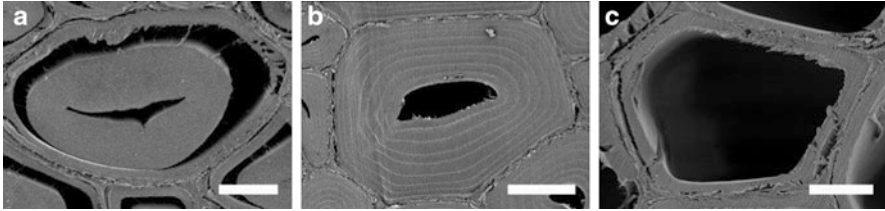
Fig. 2.14 Cross sections of tension wood (**a**) *Eperua falcata* showing a well-developed G-layer (**b**) *Laetia procera* showing a polylaminate organisation, and (**c**) *Simarouba amara* showing no difference with non-reaction wood fibres. Bars, 5 μm (from Ruelle et al. 2007a)

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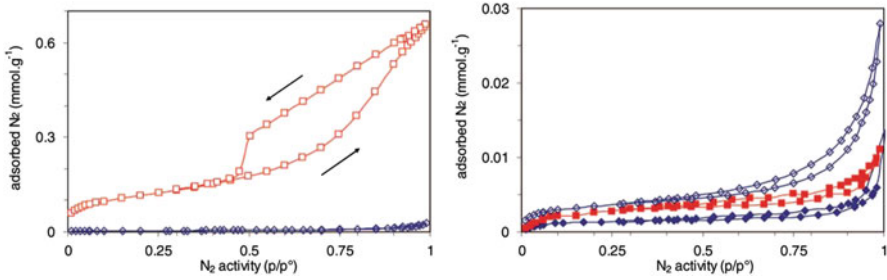
Fig. 2.15 N2 adsorption–desorption isotherms: (*left*) aerogel of tension wood (TW) and normal wood (NW); (*right*) NW and TW xerogel compared to NW aerogel. Key: *square*, TW; *diamond*, NW; *void shapes*, aerogel; *filled shapes*, xerogel

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*
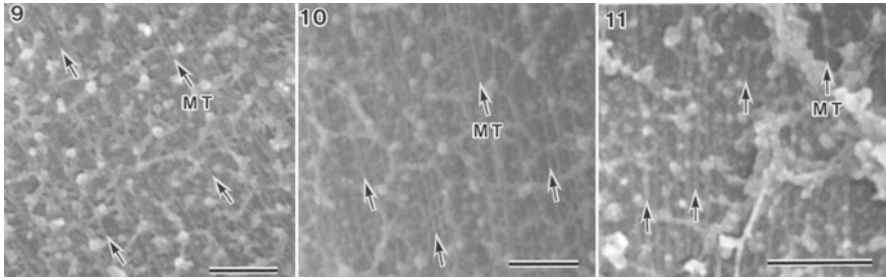
Fig. 2.16 Micrographs (FE-SEM) showing the progressive changes in orientation of MTs, with clockwise rotation (viewed from the lumen side), during formation of the G-layer in tension wood fibres of *Fraxinus mandshurica*. (*9*) The MTs (*arrows*) are oriented at an angle of about 35–40° to the fibre 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 Prodhan 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 S_2 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 (S_1 and S_2) 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 macrofibrils, 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)
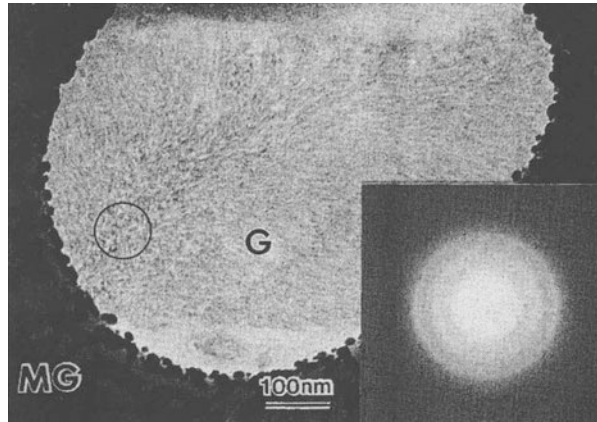
value for the S_2 layer of the same tension wood cell was 3.14 nm. Such a large value
for G-layer crystallites and the difference from that found in the S_2 layer could be
explained by two scenarios given by the authors: either cellulose biosynthesis is
considerably different between the G-layer and 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 organi-
sation (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**) S_2 and S_2L regions of *P. radiata* compression wood tracheid. Samples were coated with 12 nm of chromium. Scale bar = 0.5 μm; (**b**) G-layer of *P. deltoides* tension wood fibre. Scale bar 0.25 μm (Donaldson 2007)
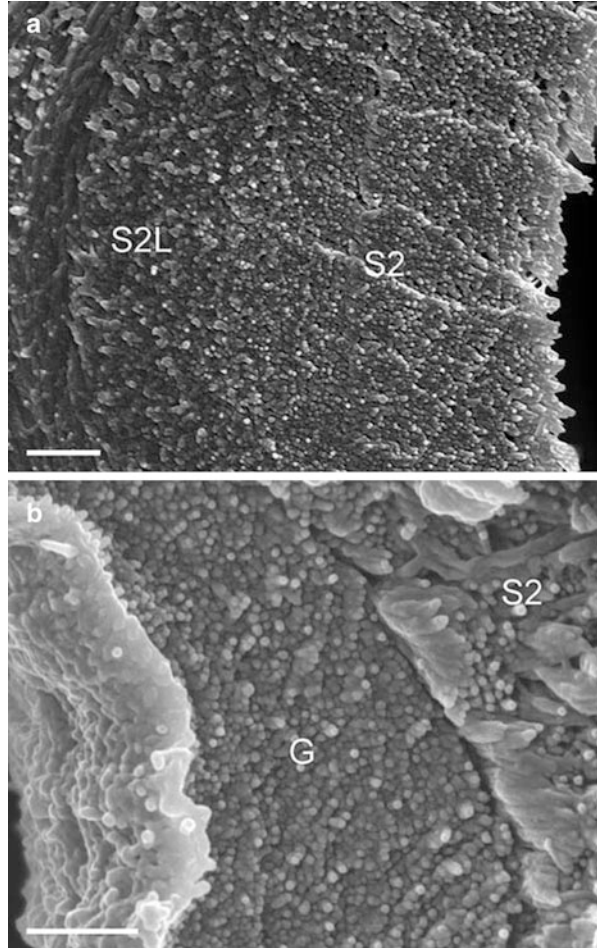
to radiata pine tracheids with an average of 19 nm. The S_2 region of compression wood tracheids had the largest macrofibrils at 22 nm diameter. The above trend suggests a relationship with the degree of lignification among these cell wall types. The G-layer of poplar tension wood is thought to be unlignified (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 S_2 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 S_2 layer from tension wood and the S_2 layer from opposite wood. For all species the macrofibrils of the G-layers and the S_2 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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Chapter 3
Cell Wall Polymers in Reaction Wood

Kurt V. Fagerstedt, Ewa Mellerowicz, Tatyana Gorshkova, Katia Ruel, and Jean-Paul Joseleau

Abstract In this chapter we concentrate on the structure of reaction wood and its functional properties. The fact that a lot more detailed information exists on tension wood rather than on compression wood, this is reflected in the amount of information in this chapter. During reaction wood formation major changes take place in the cell wall polymers and their distribution. For example, in a typical broadleaf tree G-layer almost pure cellulose is produced as a thick layer, while lignin is mainly present in the middle lamellae and primary cell walls. As reaction wood is formed in trees due to a new demand for strength, we have put some emphasis on the relations between structure and the imbued function of the newly formed polymeric structures. While our understanding of the signalling of reaction wood formation is still fragmentary, we have collected the existing information on this topic in Sects. 3.2.5 and 3.3.3.2.

K.V. Fagerstedt (✉)
Division of Plant Biology, Department of Biosciences, Helsinki University, P.O. Box 65, 00014 Helsinki, Finland
e-mail: kurt.fagerstedt@helsinki.fi

E. Mellerowicz
Umea Plant Science Center, Swedish University of Agricultural Sciences, SE901 83 Umea, Sweden
e-mail: ewa.mellerowicz@genfys.slu.se

T. Gorshkova
Kazan Institute of Biochemistry and Biophysics KSC RAS, PO Box 30, 420111 Kazan, Russia
e-mail: gorshkova@mail.knc.ru

K. Ruel • J.-P. Joseleau
Centre de Recherches sur les Macromolécules Végétales, CNRS, BP53, 38041 Grenoble, Cedex 9, France
e-mail: katia.ruel@cermav.cnrs.fr; katiaruel@yahoo.fr; jpjoseleau@gmail.com

3.1 Introduction

During reaction wood formation major changes take place in the cell wall polymers in the new wood being formed. In compression wood of conifers the amount of lignin in the cell walls increases and at the same time the proportion of carbohydrates decreases, while in broadleaf trees the opposite happens during tension wood formation. In the G-layer which is typical of tension wood in many species almost pure cellulose is produced as a thick layer, while lignin is mainly present in the middle lamellae and primary cell walls although some studies suggest that the total amount of lignin does not decrease significantly during tension wood growth. One of the cell wall characteristics typical of reaction wood which changes wood properties dramatically is the altered microfibril angle (MFA). In the hardwood G-layer the cellulose microfibrils are laid down almost vertically whereas in compression wood the MFAs are generally higher than in normal wood. Changes do also occur in other cell wall polysaccharides. This chapter concentrates on changes in the cell wall polymers cellulose, hemicelluloses, lignin, and extractives in reaction wood in comparison with the characteristics of normal wood. The information is brought together and discussed with a particular emphasis on the effect that these changes have on wood properties at larger scales.

3.2 Polymers of Tension Wood

3.2.1 Tension Wood Polymers Can Be Arranged in Two Different Types of Cell Wall Organization

Tension wood (TW) typically found on the upper side of inclined stems of hardwoods has distinct cell walls. It is defined as a type of wood characterized by a high degree of tension stress as compared with normal wood (NW) or opposite wood (OW), found in upright stems and in the opposite side of bent stems, respectively. The difference from NW or OW arises from differences in cell wall polymer composition (Table 3.1) and arrangement. Two types of cell wall organization have been recognized in tension wood fibres. The first one is similar to the arrangement found in NW and OW. It has the usual complement of two, or more secondary cell wall layers denoted as S_1, S_2, etc., deposited over a layer of primary wall (P), which in wood cells is usually considered together with the middle lamellae and for two adjacent cells has the designation "compound middle lamellae" (CML). An example of such organization is the TW of *Magnolia* species (Yoshizawa et al. 2000). The second type of cell wall organization has, in addition to the P-layer and a variable number of S wall layers, a tertiary cell wall layer called the gelatinous or G-layer (Wardrop and Dadswell 1948). The two types of organization are diagrammatically presented in Fig. 3.1, and the two types of fibres having

Table 3.1 Comparison of main polymer composition of tension wood, normal wood and isolated G-layers in *Populus alba*

Sample	% (w/w)				References
	Cellulose	Lignin	Matrix polysaccharides	Other	
NW	42.5	19.4	36.4	1.7	Baba et al. (2009)
TW	53.9	14.4	29.8	1.8	Baba et al. (2009)
G-layers	78	Not detected	19	2.6	Kaku et al. (2009)
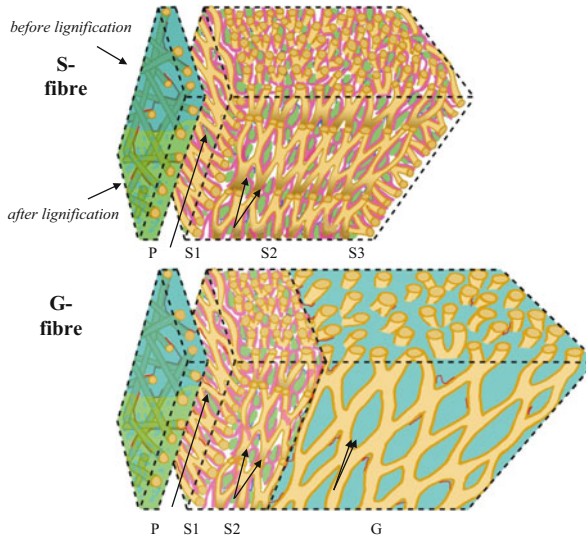
Fig. 3.1 Generalized model of cell wall structures in S-fibres (NW, OW or TW) and G-fibres (TW) of a deciduous tree. The *long arrow* indicates fibre long axis and *short arrows* show the microfibril angles. A striking difference in composition and structure between P-, S-, and G-layers is depicted. The P-layer is composed mostly of highly hydrated pectins (*blue*), cellulose microfibrils (*beige*) and xyloglucan (*red*), and it becomes filled with lignin (*green*) after the S-layers are deposited. Cellulose microfibrils run in a coordinated fashion within a single stratum of the P-layer but change orientation between strata. The S-layers are made of cellulose, xylan (*pink*) and lignin with a smaller amount of glucomannans and galactoglucomannans (*dark blue*). Cellulose microfibrils change orientation between S_1-, S_2- and S_3-layers but they follow the same direction in many strata of each S-layer. The microfibrils are aggregated forming a network of macrofibrils and creating a twisted honeycomb structure. The G-layers are mostly composed of large cellulose macrofibrils, with larger overall porosity. The pores are likely filled with pectins and arabinogalactan proteins (AGPs). Xyloglucan is present in the P- and G-layers and between different cell wall layers. Reprinted from Mellerowicz and Gorshkova (2012) with permission

these cell wall arrangements are subsequently called S- and G-fibres. The occurrence of either type of fibres in TW and the number and arrangement of different cell wall layers are genetically determined (Onaka 1949). A large comparative study of 100 families of woody plant species (trees and shrubs), mostly from

temperate zone, identified G-fibres in the TW of approximately 50 % of them (Höster and Liese 1966) whereas approximately 40 % of tropical species had TW with G-fibres (Clair et al. 2006c).

In species that form G-fibres, the mechanical behaviour of TW as a whole has been correlated with the amount of G-layer material in the wood reflected in a proportion of G-fibres and in the thickness of their G-layers (Yoshida et al. 2002a; Clair et al. 2003; Fang et al. 2008). In species that develop TW with S-fibres, the level of tension stress depends on changes in the cell wall carbohydrate composition, lignification and architecture, most of which concern the S_2-layer, which increases in thickness (Boyd 1977; Baillères et al. 1995). These observations underscore the major role of cell wall composition and organization in generation of tension maturation stress, which will be discussed later in this chapter. Contributions from other factors such as TW anatomy, cell shape, and growth eccentricity are discussed in Chap. 5 and the consequences of these stresses for wood properties are discussed in Chap. 6.

The available evidence indicates that the polymer composition and arrangement in the G-layer are fundamentally different from those found in S-layers, as is diagrammatically illustrated in Fig. 3.1 (Mellerowicz and Gorshkova 2012). The specialized cell wall structure found in the G-layer is thought to play an essential role in the development of the tensile stress in TW. In TW with S-fibres, more subtle adaptations have been recently revealed, in particular, in the cellulose fibrils. To examine these cell wall modifications, the cell wall structure and composition can be compared along the continuum of tensile stresses from low to high. These comparisons reveal trends in cell walls correlated with tension stresses developed in the wood. We will see that many features of these specially adapted S-fibres are of similar nature to the ones found in the G-layer.

There is still very little information on how a developing wood cell can regulate its tension stress. A few transcriptomic and even more scarce proteomic analyses indicate that the cell wall biosynthetic machinery is re-modelled in TW and that the polymers are modified after being deposited in the cell wall. Tension stress is created by the cell wall design and understanding the interactions between polymers in the cell wall and their modification is a key to the understanding of this process.

3.2.2 Approaches to Characterize Cell Wall Polymers

Biochemical fractionation and characterization of cell wall polymers is usually carried out using whole wood samples. These contain a complex mixture of various cell types and include different wall layers. The bulk characterization of wood reveals small differences in cell wall composition between TW, NW and OW, but this does not permit the identification of differences in the polymeric composition between cell types and cell wall layers (Table 3.1). However, comparison of TW with NW and OW provides the opportunity to characterize particular

polysaccharides whose formation was induced or repressed by gravitational stimulation (Gustafsson et al. 1952; Kuo and Timell 1969; Fujii et al. 1982; Baba et al. 2009; Hayashi et al. 2010; Brereton et al. 2011). For such a comparison the sectors of wood are dissected from corresponding side of the stem, based on detection of tension wood boundaries by its texture and appearance or by histochemical staining with Alcian blue—safranine, safranine in fluorescence, or Chlorazol Black E—safranine (Robards and Purvis 1964; Krishnamurthy 1999; Badia et al. 2005; Barbacci et al. 2008; Bond et al. 2008; Brereton et al. 2011). Bulk analysis of wood samples indicates the existence of cell wall changes but to understand their origin and to define the mode of regulation it is necessary to differentially analyse certain cell types and/or certain cell wall layers. Ideally, such differentiational analysis should be combined with in-depth characterization of chemical structures.

A way of achieving a better spatial resolution of tissue polymer analysis is the employment of a laser microdissection technique combined with cell wall analyses (Angeles et al. 2006). This method can be applied to separate groups of cells. Further development of cell separation techniques with the use of fluorescence-assisted cell sorting or tangential sectioning could boost the resolution to individual cell types. However, isolation of individual cell wall layers by this approach is not possible—it would be too tedious. Besides, the development of micro-scale polymer analysis techniques is needed to take full advantage of these methods.

The opportunity to isolate certain cell wall layer is available for G-layers of tension wood fibres: they can be released by sonication of microsections of tension wood pre-soaked in ethanol. This technique was developed in the 1960s (Norberg and Meier 1966; Furuya et al. 1970), but it is only recently that it got its "second breath" and is used to characterize G-layer components in more detail (Nishikubo et al. 2007; Kaku et al. 2009; Sandquist et al. 2010).

Most of the data characterizing individual cell types and cell wall layers have been obtained using a immunocytochemical approach, which has produced a number of very interesting results. However, antibodies are available for only a limited number of epitopes, while cell wall polymers have very complicated structures and contain many important nuances, which are not identifiable with antibodies. In addition, the qualitative determination of the abundance of various epitopes is complicated due to problems with antibody specificity and epitope accessibility. For instance, biochemical analysis of isolated G-layers from mature fibres has demonstrated the presence of xyloglucan, which could not be detected using a specific monoclonal antibody (Nishikubo et al. 2007; Sandquist et al. 2010).

An important modification of microscopical methods is their combination with various kinds of spectrometry. These techniques can offer high chemical specificity, are non-destructive, do not need preliminary labelling of tissue and permit relatively high throughput since the sample preparation is relatively simple. Information on various tissue constituents can be obtained from the same section giving opportunity to relate their distribution to anatomical structures and within the cell wall layers. Quantitative UV-microscopy has provided evidence for spectacular differences between P- and S- and G-layers in the degree of

lignification (Donaldson 2001). FTIR microspectroscopy can provide the required chemical specificity, but sufficient resolution to analyse different cell wall layers is impossible to achieve because of principal limitations due to long wavelength of infrared radiation (Rodrigues et al. 2001; Hori and Sugiyama 2003; Gorzsás et al. 2011; Foston and Ragauskas 2012). Raman microspectroscopy has excellent chemical specificity and spatial resolution (Gierlinger and Schwanninger 2006; Hänninen et al. 2011; Gierlinger et al. 2012), but Raman-scattering effect is weak when compared to infrared absorption. The effective way out of this situation can be achieved via coherent anti-Stokes Raman scattering (CARS) (Foston and Ragauskas 2012). These techniques can be used as fingerprinting tools, and in some special cases particular polymers can be determined but they have limited sensitivity to certain polymers and especially any peculiarities in their structure.

The very promising approach to obtain chemical imaging of wood tissue sections with high resolution is provided by the emerging techniques combining microscopy with mass-spectrometry. Two major types of mass-spectrometry (MS) are currently used in such type of analysis: time-of-flight secondary ion mass spectrometry (TOF-SIMS) (Tokareva et al. 2007; Jung et al. 2012) and matrix-assisted laser desorption/ionization-mass spectrometry (MALDI-MS) (Jung et al. 2010). In such a complex sample as wood, ions with the same mass (isobaric ions) originating from different polymers can be present; they can be distinguished for selective imaging by tandem MS, like MALDI linear ion trap tandem MS (MALDI-LIT tandem MS) (Lunsford et al. 2011). The resolution of these techniques is at submicrometer level, allowing the analysis of individual cell wall layers. Each of these mass-spectrometry techniques has its own advantages and limitations. MALDI-MS discriminates against high molecular weight molecules. TOF-SIMS provides chemical information directly from the surface of a cross-section without sample treatment such as bedding matrix application. Such a molecular image can be acquired to a depth of up to 10 nm from the surface resulting in a 3D stack of tissue slices analysed as shown by Jung et al. (2012) who used tension wood of poplar stems as a model substrate to investigate the application of TOF-SIMS on plant tissues. Presently, these techniques are at the stage of confirmation of their applicability and so far have demonstrated already established patterns rather than identifying new ones. The library of characteristic ions, which is necessary to identify specific cell wall components, is currently rather limited and is mainly restricted to cellulose and lignin. But such libraries are expanding (Goacher et al. 2011) and future possibilities look quite hopeful.

3.2.3 Cellulose

3.2.3.1 General Structure of Cellulose in the P- and S- Wall Layers

Cellulose consists of linear polymers of glucan [→4)-β-D-Glc$p$-(1→] that is aggregated into crystallites forming long crystalline fibrils in the plant cell wall. The

structure of these cellulose crystallites is still a matter of debate. The thickness of the smallest crystallite, which presumably corresponds to the aggregate of glucan chains produced by a single cellulose biosynthetic rosette complex, has been measured by both wide angle X-ray scattering (WAXS) and small angle X-ray scattering (SAXS) to be 2.4 nm, and by solid state ^{13}C NMR to be 2.6 nm in the primary walls (Kennedy et al. 2007). This would correspond to 25–30 glucan chains. The glucan chains interact with adjacent parallel chains via hydrogen bonds forming either a triclinic I_α or monoclinic I_β crystals. These crystal phases are interconvertible and there is a mixture of the two phases in wood cell walls with a higher proportion of I_β (Wada et al. 1995). These microfibrils are thought to aggregate to form larger entities, so-called macrofibrils, and both types of fibril are coated with matrix polysaccharides in the cell wall.

In developing xylem cells with only primary walls the cellulose microfibrils are thought to be less aggregated because of the presence of a higher proportion of matrix polymers, and they are more or less randomly oriented with some tendency to longitudinal orientation (reviewed by Mellerowicz et al. 2001; Mellerowicz and Gorshkova 2012). In the S-wall layers, it is likely that the majority of cellulose microfibrils are aggregated into macrofibrils. Microfibrils and their aggregates show a preferred orientation in each cell wall layer in relation to the cell axis. This is commonly expressed as MFA (reviewed by Donaldson 2008). MFA is an important parameter that determines wood stiffness and many other wood properties and several methods have been developed to measure the prevailing orientation of cellulose fibrils in the wood, some of which can be used to determine the orientation in particular cell wall layers. The connection between MFA and maturation strains is discussed in Chap. 5 and the impact of MFA on the physical and mechanical properties of reaction wood is discussed in Chap. 6.

3.2.3.2 Cellulose in G-Fibres

Cellulose constitutes the main polymer of the G-layer but not as much as has been repeatedly claimed based on the 98.5 % glucose content in isolated G-layers of *Populus tremula* L. (Norberg and Meier 1966). Recent estimates in the isolated G-layers of *Populus alba* indicated 78 % cellulose (Table 3.1; Kaku et al. 2009) and a 75 % value can be deduced from the results of the monosaccharide analysis published by Nishikubo et al. (2007) for the same species if it is assumed that all the glucose is present in the layer as cellulose and xyloglucan.

Early studies showed that the organization of the cellulose network is different in the G-layer compared with the adjacent S layers. A transmission electron microscopical study of tension wood of *Populus* × *euroamericana* embedded in metacrylate and swollen provides a striking picture of a structure described as "honeycomb" (Fig. 3.2), a structure which was not found in the S-layers (Sachsse 1964). However, after delignification of cell walls, a similar structure was revealed in the S-layers (Côté et al. 1969), suggesting that the honeycomb structure was typical of both S and G, but that it was masked by lignin in the S-layers. The
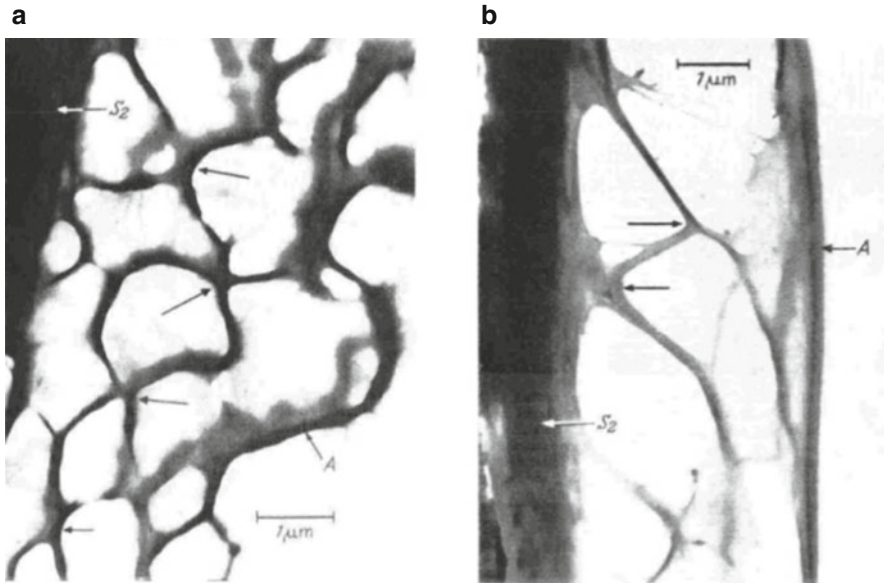
Fig. 3.2 Honeycomb structure of cellulose revealed by the direct microscopic observation of methacrylate embedded swollen TW of *Populus × euroamericana*. Cross-sectional (**a**) and longitudinal (**b**) views are shown. *A*—terminal lamella (Abschlusslamelle). Reprinted from Sachsse (1964) with permission

interconnected network of the honeycomb structure was thought to represent large cellulose aggregates, or macrofibrils coated by matrix, forming axially oriented lens-shaped compartments. The approximate dimensions of these compartments were of the order of 1/2 μm.

More recently, the porosity of the G-layers has been deduced from nitrogen adsorption–desorption experiments using supercritically dried TW (Clair et al. 2008). The drying process is thought to preserve the hydrated structure of cell walls. It was found that TW is characterized by much higher porosity (total pore area per volume) in comparison with NW. Mezopores of 2–50 nm in diameter have been found in chestnut (*Castanea sativa* Mill.) TW (Clair et al. 2008). A similar size-range for the mezopores (6–12 nm in diameter) was found in TW and NW in several tropical species, but porosity was higher in TW than in NW (Chang et al. 2009). The largest difference, up to 30-fold, was recorded for TW fibres characterized by thick G-layers. From comparisons of the porosities of TW in species with and without a G-layer the authors concluded that the high porosity is an attribute of the G-layer itself. The nanoporous structure of tension wood cell wall is also obvious on the cross-sectional surface subjected to repeated cycles of sputtering with O_2^+ (Jung et al. 2012).

Images of cellulose fibrils vary depending on the method of sample preparation and the technique used for visualization, but they consistently show the presence of microfibril aggregates in the G-layer. Field emission scanning electron microscopy

Image of cellulose
aggregates in the G and
adjacent S-layers of
Populus deltoides. Scale
bar = 0.25 μm. Reprinted
from Donaldson (2007)
with permission
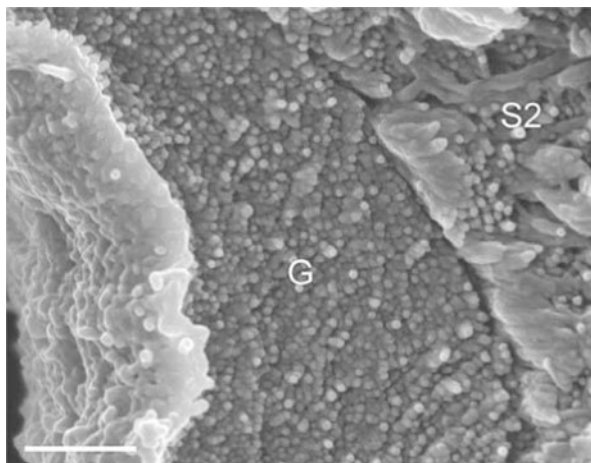
(FE SEM) using fixed, usually delignified sections (Fig. 3.3) shows macrofibrils of
diameters ranging from 10 to 60 nm in the G-layer, even allowing for the effect of
coating by chromium or other metals used during sample preparation (Daniel
et al. 2006; Donaldson 2007; Lehringer et al. 2009). Variable results have been
reported concerning the difference in macrofibril size between G- and S-layers
(in either NW or TW) (Table 3.2). For example, in a study in which macrofibril
sizes were compared between G-layers and S_2 layers of TW in three different
species, *Fagus sylvatica* showed the largest macrofibrils in the G-layer, while in
Quercus robur L. and *Acer* spp. they were found in the S layers (Lehringer
et al. 2009). In chestnut (*C. sativa*), macrofibrils of diameters up to 55 nm have
been estimated from SEM micrographs of the G-layer, and similar sizes were
deduced from porosity measurements by nitrogen adsorption–desorption of
supercritically dried TW specimens (Clair et al. 2008). In *Populus deltoides*
(Bartr. ex Marsh) larger macrofibrils diameters were observed in S-layers and
middle lamellae in NW than in the G-layers (Donaldson 2007). The apparently
larger diameters could be partially explained by the fact that the macrofibrils are
sectioned at different angles in the different cell wall layers resulting in ellipsoidal
shapes in the CML and S- layers and circular shapes in the G-layer. But in addition,
since a positive correlation was observed between lignin content and macrofibril
size in that study, it is likely that a coating of lignin and matrix carbohydrates
contributed to the diameters measured. This correlation between lignin content and
macrofibril size has not been universally observed. For example, Ruelle
et al. (2007a) observed larger macrofibril diameters in polylamellate and weakly
lignified G-layers (21.9 ± 0.8 nm) than in the heavily lignified S-layers of OW
(18.4 ± 1.6 nm) in *Laetia procera* (Poepp.) Eichl. SEM, however, does not differ-
entiate between lignin, matrix polysaccharides and cellulose so that measurements
of cellulose aggregate size using SEM will always be towards the upper limit.

Table 3.2 Cellulose macrofibril diameters as determined by different methods in G-layers compared to S-layers in tension wood (TW), S-layers in normal wood (NW) and S-layers in opposite wood (OW)

Species	Type of TW	Method	Diameter (nm)				References
			G-layer in TW	S-layer in TW	S-layer in OW	S-layer in NW	
Fagus sylvatica L.	G-fibres	FE-SEM	12.7–22[a]	7.5–15[a]	9.6–18[a]		Lehringer et al. (2009)
Quercus robur L.	G-fibres	FE-SEM	6.8–13[a]	14.5–17.4[a]	13–19[a]		Lehringer et al. (2009)
Acer spp.	G-fibres	FE-SEM	9–16.8[a]	13–21[a]	11.5–16[a]		Lehringer et al. (2009)
Populus deltoides Bartr. ex Marsh	G-fibres	FE-SEM	13–16[a]			15–18[a]	Donaldson (2007)
Populus tremula L. × tremuloides Michx.	G-fibres	FE-SEM	30–40[a]				Daniel et al. (2006)
Laetia procera (Poepp.) Eichl.	Polylamelate G-fibres	SEM	21.9 ± 0.8[b]		18.4 ± 1.6[b]		Ruelle et al. (2007a)
Populus maximowiczii Henry	G-fibres	X-ray	6.49 ± 0.24[b]	3.14 ± 0.23[b]			Müller et al. (2006)
			TW with G-fibres	TW with S-fibres	OW	NW	
Quercus acutissima	G-fibres	X-ray in wet and dry samples	Approx. 6.2–5.7[a] (wet–dry)		Approx. 3–2.9[a] (wet–dry)		Yamamoto et al. (2010)
Eucalyptus grandis	S- and G-fibres	X-ray in SilviScan 2	3.0–3.5[a]	2.7–3.0[a]	2.7–3.0[a]		Washusen et al. (2005)
Eucalyptus globulus Labill	S- and G-fibres	X-ray in SilviScan 2	3.1–3.8[a]	2.8–3.1[a]	2.8–3.2[a]		Washusen et al. (2005)
E. globulus Labill	S- and G-fibres	X-ray diffraction in SilviScan 2	3.3–3.4[a]	3.1–3.3[a,c]			Yang et al. (2006)

[a] Range
[b] Mean ± SD
[c] Sample contained both S- and G-fibers

A more precise measurement of the cellulose crystallite diameter can be obtained from X-ray diffraction analysis. Results obtained using this technique support the formation of cellulose aggregates in the G-layer. Observations on isolated single G-fibres of *Populus maximowiczii* produced distinct signals for G- and S_2-layers (Müller et al. 2006) from which crystallite diameters were calculated using [100] reflections to be 6.49 ± 0.24 nm in the G-layer and 3.14 ± 0.23 nm in the S_2 layer. Based on these diameters the G-layer crystallites can be estimated as containing on average four times more cellulose microfibrils than were found in the adjacent S_2-layers. It is still unknown how many microfibrils there are in either type as the 3.14 nm value probably reflects the average size obtained from a mixture of single and aggregated microfibrils. Similar values were obtained in oak (*Quercus acutissima*) by measuring diffraction from isolated G-layers and in bulk TW and NW powders at different moisture levels (Yamamoto et al. 2010). The crystallite size decreased a little during drying, being more pronounced in TW than in NW. Larger crystallite sizes in TW containing G-fibres compared to NW were recorded in several species (Washusen and Evans 2001; Hillis et al. 2004; Ruelle et al. 2007b; Leppanen et al. 2011) and were positively correlated with the degree of tension stress (Ruelle et al. 2007b) implying a role in tension stress generation. Crystallite size increased from 3.55 to 3.64 nm in isolated G-layers of *P. alba* after extraction of proteins with urea (Kaku et al. 2009). This indicates that proteins or other urea-extracted material could prevent cellulose crystallization by keeping the microfibrils apart.

More microfibril aggregation in TW could explain the higher crystallinity in TW compared to NW, which has been observed from the earliest studies onwards (Wardrop and Dadswell 1948, 1955). High crystallinity of G-layer cellulose was suspected as the underlying cause for the longer hydrolysis times required to release glucose from TW (Wardrop and Dadswell 1948). Recent estimates for isolated G-layers of *P. alba* using X-ray diffraction have shown 60 % crystallinity (Kaku et al. 2009), which is approximately 17 % higher than in the bulk juvenile NW in *Populus* (Coleman et al. 2009).

Cellulose crystallites in the G-layer are classified as monoclinic I_β similar to crystals found in NW cellulose (Wada et al. 1995). Their lattice dimensions were: $a = 0.802$ nm (double inter-sheet distance), $b = 0.815$ nm (between chain distance), and $c = 1.035$ nm (the repeat value along the chain corresponding to a cellobiose length). The a and b dimensions are somewhat variable and have been shown to depend on temperature and moisture content, which might indicate some degree of anisotropy in the lateral direction (Müller et al. 2006; Yamamoto et al. 2010). Interestingly, the inter-sheet distance was found to be smaller in TW or in isolated G-layers (0.391 nm) than in NW (0.397 nm) and it was more stable during drying compared to NW, indicating less anisotropy (Yamamoto et al. 2010). If the sheets of glucan are indeed closer in the G- than in the S-layer, the diameters of individual microfibrils might be smaller and the above estimate of microfibril aggregation (four times more in the G-layer) might be underestimated. In contrast, the c value does not vary with temperature, but it is affected by the stress in the wood. Clair et al. (2006b) deduced c-values from 004 X-ray diffraction patterns

observed before and after tension stress release. The stress was released by transversal cutting of the wood and resulted in slight shrinking observed macroscopically at the wood surface. The c value of 1.0035 nm was observed in cellulose under tension, and 1.0033 nm after tension stress release. In NW, cellobiose length in cellulose is usually 1.0033 nm whereas higher values have been observed in phloem fibres of ramie, in cotton "fibres" and in algal cellulose that have larger cellulose aggregates than are found in the wood. The implications of these findings are discussed below.

The degree of polymerization (DP) was compared between NW, OW and TW for NaOH-purified and isocyanate-derivatized cellulose in *P. tremula* × *alba* fractionated by gel permeation chromatography and was found to be higher in TW ($DP_w = 2,500$) than in NW ($DP_w = 2,200$) or OW ($DP_w = 2,100$) (Foston et al. 2011). This would suggest that the DP of cellulose in G-layers might be higher than in the S-layers although no measurements are yet available for separate wall layers.

MFA is one of the most important parameters determining wood tension stresses (Yang et al. 2006) since a consistent negative correlation between tension stress and MFA has been observed for all species studied so far (Donaldson 2008). A steep negative correlation between MFA and tension stress is observed below an MFA of 10° (Wahyudi et al. 2000). A variety of techniques have been employed to determine MFA, including microscopy (iodide precipitate observation by light microscopy, FE SEM, and other techniques) and X-ray techniques (diffraction analysis, WAXS, and others) (reviewed by Donaldson 2008), which all show comparable results (Prodhan et al. 1995; Ruelle et al. 2007a, b). In G-layers, small MFAs down to almost zero degrees have been reported (Table 3.3). In this respect, the G-layer in TW fibres resembles that in phloem fibres (Müller et al. 2006). The S-layer adjacent to the G-layer in G-fibres has been reported to sometimes have an MFA larger than that in the S-layers of NW (Müller et al. 2006; Goswami et al. 2008). The significance of this change in the MFA in the S-layer is not clear. In some cases, larger MFAs were observed in OW than in NW. This may be connected with the development of the compressive stresses in the OW that have been measured occasionally in angiosperms (Clair et al. 2006a).

3.2.3.3 Cellulose in TW Without Distinct G-Fibres

In species that do not form G-layers, measurement of tension stress is required to identify TW. Recently, a few studies have reported tension stresses estimated systematically in wood samples and correlated with several cell wall parameters. This approach has revealed some aspects of cellulose structure that vary along the tension stress continuum. In one study, tension stresses were measured around the stem circumference in leaning stems of three tropical species that have very different cell wall organization: *Eperua falcata* Aublet has fibres with typical G-layers, *L. procera* (Poepp.) Eichler has fibres with multiple G-layers, and *Simarouba amara* Aublet has only S-fibres (Ruelle et al. 2007b). In the three

Table 3.3 Cellulose microfibril angle (MFA) in tension wood (TW) compared to normal wood (NW) and opposite wood (OW)

Species	Type of TW	Method	MFA (°) G-layer in G-fibres of TW	S-layer in G-fibres of TW	TW	NW	OW	References
Laetia procera (Poepp.) Eichl.	Polylametate G-fibres	FE-SEM	5.2 ± 3.1[a]				17.5 ± 2.8[a]	Ruelle et al. (2007a)
Fagus sylvatica L.	G-fibres	FE-SEM	Approx. 0	32–37.5[b]			34–39.8[b]	Lehringer et al. (2009)
Quercus robur L.	G-fibres	FE-SEM	Approx. 0				22–31.6[b]	Lehringer et al. (2009)
Acer spp.	G-fibres	FE-SEM	Approx. 0	13–26[b]			24–37[b]	Lehringer et al. (2009)
Prunus spachiana Kitamura	G-fibres	X-ray diffraction	10–11[c]			24–28[c]	15–20[c]	Yoshida et al. (2000a)
Populus euroamericana	G-fibres	X-ray diffraction			Approx. 6		Approx. 17	Clair et al. (2006a)
Populus maximowiczii Henry	G-fibres	X-ray diffraction	5.41 ± 0.02[a]	20 ± 5[a]				Müller et al. (2006)
Acacia sp.	Spiral TW	X-ray diffraction in SilviScan 2			Approx. 10	Approx. 25		Hillis et al. (2004)
			G-fibres of TW	*S-fibres of TW*	*NW*	*OW*		
Liriodendron tulipifera L.	S-fibres	X-ray diffraction	–	20–21[c]	27–30[c]	29–30[c]		Yoshida et al. (2000a)
Eucalyptus grandis	S- and G-fibres	X-ray diffraction in SilviScan 2	8–10[b]	10–30[b]		35–70[b]		Washusen et al. (2005)
Eucalyptus globulus Labill	S- and G-fibres	X-ray diffraction in SilviScan 2	8–10[b]	10–20[b]		30–55[b]		Washusen et al. (2005)

[a] Mean ± standard deviation
[b] Range
[c] Range of means

species, larger cellulose crystallite size and lower MFA were observed in TW regardless of the presence or the structure of the G-layer. In *Liriodendron tulipifera* L. that does not form G-layers, cellulose content and crystallinity were strongly correlated with the tension stress developed by the leaning stems (Sugiyama et al. 1993; Yoshida et al. 2002b). The MFA in this species was lower on the TW side compared to the OW side, but the values were higher than those typically observed in the G-layers formed in other species (Yoshida et al. 2000a) (Table 3.3). Similarly, in *Magnolia* species, and several tropical species that do not form G-layers, cellulose MFA is reduced in the TW side compared to OW side (Yoshizawa et al. 2000; Ruelle et al. 2006).

In *Eucalyptus*, some species such as *E. regnans* F. Muell. and *E. gigantea* Hook produce typical G-fibres (Wardrop and Dadswell 1948), while in other species this ability is reduced or even absent. For example, in *Eucalyptus nitens*, some clones have been observed to form a small quantity of G-fibres (10 % of fibres in TW) and other clones were completely devoid of G-fibres (Qiu et al. 2008). In both types of clones, cellulose MFA showed a striking pattern: low in TW (12–25°), extremely high in OW (38–52°) and intermediate in NW (25°). Wood density was slightly higher in TW than in OW or NW due to the increased cell wall thickness. The cellulose content was also higher. Two other *Eucalyptus* species, *E. grandis* and *E. globulus* Labill, had low MFA in TW on the upper side of the branch, compared to OW, regardless of the presence of G-fibres in the TW (Table 3.3; Washusen et al. 2005). However, the regions of TW with G-fibres had a lower MFA than the regions with S-fibres. G-fibres were also characterized by larger cellulose crystallites than found in S-fibres of TW (Table 3.2). In *E. globulus* wood without detectable G-layers the tensional stress was positively correlated with cellulose microfibril size and MFA in upright 10-year-old trees (Yang et al. 2006).

The data describing the continuum of tensional stresses in the S-fibres clearly show that cellulose structure in the S_2-layers of these fibres is modified the same way as in the G-layers of G-fibres. That is, the larger the cellulose crystallites, the higher the cellulose content, the lower the MFA, and the more tension develops in the wood. Thus, similar changes in cellulose are found in S- and G-fibres during cell adaptation designed to induce high maturation stress (high tension). However, the porosity of TW with S-fibres does not differ from that in NW (Chang et al. 2009) suggesting that high porosity is the attribute of G-layers.

3.2.4 Non-cellulosic Polysaccharides of Tension Wood

3.2.4.1 Non-cellulosic Polysaccharides of the CML in Cells of Tension Wood

The outer cell wall layer actually combines the middle lamella and the primary cell wall, which are formed at early stages of cell development, during cell division and cell enlargement. As observed in toluidine-stained sections, the CML is thicker in

mature normal wood than in mature tension wood, but that could be a result of compression by mechanical stress (Bowling and Vaughn 2008).

The major non-cellulosic constituents of the CML in wood are pectins, primarily polygalacturonic acid (also called homogalacturonan) and rhamnogalacturonan I. Polygalacturonic acid is a linear homopolymer of [→4)-α-GalAp-(1→]. C-6 of galacturonic acid can be methylesterified; the degree of methylesterification and distribution of methylester groups along the molecule are important, since they affect the accessibility of the polymer to enzymes and the propensity of homogalacturonan to form intramolecular ionic bonds involving Ca^{++} ions (Fry 1988; Carpita and McCann 2000). In the CML of both mature NW and mature TW, the degree of methylesterification is low, as revealed by binding to antibodies such as JIM5, raised against de-esterified homogalacturonan (Bowling and Vaughn 2008). This is due to the activity of pectin methyl esterase (PME) the cell wall enzyme that removes methyl groups of polygalacturonic acid. PME activity influences the symplastic and intrusive growth of developing fibres (Siedlecka et al. 2008).

Rhamnogalacturonan I is built on the base of a backbone which consists of dimers [→4)-α-D-GalAp-(1→2)-α-L-Rhap(1→] and side chains of variable structure composed of galactans [→4)-β-D-Galpz(1→], arabinans [→5)-α-L-Araf-(1→] or arabinogalactans usually having terminal arabinose, and linear or branched chains of galactans, where the galactose residues are linked at C-4 (type I), or at C-3 and C-6 (type II). The presence of rhamnogalacturonan I in the CML in various cell types of TW has been demonstrated using several antibodies, specific both for the RG I backbone and for neutral side chains (Bowling and Vaughn 2008). Their distribution in CML does not differ much between NW and TW.

One other important pectin usually present in primary cell walls is a relatively small polysaccharide with a homogalacturonan backbone and a conserved complicated side chain structure called rhamnogalacturonan II (RG II) (Carpita and McCann 2000), which has not yet been characterized in wood tissues.

Xyloglucan (XG) is the cross-linking glycan present in all land plants (Popper and Fry 2004). Until recently, it was considered to be the characteristic polymer of primary cell wall necessary for effective wall expansion. However, *Arabidopsis* mutants devoid of XG were found to grow almost normally, which challenged this view (Cavalier et al. 2008). The backbone of XG is similar to that of the cellulose molecule and consists of [→4)-β-D-Glcp-(1→]. The repeating blocks of cellotetraose have the same types of side chain, which include single xylose residues attached to the C-6 of backbone glucose α-D-Xyl-(1→6), together with an additional galactose β-D-Gal-(1→2)-α-D-Xyl-(1→6), the latter dimer further substituted with fucose α-L-Fuc-(1→2)-β-D-Gal-(1→2)-α-D-Xyl-(1→6) (Carpita and McCann 2000). Chemical analyses of isolated tissues containing the developing xylem in *Populus* reveal approximately only 6 % of XG in the primary walls (reviewed by Mellerowicz et al. 2001). This is much less than is normally found in other tissues and it is probably an underestimate due to contamination of the primary-walled tissue fraction by developing xylem cells that have secondary walls.

XG is readily detected using the monoclonal antibody CCRC-M1, specific to the fucosyl residue in XG. CCRC-M1 binding is prominent in the cambial zone cells indicating that XG is a main component of primary cell walls in fusiform and ray initials in aspen (*P. tremula* L.) (Bourquin et al. 2002). During xylem development, at early stages of secondary wall deposition, XG is localized specifically in the P-layer of developing fibres seen in both NW and TW fibres (Bourquin et al. 2002; Nishikubo et al. 2007). In developing NW of aspen, the CCCR-M1 label density increases in the primary cell wall at the time of secondary cell wall deposition, indicating either an XG deposition to the primary cell wall through the S-layers or, more likely, an XG rearrangement that reveals the epitope (Nishikubo et al. 2011).

The less abundant constituents of the primary walls are mannan and glucuronoarabinoxylan, which are the main components of S layers. The general structure of these components is discussed in the following section. These components were detected by sugar and linkage analyses in the developing primary-walled NW in *Populus* (reviewed by Mellerowicz et al. 2001) and by specific probes in the sections through aspen NW, OW and TW (Kim et al. 2012a, b; Kim and Daniel 2012). Signals from monoclonal antibodies specific to mannan, LM21 and LM22, and mannan binding motif BGM-C6, were detected in CML of G-fibres in TW, whereas of these probes only LM21 reacted with the CML in the S-fibres of NW (Kim and Daniel 2012). The binding of LM22 to mannan is known to be inhibited by galactose substitution of mannan, and binding of all mannan probes is inhibited by the presence of pectin (Marcus et al. 2010). Binding of the LM11 antibody specific to xylan was observed in the CML of aspen NW fibres (Kim et al. 2012b). In contrast, the anti-xylan antibody LM10 did not react with CML in NW or TW fibres of aspen (Kim and Daniel 2012). However, this antibody appears to be specific for the xylan epitopes characteristic of secondary walls (McCartney et al. 2005).

3.2.4.2 Non-cellulosic Polysaccharides of S-Layers in Cells of Tension Wood

The basic set of non-cellulosic polymers in S-layers in cell walls of TW (both in G-fibres and S-fibres) is similar to that in normal wood. The major non-cellulosic polysaccharide is xylan [→4)-β-D-Xyl$p$-(1→]. The backbone of xylans of different origin may have branching at C-2 or C-3 with short side chains, consisting of single or several residues of α-L-Ara$f$ or a single residue of α-D-GlcAp. Depending on the presence of various side chains the polymer is named arabinoxylan, glucuronoar-abinoxylan or glucuronoxylan (Ebringerova and Heinze 2000). In the S-layers of dicotyledons there is typically glucuronoxylan (Ebringerova and Heinze 2000; Evtuguin et al. 2003; Decou et al. 2009).

Being the most abundant non-cellulosic polysaccharide, xylans are rather well characterized from the structural point of view. Glucuronic acid is attached at C-2 of the xylose residue; the molar ratio between Xyl and GlcA in wood glucuronoxylans is within the range (4–16):1 (Teleman et al. 2002; Ebringerova

and Heinze 2000; Kabel et al. 2007). Glucuronoxylan can be covalently linked with other cell wall polysaccharides like galactan side chains of rhamnogalacturonan I through the GlcA (O-2) residues (Shatalov et al. 1999; Evtuguin et al. 2003). An important feature of cell wall polysaccharides is the modification of sugar residues with methyl or acetyl groups. Glucuronic acid in xylans is typically etherified at O-4 with the methyl group, giving 4-O-Me-GlcA (Azuma et al. 1983; Decou et al. 2009). The xylose residues, which are branched with GlcA, are usually additionally acetylated at C-3. In addition, acetyl groups are often present in other xylose residues at C-2 or 3-C, or both (Teleman et al. 2002; Evtuguin et al. 2003). The ester linkage of the acetyl group is broken by saponification during alkali extraction, which is often used to isolate xylan. For this reason few papers describe distribution of acetyl groups in xylan molecules. Detailed characterization of xylan from wood of *Eucalyptus globulus* Labill by ^{1}H NMR has showed that 34 % of the xylose residues contained acetyl groups at O-3, 15 % at O-2, 6 % both at O-2 and O-3, giving in total the molar ratio between Ac and Xyl of 0.61 (Evtuguin et al. 2003). Xylan from various sources may have different distributions of side chains and modifying groups: random, block-wise or regular along the whole backbone (Ebringerova and Heinze 2000).

Wood xylans have relatively low molecular mass according to gel-chromatography data. Their M_w is between 8 and 40 kDa as found in several hardwood species (Teleman et al. 2002; Evtuguin et al. 2003; Kabel et al. 2007), which roughly corresponds to the degree of polymerization of 50–250 units.

Xylan molecules are able to self-associate and to interact with cellulose (Kabel et al. 2007; Patel et al. 2007; Köhnke et al. 2008). This ability is influenced by the amount and distribution of side chains and modifying groups. The presence of acetyl groups and especially of arabinose residues weakens such interactions (Kabel et al. 2007). Xylan is sometimes considered as the "twisting agent" helping to form the helicoidal orientation of cellulose microfibrils (Reis et al. 1991; Reis and Vian 2004). Glucuronoxylan located at the surface of cellulose micro- and macrofibrils gives the latter a negative charge, which can be revealed using cationic gold particles (Reis and Vian 2004). This prevents adhesion of cellulose fibrils to each other and helps their parallel arrangement.

Glucuronoxylan is considered an interface between cellulose macrofibrils and lignin (Reis and Vian 2004; Dammström et al. 2009). Acetylated 4-O-methylglucuronoxylan is the major polysaccharidic component of lignin-carbohydrate complexes, at least in hardwoods (Yuan et al. 2011). Such a complex is a fundamental element of S-layers as evidenced, for instance, in the *Arabidopsis* mutant irx3, which is defective in cellulose synthesis but still contains the glucuronoxylan–lignin complex (Ha et al. 2002). It is suggested that the presence of lignin influences the self-association of xylan molecules (Westbye et al. 2007).

Covalent linkages between xylan and lignin are largely formed through the residue of 4-O-Me-GlcA: its carboxylic group forms ester linkage at γ-position of aromatic ring side chain (Balakshin et al. 2011). This linkage is alkali-sensitive. Quantification of different linkages in lignin–carbohydrate complexes is still quite complicated (Yuan et al. 2011). Very rough estimation, based on the data of HSQC

2D spectra from high field NMR (Balakshin et al. 2011), indicate that in birch (*Betula* spp.) around 1 % of lignin monomers are ester-linked to xylan. The significant proportion of glucuronic acid, which is attached to the xylan backbone, is not linked to lignin, while in pine (*Pinus* spp.) almost all GlcA is esterified (Balakshin et al. 2011).

Another type of linkage between xylan and lignin is benzyl ether linkage at α-position of aromatic ring side chain with the C2 or C3 of xylose of the polysaccharide backbone. Quantification of such linkage in birch wood gave the value below 0.1 % (Balakshin et al. 2011). Phenyl glycoside linkages with C1 of sugar moiety are also observed in lignin–carbohydrate complexes. It is rather difficult to directly determine the sugar involved in this type of bond. Based on the monosaccharide composition of fraction enriched in lignin–carbohydrate complexes (90 % xylose) in birch (Balakshin et al. 2011), it can be suggested that xylan residues are involved in phenyl glycoside linkages. Their amount would be at the level similar to that of ester linkages of 4-*O*-Me-GlcA at the γ-position.

Immediately after secretion, xylan can interact with cellulose in the periplasmic space outside the plasma membrane. However, the intensity of antixylan antibody labelling of S-layers distant from the plasma membrane increases with time, suggesting that additional xylans are inserted into layers of the secondary cell wall deposited earlier or that the xylan epitopes are revealed during cell wall maturation (Awano et al. 1998, 2000; Ruel et al. 2006). Based on several lines of evidence it has been proposed that two fractions of xylan co-exist: the first is the low substituted xylan bound to cellulose fibrils in the course of their formation and coating the microfibrils as a thin layer, and the second fraction comprises more substituted xylan that interacts with lignin (Reis and Vian 2004; Dammström et al. 2009). Upon delignification a fraction of xylan (presumably the second fraction) forms globular structures, which can be destroyed with xylanase and are seen easily on electron micrographs (Awano et al. 2002). Similar globular structures of nanometer size, which are a result of self-association of xylan molecules, are formed in vitro in the presence of bacterial cellulose (Linder et al. 2003).

It has been observed that the distribution and/or structure of xylan in different S-layers are not uniform (Vian et al. 1992; Awano et al. 1998, 2000; Ruel et al. 2006; Bowling and Vaughn 2008). The antibodies raised against the xylan, and recognizing mainly unbranched backbone of xylan, revealed variable epitopes distributions. Preferential labelling in the S_1- and S_3-layers was observed in poplar fibres (Ruel et al. 2006), but uniform labelling was also reported in this species (Koutaniemi et al. 2012), and the labelling primarily in the S_2-layer was reported in Japanese beech (*Fagus crenata* Blume) fibres (Awano et al. 1998, 2000). Antibodies LM10 and LM11 were raised against penta-1,4-xyloside but LM10 is considered to bind to unsubstituted xylan, whereas LM11 binds to weakly substituted xylan (McCartney et al. 2005). In aspen fibres, LM10 signals were more intense in the outer S layers, whereas LM11 labelled all S-layers uniformly (Kim et al. 2012b). Recently, the UX antibody specific to the glucuronate side chain on xylan molecule has been developed (Koutaniemi et al. 2012). Poplar fibres had inner S-layer highly reactive to this antibody. Following the NaOH treatment that

removes ester groups including acetyl esters from xylan, the UX labelling became uniform, indicating that the acetylation of xylan or other esterification might differ within the secondary wall. However, birch fibres did not exhibit such labelling pattern. Clearly, much needs to be still learnt on the xylan recognition in wood fibres using these probes.

Changes in enzymatic activities of nucleoside phosphate interconverting enzymes show that transition from primary cell wall to S-layer formation is coupled with activation of xylan synthesis and depression of the formation of pectins (Dalessandro and Northcote 1977). Several genes responsible for the formation of substrates of cell wall polysaccharide synthesis are activated including genes encoding UDP-Glc-dehydrogenase, UDP-Xyl-synthase, and UDP-Glc-pyrophosphorylase (reviewed by Mellerowicz et al. 2001; Hertzberg et al. 2001; Milioni et al. 2002; Meng et al. 2007). Also several genes directly involved in xylan biosynthesis show a sharp upregulation at the transition from primary to secondary wall biosynthesis in *Populus* (Mellerowicz and Sundberg 2008) and are probably regulated by the master switches of the secondary wall program (Winzell et al. 2010), as found in *Arabidopsis* (Lee et al. 2011).

Recently, the example of glucuronoxylan has evoked discussions on the mechanism of cell wall polysaccharide synthesis (York and O'Neill 2008). The stimulus for such discussions was the presence of the specific oligomer →4)-β-D-Xyl*p*-(1→3)-α-L-Rha*p*-(1→2)-α-D-GalA*p*-(1→4)-D-Xyl at the reducing end of glucuronoxylan molecule in various plant species, including birch (*Betula verrucosa* Ehrh.), spruce [*Picea abies* (L.) Karst.] and *Arabidopsis* (Johansson and Samuelson 1977; Andersson et al. 1983). This reducing end sequence was suggested to be involved in controlling the DP of dicot glucuronoxylans (York and O'Neill 2008). However, this sequence is absent in grass arabinoxylans, including the ones from the secondary cell wall (Kulkarni et al. 2012).

Glucuronoxylan content in total cell wall dry mass is reduced in TW as compared to NW (Baba et al. 2009; Hedenström et al. 2009; Foston et al. 2011) (Tables 3.4 and 3.5). This effect is due to a lower proportion of S layers in the total cell wall mass in TW. TEM study in aspen indicated almost identical labelling of xylan in the S-layers of G-fibres compared to S-fibres of NW and OW with LM10 (Kim and Daniel 2012).

It has not yet been fully investigated whether the details of the polysaccharide structure, such as the degree of backbone branching, composition of side chains, and presence of acetyl or methyl groups are the same in S-layers of G-fibres, S-fibres of TW and S-fibres of NW or OW. In a study comparing xylans extracted by 1 % KOH from cell walls of TW, OW and side wood of Japanese beech all the extracted xylans were similar: all had molecular masses in the range 20–22 kDa as estimated from gel-filtration, and their methylation analysis and ^{13}C NMR study confirmed the same monosaccharide composition, type of linkages, anomeric configuration and position of *O*-glycosylation (Azuma et al. 1983). Recent 2D NMR study revealed significant increase in the C2-acetylated xylopyranoside:C3-acetylated xylopyranoside ratio in TW and OW compared to NW (Foston et al. 2011).

Table 3.4 Neutral monosaccharide composition (% of total neutral sugars) of tension and opposite woods of *Fagus crenata* Blume (Fujii et al. 1982)

Sample	Rha	Ara	Gal	Glc	Xyl	Man
Tension wood	1.1	1.7	2.3	71.7	21.8	1.5
Opposite wood	1.0	1.1	1.5	65.5	26.2	4.8

Table 3.5 Composition of tension and normal wood in poplar (mg/100 mg wood) (Baba et al. 2009)

Component		Tension wood	Normal wood
Cellulose		53.9	42.5
Lignin		14.5	19.4
Hemicellulose		29.8	36.4
Fucosyl	terminal	0.5	0.6
Arabinosyl	terminal	0.6	1.3
Arabinosyl	5-Linked	1.2	2.2
Xylosyl	terminal	3.6	3.6
Xylosy	2- or 4-Linked	12.1	20.2
Glucosyl	4-Linked	2.5	1.2
Glucosyl	4,6-Linked	3.8	0.6
Galactosyl	terminal	0.5	1.2
Galactosyl	2-Linked	2.3	2.2
Galactosyl	4-Linked	1.2	1.2
Galactosyl	3,6-Linked	0.9	0.7
Mannosyl	4-Linked	0.6	1.2

It has been suggested that xylans in S-layers of TW have a different orientation of molecules relative to cellulose microfibrils: polarization FTIR microspectroscopy revealed that in tension wood absorption peaks normally assigned to xylan exhibited a 90° change in the orientation dependence of the vibrations as compared with normal wood (Olsson et al. 2011). However, the authors mentioned that the signals in TW could be due to some other pentose present in G-layer and not the xylose from S-layer.

A less abundant component in S-layers of cell wall in dicotyledons is glucomannan, which is the predominant hemicellulose in softwoods. This polymer is composed of a mixture of oligomers of [→4)-β-D-Man*p*-(1→] and [→4)-β-D-Glc*p*-(1→] in a molar ratio of 2:1 (Mellerowicz et al. 2001). The polymer is acetylated: acetyl residues are attached at the C-2 or C-3 position of randomly distributed mannose residues with a total degree of polysaccharide acetylation around 0.3 (Teleman et al. 2003). Genes encoding mannan synthase (CSLA3) and glucomannan synthase [CSLA1 (also known as GT2A)] have been identified in *Populus* (Suzuki et al. 2006). The CSLA1/GT2A is highly upregulated during the transition from primary to secondary walls (Geisler-Lee et al. 2006).

The amount of mannose per cell wall dry mass is two to three times lower in TW than in NW (Fujii et al. 1982) (Table 3.4), this mannose comes from mannan as indicated by linkage analysis (Baba et al. 2009) and an NMR study (Hedenström

et al. 2009) (Table 3.5). Part of this effect, as is the case for xylan, originates from the development of highly cellulosic G-layer. However, immunolabelling with several antibodies in aspen also showed significant decrease of glucomannan signals in the S-layers of TW as compared to OW or NW fibres (Kim and Daniel 2012). This was specifically observed in fibres, while labelling of vessels was similar in TW, NW and OW (one of the limited examples demonstrating cell type-specific differences in S-layers of TW and NW). Data from transcript profiling also indicated decrease of carbon-flux to mannans in TW (Andersson-Gunnerås et al. 2006).

3.2.4.3 Non-cellulosic Polysaccharides of G-Layers Are Different from Those of S-Layers

Although bulk analyses of TW, OW and NW have revealed rather small (but consistent) changes in the neutral sugar composition between TW having G-fibres and OW or NW having S-fibres (for example, see Tables 3.4 and 3.5), the data obtained by a variety of approaches indicate that the types of non-cellulosic constituents of G-layers are different from those of S-layers.

Xylan, the main non-cellulosic polysaccharide of the S-layers, is absent in the G-layers of TW fibres. This absence has been demonstrated in several plant species by immunocytochemical analysis of wood sections: antixylan antibodies, which label S-layers heavily, did not bind to G-layers (Bowling and Vaughn 2008; Decou et al. 2009). However, xylose is known to be present in G-layers isolated from poplar tension wood (Table 3.6; Nishikubo et al. 2007; Kaku et al. 2009), but its content is considerably lower than in the S-layers where it constitutes usually between 20 and 25 % of cell wall dry mass. Linkage analysis (Table 3.7) is not able to distinguish between 2- and 4-linked xylose in order to differentiate between XG- and xylan-derived xylose, respectively, but it has revealed a set of other linkages characteristic for XG, the presence of which has been confirmed by a number of other approaches (Nishikubo et al. 2007). Consistent with these data, a sharp decline in xylan biosynthesis and processing during TW development in association with G-layer initiation is suggested by changes in transcriptome as will be discussed in Sect. 3.2.5.

Moreover, in an extensive study, with a wide set of antibodies raised to non-cellulosic polymers, performed by Bowling and Vaughn (2008) on fibres of tension wood in sweetgum (*Liquidambar styraciflua* L.; Hamamelidaceae) and taxonomically distinct hackberry (*Celtis occidentalis* L. Ulmaceae) none of the antibodies labelled both the S- and the G-layers. The polysaccharide components of these layers appeared to be completely different. One exception to this rule is probably mannan. Though Man and 1,4-β-mannan amounts are approximately reduced by a factor of 2–3 in TW with G-fibres compared to NW or OW with S-fibres (Tables 3.4 and 3.5), mannose was detected among the monomers of polysaccharides present in isolated G-layers (Furuya et al. 1970; Nishikubo et al. 2007) (Table 3.6) and the linkage analysis detected the (1→4)-type of linkage

Table 3.6 Neutral monosaccharide composition of isolated G-layers in *Populus* spp.

Species	Fuc	Rha	Ara	Gal	Glc	Xyl	Man	Unknown	References
P. euroamericana 2-year-old (%)			0.6	8.7	75.6	14.2	0.5	0.4	Furuya et al. (1970)
P. euroamericana 12-year-old (%)			0.2	7.2	71.3	18.9	3.1	0.2	Furuya et al. (1970)
P. alba (mol%)	1.3	0.6	0.7	1.1	88.6	5.6	2.1		Nishikubo et al. (2007)

Table 3.7 Types of linkages present in neutral monosaccharides of cell wall polymers in isolated G-layers (Nishikubo et al. 2007)

Sugar residue	Proportion in polysaccharides of G-layer (mol%)	Potential components
Xylosyl terminal	1.2	Xyloglucan
Xylosyl 2- or 4-linked	3.0	Xyloglucan, xylan
Fucosyl terminal	0.5	Xyloglucan
Glucosyl terminal	1.9	Cellulose, xyloglucan, glucomannan
Glucosyl 4-linked	75.6	Cellulose, xyloglucan, glucomannan
Glucosyl 4,6-linked	9.0	Xyloglucan
Galactosyl 2-linked	0.9	Xyloglucan
Galactosyl 3,6-linked	5.5	Arabinogalactan II
Mannosyl 4-linked	2.2	Glucomannan

between Man residues (Nishikubo et al. 2007) (Table 3.7). Glucomannan was also demonstrated to be present in G-layers by immunolabelling, though its intensity was rather low (Kim and Daniel 2012).

Analyses in poplar suggested that the polymers of TW (XG, RG I or mannan) must be heavily acetylesterified because the acetyl level was not reduced in TW compared to NW in spite of the reduction of glucuronoacetoxylan in the G-layers (Gou et al. 2008).

3.2.4.4 Content and Monosaccharide Composition of Non-cellulosic Cell Wall Polysaccharides in Tension Wood G-Layers

Ever since the publication of the monosaccharide composition of isolated G-layers of aspen (*P. tremula* L.) having 98.5 % glucose and 1.4 % xylose, based on quantitative paper chromatography (Norberg and Meier 1966), G-layers have been considered to be purely cellulosic. This idea has persisted despite the report of Furuya et al. (1970) who found xylose, mannose, galactose and arabinose, in

addition to 75 mol% of glucose, after complete hydrolysis of G-layers of *Populus euroamericana*. Galacturonic acid residues detected after pectinase treatment, ruthenium red staining, and IR spectra indicated the presence of pectic substances in G-layers (Wardrop and Dadswell 1948; Furuya et al. 1970; Scurfield 1973). Several authors have reported that TW contains two to four times more galactose than NW. This has been found in *Eucalyptus goniocalyx* (7.5 % of Gal as compared to 2.5 %) (Schwerin 1958), *Betula pubescens* (11.6 % versus 2.6 %), *B. verrucosa* (8.0 % versus 2.3 %) (Gustafsson et al. 1952) and *F. sylvatica* (4.9 % versus 1.3 %) (Meier 1962a, b). The content of galactose in *F. sylvatica* has also been suggested as an indicator of the G-fibre content (Ruel and Barnoud 1978). This galactose comes from the galactan which is unique among wood polysaccharides in its structural complexity and high degree of branching (Meier 1962a, b; Kuo and Timell 1969; Azuma et al. 1983) as will be discussed in Sect. 3.2.4.7. However, several decades after publication of this work, the notion that G-layers are virtually pure cellulose was so widely held that one of the papers which recently described the presence of other cell wall polysaccharides was titled "Gelatinous fibres contain more than just cellulose" (Bowling and Vaughn 2008).

Recent investigations using *Populus* showed that matrix polysaccharides account for approximately 20 % of the G-layer dry weight (Kaku et al. 2009; Nishikubo et al. 2007; Table 3.1). The remaining constituents are 78 % cellulose and around 3 % protein; lignin has not been detected (Kaku et al. 2009). This is very different from NW containing S-fibres (CML and S-layers), which in various species contain around 50 % cellulose, 30 % hemicelluloses (including 25 % xylan and 5 % glucomannan) and 20 % lignin (Timell 1967; Mellerowicz et al. 2001; Awano et al. 2002).

3.2.4.5 Individual Polysaccharides of the G-Layer: Xyloglucan

The most complete data describing an individual polysaccharide within the G-layer have been obtained for xyloglucan (XG). Its presence in the G-layer has been proved by (1) biochemical analysis of isolated G-layers, (2) labelling with corresponding antibodies and (3) the presence of active xyloglucan-endo-transglycosylase (XET) enzyme, specific for XG (Nishikubo et al. 2007; Mellerowicz et al. 2008; Baba et al. 2009; Kaku et al. 2009).

Linkage analysis of isolated G-layers has revealed that the most abundant component besides 4-Glc was 4,6-Glc, which among cell wall polymers is characteristic for XG. All other components of fucosylated XG, including *t*-fucose, *t*-xylose, 2-galactose and 2-xylose (though not distinguished from 4-xylose), were present (Table 3.7). The proportion of sugars indicates that XG is by far the major non-cellulosic component of G-layers in *P. alba*, comprising 10–15 % of G-layer dry mass (Nishikubo et al. 2007; Mellerowicz et al. 2008; Kaku et al. 2009).

Further evidence for the presence of XG in G-layers of tension wood fibres has been obtained by the analysis of XET activity; an enzyme, which requires both an XG donor and an XG [or xylogluco-oligosaccharide (XGO)] acceptor to form a

glycosyl linkage at a new position (Fry et al. 1992). Labelling of developing G-layers after incubation of tension wood sections with sulforhodamine-labelled XGO acceptor has indicated the presence of both active enzyme and donor molecules of XG (Nishikubo et al. 2007). G-fibres have also been labelled at maturity (but with a different pattern as explained below), whereas adjacent ray parenchyma and vessel elements, and even S-type fibres, did not show any label incorporation into the cell wall. The presence of XET in developing G-layers has been confirmed using the polyclonal antibody XET16A and genes encoding several XET isoforms are upregulated at this stage (Nishikubo et al. 2007). The monoclonal antibody CCRC-M1 that binds the fucose side chains of xyloglucan has revealed this polymer to be present within developing G-layers.

The distribution of the label within cell wall layers for the xyloglucan itself (CCRC-M1), for XET protein (XET16A antibody) and for XET activity (labelled substrates) has revealed some discrepancies in G-fibres of tension wood, which lead to interesting conclusions. Within the G-layer, both in poplar and in sweetgum, CCRC-M1 detects xyloglucan mainly at the innermost edge of the G-layer, which contains the most recently deposited material, while in mature fibres labelling was present only in the CML, as in normal wood (Nishikubo et al. 2007; Bowling and Vaughn 2008; Baba et al. 2009). The presence of xyloglucan in mature G-layers has been demonstrated after their isolation and biochemical analysis (Nishikubo et al. 2007; Baba et al. 2009).

The XET16A antibody could detect signals also from entire mature G-layers indicating the presence of the XET enzyme. Consistently, XET protein has been identified among G-layer proteins (Kaku et al. 2009; Baba et al. 2009). XET activity, determined with labelled XGO, shifts from developing G-layers in differentiating G-fibres into the directly adjacent S_2-G boundary in mature G-fibres. There, label incorporation activity could be detected several years after G-layer formation even though the fibre cells were dead (Nishikubo et al. 2007).

These discrepancies are explained by the disappearance of accessible XG in the G-layer during the course of its maturation. Evidence that active XETs are present not only at the periphery but also within the mature G-layers, and that the accessible XG donor is no longer present, comes from tests with labelled long chain XG (Baba et al. 2009). With this substrate it has been possible to detect the incorporation of the label into mature G-layers even though fluorescently labelled heptasaccharide XGO was not incorporated. Since XG but not XGO could serve both as a donor and an acceptor (Saura-Valls et al. 2006), this result has confirmed the presence of XET activity and the lack of an accessible XG donor in mature G-layers, consistent with XET16A antibody labelling. Thus, XG becomes inaccessible to antibodies and enzymes during the course of G-layer maturation. Future research needs to work out the mechanisms leading to this interesting fact.

3.2.4.6   Individual Polysaccharides of the G-Layer: Arabinogalactan Protein

Arabinogalactan proteins (AGPs) belong to cell wall components with highly variable structure. The backbone is made of protein, while up to 95 % of the molecule can consist of carbohydrate, which is sometimes referred to as arabinogalactan II. This is because the glycan part of AGPs has chains of [→3]-β-D-Galp-(1→] and [→6]-β-D-Galp-(1→] units, which often have terminal arabinose residues and are connected to each other by (1→3, 1→6)-linked branch points, the presence of which is indicative for AGPs. Another characteristic of all AGPs is their ability to bind the Yariv reagent, a β-D-Glc derivative of phloroglucinol (Carpita and McCann 2000).

The protein backbones of AGPs can be of diverse structure and are subdivided into several classes (Gaspar et al. 2001). Some of them contain domain(s) similar to the protein of *Drosophila* fasciclin and such AGPs are designated fasciclin-like (FLA). At the C terminus some AGPs have covalently attached glycosylphosphatidyl inositides (called GPI anchors), which may anchor the polymer in the plasma membrane and can later be cleaved off (Carpita and McCann 2000). AGPs are highly water soluble although they are sometimes tightly fixed within the cell wall, indicating covalent linkage with other cell wall constituents. AGPs are present in all types of plant tissues at all stages of their development, but no functional role for any AGP has yet been elucidated.

The presence of AGPs in G-layers has been demonstrated by monosaccharide linkage analysis in isolated G-layers (Nishikubo et al. 2007; Kaku et al. 2009) and immunochemically by labelling with antibodies (Lafarguette et al. 2004; Bowling and Vaughn 2008) in all species investigated so far. The 3,6-linked galactosyl residues characteristic of AGPs have been determined biochemically in isolated G-layers in poplar (Nishikubo et al. 2007; Kaku et al. 2009). The content of this polymer in isolated G-layers is estimated to be around 2 % (Mellerowicz et al. 2008) based on data shown in Table 3.7 but this value might be an underestimate due to the high water-solubility of AGPs. Rocket electrophoresis in agarose gels containing β-glycosyl Yariv reagent has demonstrated that AGPs accumulate in poplar TW (Lafarguette et al. 2004). Western blotting of these proteins probed with the monoclonal antibody JIM14 has revealed polypeptides with apparent molecular masses of 100 and 200 kDa. They were present in both TW and OW but appeared much more abundant in TW. Gene expression analyses have led to the identification of classes of highly expressed AGPs that are specifically and very strongly upregulated during TW formation in poplar (Déjardin et al. 2004; Lafarguette et al. 2004; Andersson-Gunnerås et al. 2006) and in *Eucalyptus* (Qiu et al. 2008).

The distribution of AGPs has been studied by labelling with JIM14 antibody in sweetgum and hackberry (Bowling and Vaughn 2008) and in hybrid poplar (*P. tremula* × *P. alba*) (Lafarguette et al. 2004). This has revealed AGPs throughout the entire G-layer but the highest concentration is in the outer part of the G-layer
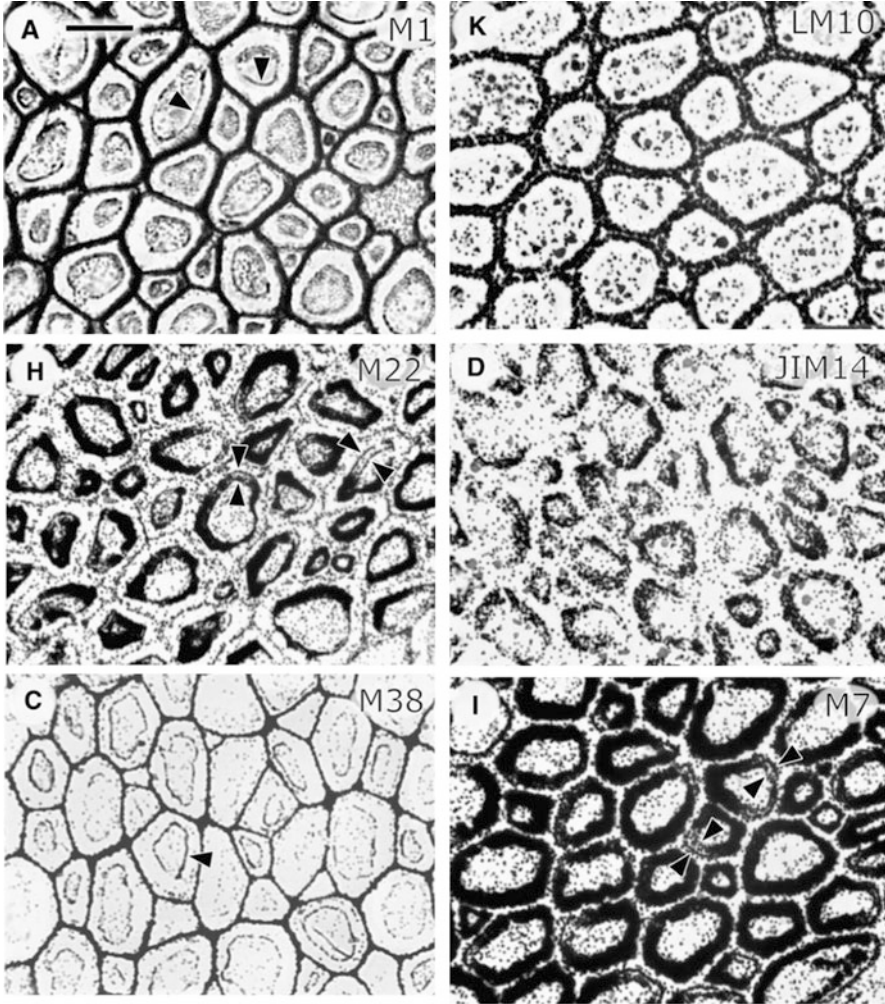
Fig. 3.4 Immunoprofiling of G-layers in sweetgum (*Liquidambar styraciflua*) with monoclonal antibodies recognizing various epitopes. *M1* xyloglucan, *LM10* xylan, *M22* RG I, *JIM14* AGPs and RG I, *M38* mucilage, *M7* RG I. Reprinted from Bowling and Vaughn (2008) with permission

in sweetgum and hackberry (Fig. 3.4) (Bowling and Vaughn 2008) or conversely, mainly in the inner part in hybrid poplar (Lafarguette et al. 2004). The latter result was obtained using secondary antibodies labelled with 15 nm gold and could reflect differences in accessibility of AGP epitopes within the layer for such a large antibody-gold complex. Though the genes coding for the protein backbone of tension wood AGPs are well characterized, nobody has analysed the structural details of their glycan part.

3.2.4.7 Individual Polysaccharides of the G-Layer: Rhamnogalacturonan I

An indication of the presence of some acidic polymers in G-layers comes from simple histochemical staining, which was first demonstrated by Wardrop and Dadswell (1948), and later reported in several hardwood species (Furuya et al. 1970; Scurfield 1973; Bowling and Vaughn 2008). G-layers of tension wood fibres are stained pink-red by Toluidine blue O, or vivid-red with ruthenium red, which reveal the presence of acidic polysaccharides (Krishnamurthy 1999). Application of specific antibodies (JIM5, JIM7) did not reveal any homogalacturonan in G-layers (Bowling and Vaughn 2008). However, antibodies to the rhamnogalacturonan I (RG I) backbone (CCRC-M10), and to de-arabinosylated RG I (CCRC-M22), were found to be heavily bound to G-layers in TW fibres of two unrelated plant species, sweetgum and hackberry (Bowling and Vaughn 2008), and indicated the presence of RG I in the G-layer. So far only one study (in poplar) has shown the presence of rhamnose in isolated G-layers (Nishikubo et al. 2007) and no galacturonic acid was reported. According to the monosaccharide analysis, rhamnose constitutes 0.2 % of total cell wall sugars. However, a member of the PL4 family of RG I lyases has been reported as highly upregulated during TW formation (Andersson-Gunnerås et al. 2006), indicating that the substrate for this enzyme must be present in TW.

G-layers have also been labelled with antibodies specific for neutral polysaccharides such as galactan or arabinogalactan, which are usually present as side chains attached to the RG I backbone, β-(1→4)-galactan was found with LM5 antibody in hybrid poplar (*Populus trichocarpa* × *P. koreana*) (Arend 2008). Labelling with CCRC-M7, which binds to RG I/AG/AGP, has indicated the possibility of arabinogalactan attached to the rhamnogalacturonan I backbone in sweetgum and hackberry (Fig. 3.4), but no LM5 labelling has been detected in these species (Bowling and Vaughn 2008).

Galactans with a complicated structure have long been considered among the polysaccharides peculiar to tension wood. It was reported that TW of American beech (*Fagus grandifolia* Ehrl.) contains 6.0 % galactan compared with only 1.6 % for NW. The galactan from TW was isolated and characterized (Kuo and Timell 1969) and was and is still considered to be unique among structural wood polysaccharides, both in its complexity and its high degree of branching, being composed of β-(1→4)- and β-(1→6)-galactopyranosidic linkages (Meier 1962a, b; Kuo and Timell 1969; Azuma et al. 1983). The tension wood fraction enriched in galactan contained also galacturonic acid and rhamnose, with the disaccharide 2-*O*-α-D-GalA*p*-L-Rha being identified and indicating RG I. However, it was not possible to determine whether the fraction contained an individual polysaccharide or a mixture of several (Meier 1962a, b). In Japanese beech one of the fractions of cell wall alkaline extracts separated on Sepharose 4B column was enriched in galactose and rhamnose which amounted, correspondingly, to 33.9 and 9.1 % of the fraction in tension wood, as compared to 9.5 and 6.5 % in normal wood; the

yield of the fractions was similar (Azuma et al. 1983). Later analyses on isolated G-layers from poplar found no evidence of 4-linked galactose (Nishikubo et al. 2007; Kaku et al. 2009), which might be due to technical problems with its identification. The possibility that the pectic molecules were lost during isolation of G-layers was suggested (Bowling and Vaughn 2008).

It should be noted that in G-fibres found in the phloem, which have much in common with TW G-fibres (Gorshkova and Morvan 2006; Gorshkova et al. 2010), galactans with a rhamnogalacturonan I backbone are the major non-cellulosic polysaccharides and they play a prominent role (Gorshkova et al. 2010). However, in tension wood the presence of RG I has been shown only by immunohistochemical analysis (Fig. 3.4).

3.2.5 Reprogramming of Synthesis and Modification of Cell Wall Polysaccharides Upon the Induction of Tension Wood

Formation of TW can be induced by inclining or bending the tree. This provides a nice model system, which has been used widely to study the process at molecular level, including using transcriptomics (Pilate et al. 2004a, b; Paux et al. 2005; Andersson-Gunnerås et al. 2006; Qiu et al. 2008), metabolomics (Andersson-Gunnerås et al. 2006) and proteomics (Baba et al. 2000; Plomion et al. 2003; Kaku et al. 2009). These approaches have become especially effective in conjunction with sequencing programs in woody species, including *Populus* and *Eucalyptus*. However, only a few genes involved in cell wall polysaccharide synthesis during the transition from NW to TW have so far been characterized. This can be explained partly by poor understanding of glycosyltransferase genes involved in the synthesis of cell wall polysaccharides (Perrin et al. 2001). Another reason is the low abundance of mRNAs for such enzymes, even if the synthesis of the corresponding polysaccharide proceeds intensively (Dhugga 2005). As a consequence, some important genes for the process could have been omitted in the microarrays. Nevertheless, preliminary surveys have led to the identification of some important pathways and regulons involved in TW formation.

The reprogramming of cell wall biosynthetic machinery during TW induction has been followed in species having TW with G-layers, primarily in *Populus* and *E. globulus* (Pilate et al. 2004a, b; Paux et al. 2005; Andersson-Gunnerås et al. 2006; Jin et al. 2011), as well as in *E. nitens*, which only occasionally forms G-fibres (Qiu et al. 2008). The comparison between these two systems has provided some interesting insights.

First, in both types of TW global gene expression analyses have provided a clear indication of an increased carbon sink during TW formation. In *Populus*, it is exemplified by a high expression and upregulation of genes encoding key sucrose metabolism enzymes such as sucrose synthase (SUS) and UDP-glucose

pyrophosphorylase (UGP) (Déjardin et al. 2004; Andersson-Gunnerås et al. 2006). This upregulation may be related specifically to an increased flow of carbon to cellulose biosynthesis. The overexpression of genes encoding these enzymes, including cotton SUS (Coleman et al. 2009) or microbial UGP (Coleman et al. 2007) in poplar has led to increased wood cellulose content in transgenic plants by 5–6 % as compared to the wild type (WT). SUS provides UDP-glucose for the cell wall carbohydrate biosynthetic machinery. A plasma membrane (or particulate) associated SUS isoform has been postulated to be metabolically coupled to the cellulose biosynthetic complex (Haigler et al. 2001). The poplar genome contains 11 SUS genes (Geisler-Lee et al. 2006) and transcripts of two of them, *SUS1* and *SUS2*, are upregulated in developing TW in comparison with NW and are among the most abundant transcripts (Andersson-Gunnerås et al. 2006). These genes are also highly expressed during NW formation and thus are probably involved in cellulose biosynthesis both in NW and TW.

Of the two annotated *Populus* UGP genes with tissue-specific expression patterns (Meng et al. 2007), *UGP2* transcripts have been shown to be induced in developing TW (Andersson-Gunnerås et al. 2006). UGP is a key regulator of UDP-glucose in the plant cell. It has been proposed that it is involved in recycling of fructose produced by SUS during production of UDP-glucose from sucrose. However, knocking down the two UGP genes that resulted in only 15 % of WT UGPase activity in *Arabidopsis* did not affect the cellulose content (Meng et al. 2009), indicating other routes for fructose recycling.

Another enzyme postulated to be involved in fructose recycling during cellulose biosynthesis is fructokinase (Andersson-Gunnerås et al. 2006). This enzyme converts fructose to fructose-1-phosphate that can be further metabolized in the cytosol or in plastids. Three fructokinase genes have been shown to be highly upregulated during TW development indicating their importance for cellulose biosynthesis (Pilate et al. 2004a, b; Andersson-Gunnerås et al. 2006). One of them, FRK2, has been shown to be essential for cellulose synthesis in aspen wood (Roach et al. 2012).

Compared to the above carbon-flux related genes, only relatively minor changes have been noted in genes encoding the cellulose biosynthetic complex during TW formation in a microarray analysis (Andersson-Gunnerås et al. 2006). Two of the 17 *Populus CesA* genes, *CesA1-A* and *CesA8-B* (*Populus* gene nomenclature is normalized to *Arabidopsis* genes as proposed by Kumar et al. 2009), have been suggested to be upregulated while one, *CesA4*, downregulated in TW. Quantitative RT-PCR revealed small upregulation of *CesA8-A*, *CesA8-B*, and *CesA7-A*, and downregulation of *CesA6-A*, *CesA6-C*, and *CesA1-B* in TW (Djerbi et al. 2004). Based on extensive evidence from *Arabidopsis*, the cellulose synthase complex has been proposed to contain three different CesA isoforms encoded by either "primary wall CesA genes" i.e. *CesA1*, *3* or *6*-related, or "secondary wall CesA genes" comprising *CesA4*, *7*, and *8*. Therefore, a coordinated regulation of the corresponding triplets is expected. The observed changes in TW are difficult to reconcile with the proposed rosette models; they suggest a need for closer analysis of rosette complexes at the protein level. In contrast to *Populus*, in *E. nitens*, which

rarely forms G-fibres in TW, an upregulation of a secondary wall type related to *Arabidopsis CesA4* (*EgCesA2*) has been observed by microarray analysis (Qiu et al. 2008). This has been observed also in *Eucalyptus grandis*, in which the expression of types related to *AtCesA4*, and *8* (*EgCesA2* and *EgCesA3*, respectively) is stimulated in the upper compared to the lower side of inclined stems as shown by in situ hybridization and by promoter-GUS reporter gene expression in tobacco (Lu et al. 2008). Although the formation of G-fibres was not reported in that study, the comparison between *Eucalyptus* species that are known to form largely S-fibres in TW (Washusen et al. 2005) and *Populus* which forms G-fibres, suggests that biosynthesis of cellulose in the G-layer may involve a special type of rosette.

The *Populus* orthologue of *KORRIGAN1*, *Cel9A1* (Takahashi et al. 2009), encoding a membrane-bound cellulase, is highly expressed but not unregulated in TW compared to NW (Geisler-Lee et al. 2006; Andersson-Gunnerås et al. 2006; Bhandari et al. 2006). This cellulase is necessary for cellulose biosynthesis in primary and secondary walls but it negatively affects wood cellulose crystallinity (Takahashi et al. 2009; Maloney and Mansfield 2010). Therefore, a lack of its induction in TW is perhaps not surprising.

Cellulose-synthesizing rosette complexes move in an organized fashion along cortical microtubules (MTs) and are thought to be influenced by and to influence the MT network. In developing TW, cortical microtubules assume an axial orientation parallel to axial cellulose microfibrils (Prodhan et al. 1995; Chaffey et al. 2002). Significant changes in several genes encoding both microtubule sub-units, alpha and beta, and in key microtubule-organizing network proteins, including different microtubule associated proteins and kinesin motor proteins, have been observed in developing TW as compared with NW (Andersson-Gunnerås et al. 2006) or OW (Paux et al. 2005). These changes may be related to a high rate of cell division activity and a high rate of cellulose formation, as well as to the establishment and maintenance of the axial MT orientation in TW. In *Eucalyptus* spp., the beta tubulin *TUB1* gene has been found to be upregulated in TW and downregulated in OW as compared to NW, regardless of the presence or absence of G-fibres (Spokevicius et al. 2007; Qiu et al. 2008). Thus, high *TUB1* expression is correlated with low MFA. Moreover, in fibre sectors stably transformed with the *EgrTUB1* gene driven by the 35S CMV promoter in *E. globulus*, which supposedly had decreased *TUB1* expression via a co-suppression effect, a higher MFA has been observed (Spokevicius et al. 2007). This demonstrates that modification of the MT network affects the cellulose deposition pattern in wood. Moreover, the upregulation of a kinesin motor protein FRA1 in *Arabidopsis* has been shown to increase the number of secondary cell wall layers and axial cellulose MFA in stem fibres (Zhou et al. 2007), demonstrating the importance of the MT network organization for cellulose formation in secondary walls.

The most dramatic response in the transcriptome associated with TW induction in both types of TW, with or without G-layers, is the induction of AGPs (Déjardin et al. 2004; Lafarguette et al. 2004; Paux et al. 2005; Andersson-Gunnerås et al. 2006; Qiu et al. 2008). The AGPs induced in tension wood belong to the subgroup A of fasciclin-type AGPs. The involvement of this class of proteins in

secondary wall cellulose biosynthesis has been postulated based on the co-expression of several genes from this subgroup with secondary wall CesA genes. The AGPs of subgroup A have an N-terminal secretion signal and a fasciclin domain flanked by two AGP domains rich in Ala, Pro, Ser and Thr (Lafarguette et al. 2004). The phylogenetic analysis showed that *Arabidopsis* genes *AtFLA7*, *AtFLA11* and *AtFLA13* had two putative poplar orthologs, whereas *AtFLA12* had as many as 22 similar genes in poplar that were subsequently renamed as poplar *FLA12A-FLA12V* (Andersson-Gunnerås et al. 2006). Interestingly, all poplar *FLA12* genes that are upregulated in TW show also high expression in NW after the main period of secondary wall differentiation, whereas the *FLA12* genes that are not induced in TW are expressed in NW during the main secondary wall formation phase (Andersson-Gunnerås et al. 2006). This expression pattern suggests that the two groups of *FLA12* genes have distinct functions, the first one in fibre maturation and G-layer differentiation, and the second one in S-layer biosynthesis. It is interesting that the *Eucalyptus FLA12* genes, *EgrFLA1* and *EgrFLA2*, strongly upregulated during TW S-fibre formation in *E. nitens*, are more closely related to the second group. It is therefore conceivable that *FLA12* family in poplar evolved and diversified in adaptation to G-layer formation.

The transcriptome and metabolome analyses have suggested decreased activity of the pathway for C flux through guanosine 5′-diphosphate (GDP) sugars to mannans in TW as compared to NW. Gene encoding GDP-mannose-pyrophosphorylase (an essential enzyme for the biosynthesis of GDP-mannose) has been reported downregulated in *Populus* tension wood (Andersson-Gunnerås et al. 2006). Fourfold decreased abundance of mRNA for mannan synthase as compared to NW control was also observed in that study, suggesting that genes in the mannan biosynthesis pathway have been co-ordinately downregulated in TW. Similarly, there were significant decreases in transcripts encoding enzymes involved in the formation of nucleotide-sugar precursors for xylan and arabinan: UDP-D-glucuronic acid decarboxylase (UDP-xylaose synthase) and UDP-xylose 4-epimerase, suggesting a decreased C flow to xylan and arabinan. Moreover, a lower xylan biosynthetic activity in TW was suggested by a decrease in several glycosyl transferase genes including *GT8D* (*Arabidopsis IXR8/GAUT12*), *GT8E/D* (*PARVUS/GATL1*) and *GT47C* (*FRA8*), thought to be involved in xylan biosynthesis in poplar (Kong et al. 2009; Lee et al. 2009a, b, c).

Interestingly, upregulation of two closely related GT31 genes putatively encoding galactosyltransferase, and some other glycosyl transferase and glycoside hydrolase genes that have been classified into CAZy families, have been noted in TW but their precise functions have not been determined (Andersson-Gunnerås et al. 2006). These genes might be involved in the biosynthesis and restructuring of G-layer matrix components, RG I, AG II and XG.

Genes for some of the enzymes for post-deposition modifications of secondary cell wall polymers have been shown to be differentially regulated upon tension wood initiation. The best characterized is the activity of xyloglucan-endo-transglycosylase enzyme, specific for XG (Nishikubo et al. 2007; Mellerowicz et al. 2008). In accordance with the importance of XET, the expression of the corresponding

genes increases upon induction of tension wood in the tissue forming G-layers (Nishikubo et al. 2007). Transcription of the *PtaBXL1* gene, encoding β-1,4-xylosidase and involved in xylan modification, is downregulated in poplar tension wood (Decou et al. 2009), while a member of the family PL4 of RG I lyases is highly upregulated (Andersson-Gunnerås et al. 2006). Changes in transcripts encoding pectin/pectate degrading enzymes such as PME, polygalacturonase and pectin/pectate lyase have been observed also with specific genes up- or downregulated, pointing to a change in pectin metabolism in developing TW. It should be borne in mind that these changes could be related to general activity of cell production known to be stimulated in TW and inhibited in OW. Indeed, in *E. nitens* OW, pectate lyases, polygalacturonases and beta tubulin genes have been shown to be downregulated as compared to NW (Qiu et al. 2008). Interestingly, one isoform of PME is highly upregulated in OW, which could indicate that cell walls are being modified in that tissue. The involvement of any of these genes in the process of G-layer formation and maturation needs to be addressed by the reverse genetics approach.

The above discussion of gene expression strongly suggests that there is a coordinated activation of entire arrays of genes encoding specific polymers that build the G-layer during the transition from S- to G-layer biosynthesis. Such transcriptional regulation is known to occur during the P- to S-layer biosynthesis in xylem fibres, where it involves master switches from the NAC transcription factor family, including SND1 and NST1 acting either directly on the genes involved in biosynthesis of different cell wall polymers, or via certain MYB transcription factors (Mitsuda et al. 2005, 2007; Zhong et al. 2006, 2007a, b, 2008, 2010; McCarthy et al. 2009). There is an indication that at the same time as secondary wall program is induced, there could be a downregulation of some genes involved in the primary wall biosynthesis via siRNA (Helda et al. 2011). The regulation of G-layer formation is less understood, but several transcription factors were up- or downregulated in TW transcriptomes as compared to NW or OW transcriptomes (Déjardin et al. 2004; Paux et al. 2005; Andersson-Gunnerås et al. 2006). Some of them potentially could trigger the formation of the G-layer or suppress the formation of the S-layer, but they have not yet been functionally analysed.

3.2.6   Role of Cell Wall Polysaccharides in Tension Stress Generation

There is overwhelming evidence that in TW with G-fibres, it is the G-layer that is the driving force in tensile stress generation (Yamamoto et al. 2005; Fang et al. 2008) but the mechanism of this phenomenon is still unknown. Many different proposals have been considered (discussed in Chap. 5), and presently two hypotheses are considered in the literature, referred to as the radial swelling and the

longitudinal shrinking hypotheses (reviewed by Mellerowicz and Gorshkova 2012). The radial swelling hypothesis suggests that the G-layer swells during maturation exerting an outward pressure on the adjacent S layers that cause their circumferential expansion and longitudinal shrinkage (Burgert and Fratzi 2009) (Fig. 3.5a). Direct support for this idea comes from measurements of strains in TW after enzymatic removal of G-layers (Goswami et al. 2008). When this happens, the remaining S-layers shrink tangentially indicating that the G-layers normally exert a pressure on adjacent S-layers. The thickness of the compound middle lamella is visibly reduced in TW compared to NW which is in line with the high outward radial pressure in TW (Bowling and Vaughn 2008). TW cell wall architecture and G-layer composition have certain other features compatible with this idea. The outer cell walls have high MFA (Sect. 3.2.3.2) causing longitudinal shrinking when tangentially expanded (Goswami et al. 2008). Since G-layers have gel-like properties defined by a paradoxical shrinking behaviour during drying and by high porosity (Clair et al. 2008; Chang et al. 2009), it has been speculated that this structure holds a lot of water. Indeed it contains RG I which has mucilage-like properties, and highly hydrophilic AGPs (Sects. 3.2.4.6 and 3.2.4.7). The existence of hygro-sensible regions within the gelatinous layer has been suggested after analysis of the effects of boiling and drying treatments on the behaviour of TW in *Zelkova serrata* (Abe and Yamamoto 2007). However, it is not certain that the G-layer imbibes water during maturation as postulated by the original swelling hypothesis. The direct measurement of water content in TW and NW kept at the same air humidity indicates a lower water content in TW than NW (Wardrop and Dadswell 1955; Tarmian et al. 2009). Moreover, radial pressure in G-layers seems to develop during drying rather than during imbibition as suggested by the outward expansion of G-layers and their tight appression to the S-layers (Fang et al. 2007).

The longitudinal shrinkage hypothesis postulates that the G-layer itself generates the longitudinal tensional stress that is then transmitted to the outer cell wall layers (reviewed by Mellerowicz et al. 2008). The source of the stress lies within cellulose crystals, and it has been measured as a released lattice strain following the transversal cutting of wood (Clair et al. 2006b). The measured difference in cellobiose spacing within cellulose crystallites, 1.0035 nm versus 1.0033 nm, corresponds to the values recorded macroscopically at the surface of the log implying that the crystallite shrinkage alone could account for the strain of TW. Interestingly, lattice contraction is not observed during TW drying in spite of massive longitudinal macroscopic shrinkage observed during the drying process. In *Z. serrata* longitudinal shrinkage has been observed also during boiling of TW in a wet state but no change in 004 diffraction pattern accompanied the shrinkage (Abe and Yamamoto 2007).

The mechanism of the cellulose lattice shrinkage during maturation is unknown but has been proposed to involve the formation of cellulose aggregates in the G-layer (Mellerowicz et al. 2008). In the absence of large amounts of lignin and xylan, microfibrils of cellulose interact laterally, entrapping some matrix polysaccharides. Cellulose aggregation is a distinctive feature of G-layer cellulose and numerous studies have demonstrated that a strong positive correlation exists
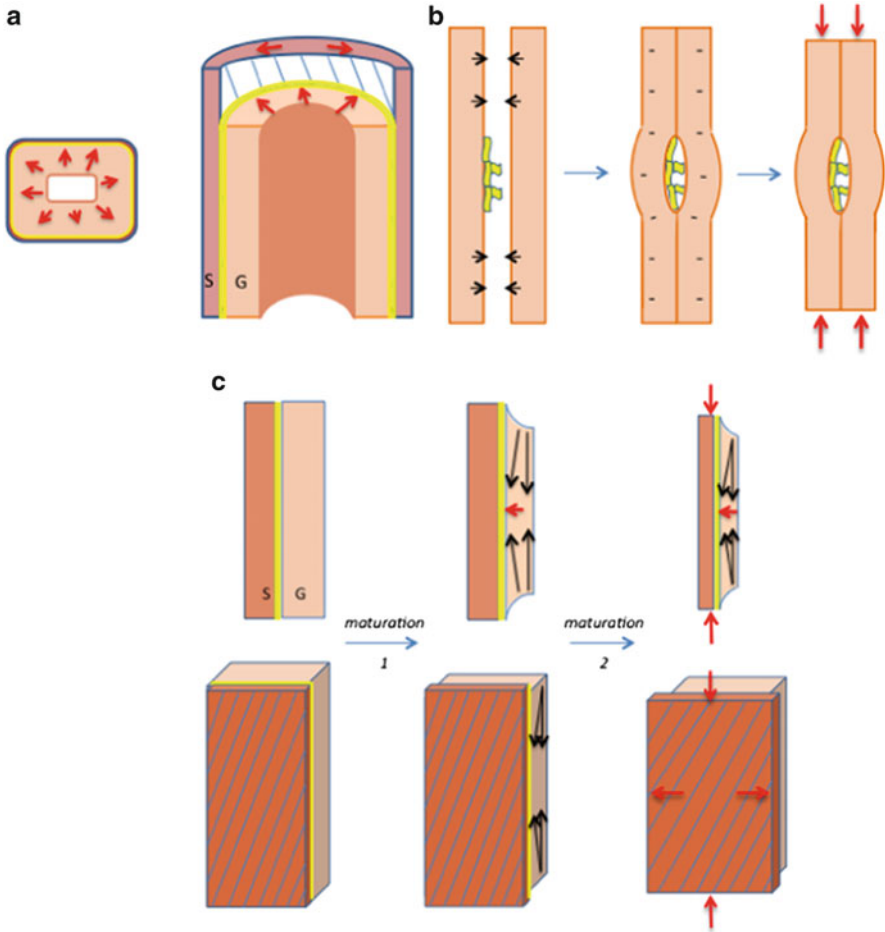
Fig. 3.5 (**a–c**) Mechanisms for tensile stress generation in TW. (**a**) G-layer swelling hypothesis (Burgert and Fratzi 2009). By exerting an outward pressure the G-layer causes the outer cell wall layers to longitudinally shrink due to their high MFA. (**b**) G-layer longitudinal shrinkage hypothesis (Mellerowicz et al. 2008). Tensile stress is generated by crystallization of adjacent microfibrils with a short and bulky polysaccharide entrapped between them, causing tension (marked by -) in the microfibrils, and their subsequent shrinkage upon tension release. XG (*yellow* layer in **a** and **c**) is proposed as cross-linking cellulose fibrils in the G-layer to those in the S-layer. (**c**) Proposed combined mechanism of TW generation by longitudinal shrinkage (*1*), leading to radial pressure (*2*) as an accessory mechanism. The *top row* is a side view and the *bottom row* is a view of the cell wall from outside, showing the S- and G-layers. The longitudinal tensional stress within the G-layer arising by the mechanism B leads to shortening of the G-layer distant from the attachment to the S-layer. This in turn leads to a pressure from G-layer to the adjacent S-layer. This pressure can contribute to S-layer shortening in step (*2*), by mechanism A proposed in the swelling theory. Note that longitudinal shrinking of the G-layer creates an expansive stress in the S-layer (*axial red arrows*) that has been shown after the removal of the G-layer (Goswami et al. 2008). The figure was reprinted from Mellerowicz and Gorshkova (2012) with permission

between aggregate formation and tensional stress as discussed in Sect. 3.2.3.2. Moreover, among several wood parameters measured by SilviScan-2, the average crystallite size, as well as MFA, was most strongly associated with tensional strain (Yang et al. 2006). The high correlation between crystallite size and tensional stress, and low MFA and tensional stress, regardless of the presence of absence of the G-fibres (Yang et al. 2006; Ruelle et al. 2006, 2007b), indicates that the formation of axial cellulose aggregates is crucial for maturation stress development in the wood. The proposed mechanism of maturation stress assumes that short molecules of cellulose-binding glycans, for example XG, become trapped within coalescing microfibrils during maturation, thus creating pockets of anisotropy where the cellulose chains must stretch to account for increased distance around the obstacles (Fig. 3.5b). Thus, the presence of the entrapped polysaccharide during lateral interaction of axially oriented microfibrils causes longitudinal tensile stress in the cellulose (Mellerowicz et al. 2008), which upon release would shorten the macrofibril to accommodate the presence of XG in the pocket, with a conformational change of the lattice as observed experimentally. A similar role has been proposed for specific RG I galactan with complicated conformational arrangements in flax fibres, which also develop a cell wall of gelatinous type. This polymer plays a crucial role in the remodelling of already deposited layers of the gelatinous cell wall (Gorshkova et al. 2010; Roach et al. 2011).

If this model is correct, an intimate contact between the G-layer and adjacent S-layers is required to pass the tensional stress into entire fibre, and finally to the entire tissue. The different cell wall layers contain cellulose fibrils of different orientations and thus cross-linking glycans or lignin are needed to bind these layers together. One candidate for such a binding molecule is XG. Its presence has been documented between primary cell wall and S- layers, S_2- and G-layer and within the G-layer (Bourquin et al. 2002; Nishikubo et al. 2007; Baba et al. 2009; Sandquist et al. 2010). Moreover, a transglycosylating activity associated with XG has been found between the G- and S_2-layers (Nishikubo et al. 2007). Therefore, it has been proposed that XG plays a crucial role in the bending mechanism and this has been confirmed by the observation that a fungal xyloglucanase introduced to poplar stops the stem righting mechanism (Baba et al. 2009). XET activity may repair G–S_2 connections that might have been broken during the shrinking of the G-layer. In this context it is interesting to recall that XET activity in the G-fibres is long-lived and can be observed in dead fibres. This would ensure the integrity of the wood tissues; an important aspect considering the weak lignification of TW.

The requirement for XG, a cross-linking agent, is difficult to explain on the grounds of the swelling theory alone. The swelling does not require any tight connection between G- and S-layers, since the main tensile pressure is within the S-layers. Such requirement supports the idea that the longitudinal shrinking of the G-layer plays an essential role in tension generation. Moreover, the longitudinal strain in the G-layer alone can explain the origin of radial pressure of the G-layer on adjacent layers, and the observed longitudinal expansion of S-layers after enzymatic removal of the G-layer (Fig. 3.5c; Mellerowicz and Gorshkova 2012). We propose that the two theories could be combined and that the G-layer induces

longitudinal shrinking of TW during maturation by two different mechanisms playing roles at different developmental stages:

1. First, cellulose aggregation induces cellulose tensional stress and longitudinal shrinkage (Fig. 3.5b), which is transferred to adjacent layers by the wall cross-linking activities involving XET and XG.
2. Second, G-layer shrinkage creates increasing radial pressure on the adjacent S-layer (Fig. 3.5c). This in turn leads to circumferential expansion and longitudinal shrinking of the S-layers (Fig. 3.5a).
3. The latter mechanism might be of particular importance at the later stages of TW action, far away from the cambium when the XG cross-links might be difficult to maintain.

At least two roles can be proposed for AGPs in TW. AGPs are considered as likely candidates for in vivo XET activity regulation (Takeda and Fry 2004) and they could therefore help to maintain the activity of XET for a long time. Alternatively, AGPs could be involved in the regulation of cellulose MFA. AGPs were recently reported to influence the organization of cortical microtubules (Sardar et al. 2006; Nguema-Ona et al. 2007). This makes it possible to speculate that AGPs may be involved in determining the trajectories of the cellulose-synthesizing rosettes, resulting in a very low angle between cellulose microfibrils and the cell longitudinal axis which is characteristic for G-fibres.

It should be pointed out that in NW, there must exist similar mechanisms to those in TW, which generate growth stresses of a smaller magnitude than in TW. It is likely that in these cases, as well as in TW with S-layers, the role of the G-layer is taken by the S_2-layer. The positive correlation with tensional stress and crystallite size and a negative one with cellulose MFA (Sect. 3.2.3.2) indicate that these two parameters are also involved in stress generation in S-fibres.

Summarizing the data presented above we can state that it is still not clear if the set of non-cellulosic cell wall polymers is the same in tension wood of different species, and which characteristics of their structure are related to their participation in tension wood formation and function. The interesting hypotheses suggested on the particular role of each of the polymers have to be fully confirmed and probably widened. However, it is already clear that during the last few years, ideas on non-cellulosic polymers in G-layers have changed dramatically: from rejecting their presence to ascribing a major role in tension wood properties and function.

3.2.7 Lignin Content, Distribution and Biosynthesis in Tension Wood

Metabolic profiling and transcriptomic analyses on *Populus* and *Eucalyptus* have revealed that when TW is induced, lignin and hemicellulose biosyntheses are reduced whereas cellulose biosynthesis continues (Plomion et al. 2003; Paux

et al. 2005; Andersson-Gunnerås et al. 2006; Mellerowicz and Sundberg 2008). Cell wall composition also differentiates TW from NW. In TW fibre walls the proportion of cellulose is generally higher, and that of lignin considerably lower (Timell 1969; Wada et al. 1995; cf. Table 3.1).

Tensile growth stress has generally been explained by the contraction of cellulose microfibrils parallel to the fibre axis (Yamamoto 1998; Yamamoto et al. 2005) and models of contracting mechanisms have been proposed (see discussion of these topics in Sect. 3.2.6 above). A possible role of lignin in TW (Okuyama et al. 1994, 1998; Yoshizawa et al. 2000; Qiu et al. 2008), and other types of reaction wood (Baillères et al. 1997) has been described but to propose a mechanism of its action we need a better understanding of its distribution within different cell wall layers, and its peculiar chemistry in TW.

3.2.7.1   Lignin Content in Tension Wood

Early studies have shown that TW had lower lignin content and higher syringyl/guaiacyl ratio than NW (Bland and Scurfield 1964; Sarkanen and Hergert 1971). The use of modern analytical methods such as (pyrolysis-GC-MS) and thioacidolysis coupled to derivatization followed by reductive cleavage provides the detailed H/G/S monomeric composition of lignins. However, thioacidolysis liberates monomers preferentially from the non-condensed fraction of lignin (Lapierre 1993; Lu and Ralph 1997). Recent development in two-dimensional NMR (2D) ^{13}C-^{1}H-correlated spectroscopy allows determining the lignin units and the interunit patterns (Ralph and Landucci 2010). Applied to NW and TW in poplar, this multivariate analysis method confirmed the qualitative difference in the composition of NW and TW lignins (Hedenström et al. 2009) with a higher relative amount of syringyl lignin and p-hydroxybenzoates in TW relative to NW, and demonstrated the usefulness of this approach for "fingerprint" analysis.

Another interesting chemical feature that differentiates TW lignin stereochemical structure from NWs is its higher ratio of erythro to threo forms of β-O-4 sub-structures found by ozonation analysis in yellow poplar (*L. tulipifera*) (Akiyama et al. 2003). This may be seen as a stereochemical control of lignin formation.

3.2.7.2   Occurrence of Lignin in the G-Layer

The occurrence of lignin in the G-layer has been debated for a long time. As early as the 1960s Scurfield and Wardrop (1963), using UV-microscopy and histochemical staining techniques, observed traces of lignification in the G-layer, as opposed to heavy lignification of adjacent S layers in G-fibres, of several hardwood species. It is noteworthy that the occurrence of a non-lignified or weakly lignified G-layer specifically concerns fibres. This is usually observed in the xylem fibres but was also characterized in different G-fibre types of the phloem (Nakagawa et al. 2012).

Early studies of lignin distribution in TW (Norberg and Meier 1966) and substractive experiments by Bentum et al. (1969), in which polysaccharides were removed by hydrofluoric acid, revealing the remaining lignin skeletons led to the conclusion that there was no noticeable difference between NW and TW S-layers, whereas the G-layer of the TW G-fibres was non-lignified. In the G-fibres of TW the S_2-layers exhibited a high level of lignification and the lignin followed the orientation of cellulose microfibrils. On the other hand, also using a kind of subtractive method with the wood of *Populus tremuloides* and *Acer rubrum* degraded by white and brown rot fungi, Blanchette et al. (1994) visualized the remaining lignin distribution with potassium permanganate staining, and described the G-layer as unlignified. They also found a normal distribution of lignin in the S_2-layer. A more recent study of the effect of fungal degradation by Baum et al. (2000) indirectly showed the presence of polyphenolics in the G-layer of beech TW fibres. Using confocal fluorescence microscopy of TW of *Populus nigra*, Donaldson (2001) concluded that lignin was absent from the G-layer. All these results concerning the absence of lignin in the G-layer were limited by the resolution of light microscopy. As for the chemical analyses of TW tissues, they were carried out on whole tissues and could not precisely ascribe the difference in lignin content specifically to the G-layer. The absence of lignin in the G-layer was also claimed at the ultrastructural scale of observation (Yoshinaga et al. 2012). Here again, the use of potassium permanganate as staining reagent applied directly on ultrathin sections cannot lead to decisive results regarding the presence of low amounts of lignin.

On the other hand, working at the ultrastructural level, and using potassium permanganate in the conditions specified by Kerr and Goring (1975) as a fixative and general electron-dense staining agent for lignin, several authors (Hepler et al. 1970; Bland et al. 1971; Araki et al. 1982) showed the presence of a low amount of lignin in the G-layer of various hardwoods. Prodhan et al. (1995) investigating *Fraxinus mandshurica* with KMnO4 also found evidence of the occurrence of lignin at some sites in the G-layer. Gierlinger and Schwanninger (2006) using confocal Raman spectroscopy showed the presence of a small (0.5 μm) lignified border toward the lumen in the gelatinous layer of poplar TW and in some cases aromatic structures that extended into the G-layer toward the S_2, preferentially in the cell corners.

In those trees where a typical G-layer was not found, thin layers with a high lignin content and thick layers with lower lignin content have been reported to alternate in areas under tensile stress in the wood of the neo-tropical forest species *L. procera* (Poepp.), belonging to Salicaceae (Ruelle et al. 2007a, b).

Thanks to the emergence of new and more powerful techniques (Ruel 2003; Fackler and Thygesen 2013) the presence of lignin in the G-layer has been unequivocally proven. Using immuno-gold labelling in TEM (Joseleau et al. 2004b) provided in situ ultrastructural evidence that the G-layer of TW fibres of *P. deltoides* harboured a discrete but significant proportion of guaiacyl–syringyl lignin. In this approach, three polyclonal antibodies raised against synthetic lignin polymers (Ruel et al. 1994; Joseleau and Ruel 1997; Joseleau et al. 2004a) were used. The specificity of the antibodies allowed differentiation between

non-condensed and condensed linkages (Joseleau and Ruel 2007). Moreover, the interest in using these immunological probes is to reveal the ultrastructural localization of the different types of lignin (relative to their monomer composition) and interunit structures. Thus, a strong positive response for the presence of lignin was observed in the S_2-layer together with a weaker but conclusive labelling in the G-Layer. In addition, the immuno-gold labelling approach operated at the high resolution of TEM has allowed semi-quantitative estimation of the distribution of guaicyl, syringyl and mixed guaiacyl–syringyl epitopes, respectively, in the middle lamella, in S_1-, S_2- and in the G-layer.

Recently, Lehringer et al. (2008) using Raman spectra analysis demonstrated that the G-layer of three hardwoods: maple (*Acer* spp.), beech (*F. sylvatica*) and oak (*Q. robur*) contained lignin. In *Q. robur*, they showed the presence of a concentric sub-layering of electron-dense material in the G-layer. There was an accumulation of aromatic compounds in the innermost part of the G-layer up to 50 % of that detected in the secondary wall. Recent studies with TEM/FE-SEM confirmed these results (Lehringer et al. 2009). Polarization FT-IR of hybrid aspen normal and tension wood showed a high degree of alignment of xylan, cellulose and lignin in NW and lignin and cellulose in TW (Olsson et al. 2011).

3.2.7.3 Variation in Lignin Structure and Distribution According to Cell Types

It has been well documented using various techniques that lignin structure and composition varies in normal wood between cell types and cell wall layers (Campbell and Sederoff 1996; Joseleau and Ruel 1997, 2005; Ruel et al. 1999; Donaldson 2001; Koch and Kleist 2001; Joseleau et al. 2004a; Prislan et al. 2009; Stevanic and Salmén 2009; Gierlinger et al. 2010; Weng and Chapple 2010). In particular, recent papers confirmed the differences in the composition of lignin from fibres and vessel cell walls showing that the former were richer in syringyl units than the latter (Yoshinaga et al. 1997; Watanabe et al. 2004; Ruel et al. 2009). Such differences between vessel elements and fibres were also found in TW cell walls (reviewed by Aguayo et al. 2010; Neutelings 2011).

At the ultrastructural scale, significant variations in the nature and distribution of lignin in TW and NW have long been recognized (Lange 1954; Timell 1969). Typically TW has a lower amount of lignin, and based on global analysis of NW and TW samples, the lignin in TW contains a higher proportion of syringyl units (S units) (Bland and Scurfield 1964). The decrease in lignin content and increased S/G lignin ratio in TW was also determined in situ by UV microspectrophotometry in *Magnolia* species and yellow poplar (*L. tulipifera*) where G-fibres do not develop (Takeda et al. 1998; Yoshizawa et al. 2000; Yoshida et al. 2002b). Few studies have analysed lignins from NW versus TW. The lower lignin content and higher S/G ratio in TW lignin have been found in various hardwood species such as trees belonging to the *Magnoliaceae* (Okuyama et al. 1998), in *Eucalyptus* (Aoyama et al. 2001) and in *Robinia pseudoacacia* (Yoshida et al. 2002a). Recently,

modifications of lignin composition in TW of aspen have been assessed by chemometric analysis of 2D NMR spectra and multivariate data analysis on dissolved acetylated NW and TW cell wall material (Hedenström et al. 2009). Although changes in lignin composition could not be deduced from the principal component analysis (PCA) of the full spectral range, the region of the aromatic peaks confirmed the higher amounts of S-lignin and *p*-hydroxybenzoates, and the relative decrease in G lignin in TW. Also, recent methods such as whole cell ionic liquid and other solid-state NMR analysis detailed the structure of lignin and hemicelluloses in the samples, confirming the presence of variations in lignin and hemicellulose sub-units, linkages and relative amounts of syringyl (S), guaiacyl (G) and *p*-hydroxybenzoate (PB) monolignol units. It was confirmed that TW displayed an increase in PB or H-like lignin and the S/G ratio from 1.25 to 1.50 when compared to the NW sample (Foston et al. 2011). Such enrichment in sinapyl alcohol units has been suggested to convey superior mechanical support properties (Li et al. 2001; Weng and Chapple 2010).

In species which do not develop a characteristic individualized G-layer, such as *Eucalyptus gundal*, it is interesting that TEM coupled to immunolabelling of lignin sub-units revealed variations in the distribution of lignin sub-units in tracheids exhibiting high values of longitudinal residual deformation (*DRLM*), and an increase in the non-condensed sub-units, with respect to normal wood (from Ruel and Joseleau unpublished).

3.2.7.4 Lignin Biosynthesis During TW Formation

The metabolic pathway identifying the enzymes and the corresponding genes leading to the production of lignins has been studied for many years and is now relatively well characterized (recently reviewed by Bonawitz and Chapple 2010; Neutelings 2011). Transcriptomic investigations have correlated expression of lignin synthesis genes and TW formation (Pilate et al. 2004a, b; Lapierre et al. 2004; Koehler and Telewski 2006; Andersson-Gunnerås et al. 2006). In a transcript profiling study Paux et al. (2005) found that 196 genes out of the 231 expressed in differentiating *Eucalyptus* xylem were differentially regulated when the tree was artificially bent.

Interestingly, the genes encoding laccases were significantly downregulated in bending-induced TW in *L. tulipifera* (Jin and Kwon 2009), indicating a modification in the conditions for monolignol polymerization. The increased synthesis of S-lignin in TW has been correlated with the enhanced activity of wall-bound peroxidases (Aoyama et al. 2001; Tsutsumi et al. 1998). Indeed, in a recent study on the formation of TW in a bent stem of yellow poplar (Jin and Kwon 2009) it was found that there is a decrease in the synthesis of not only guaiacyl and *p*-hydroxyphenyl units but also syringyl units.

Lignin biosynthesis responds to several developmental and environmental cues under the control of many transcription factors such as MYBs and NAC domain which act as "master switches" activating a suite of downstream transcription

factors modulating secondary wall biosynthesis (Goicoechea et al. 2005; Zhao and Dixon 2011; Zhong et al. 2011).

3.2.7.5   Conclusions

The occurrence of a type of syringyl-rich lignin in the G-layer could contribute to the variation in the S/G ratio observed in TW by several methods. However, the fact that a similar variation of S/G has been observed in hardwood species that do not develop a typical G-layer (Yoshizawa et al. 2000) is indicative of modification of lignin metabolism affecting the formation of S_1- and S_2-layers in TW. Such a TW-specific modification is supported by the results of functional genomics (Pilate et al. 2004a, b; Koehler and Telewski 2006; Qiu et al. 2008) demonstrating changes in the transcriptional regulatory network of genes (Paux et al. 2005; Li et al. 2006) that lead to the peculiar differentiation programme in TW. Similar conclusions about changes in lignin composition in compression wood have been suggested (Plomion et al. 2000; Gindl 2002; Koutaniemi et al. 2007; Yamashita et al. 2008). All this is consistent with the modulation of lignin metabolism in the response of trees to gravitational stresses.

3.3   Polymers of Compression Wood

3.3.1   Cellulose

Compression wood (CW) has a lower content of cellulose compared to NW. After careful dissection of tracheids with a micromanipulator followed by chemical analysis of the fractions, Côté et al. (1968) calculated that about 40 % of cellulose was located in the inner S_2-layer of the CW tracheids of *Abies balsamea*. No difference in cellulose molecules between CW and OW has been reported, but CW cellulose has a lower degree of polymerization and is less crystalline (Lee 1961; Tanaka et al. 1981). The most characteristic feature of cellulose in CW is the realignment of microfibrils with respect to the axis of the stem. In general, MFA shows a good correlation with tensile and compressive stresses, although other factors may be involved (Donaldson 2008), but the exact role of MFA orientation is not completely understood. The pattern of variation in MFA measured by confocal laser scanning microscopy (CLSM) in NW and CW has been shown to be non-uniform in a single tracheid, with the extent of variation decreasing from earlywood to latewood (Sedighi-Gilani et al. 2005). A relative agreement has been found between the MFA values measured in 12 spruce and larch compression latewood samples and the orientation of their helical cavities. The results of measurements by X-ray diffraction technique and by CLSM show some discrepancies due to the fact that in the X-ray method the measured MFA corresponds to

the mean value of several adjacent tracheids, while in CSLM local microstructural MFA in the S_2-layer of a single tracheid is measured (Sedighi-Gilani et al. 2005).

In normal tracheids, cellulose microfibrils are considered to be relatively "flat", 70–50° to the longitudinal axis of the cell in the S_1- and S_3-sublayers, whereas they are relatively "steep" in the S_2-sublayer, 45–10° (Scurfield 1973). In contrast, microfibrils in CW are aligned more or less transverse in the S_1 but at an angle of 30–45° in the S_2-sublayer (Andersson et al. 2000; Kwon et al. 2001). **MFAs values vary according to the measurement method used and also to cell type** (Gierlinger et al. 2010). MFA values of 30–45° in relation to the cell axis have been reported for the microfibrils of CW and of 5–30° for NW. Such differences in MFA may be related to mechanical properties (Lichtenegger et al. 1999; Yoshida et al. 2000b). The strong correlation between average MFA and average longitudinal shrinkage observed in *Pinus radiata* suggests a significant influence of MFA on the severity of the impact of compression wood (Donaldson 2008; Xu et al. 2009). It has been suggested that the much higher MFA-value in CW is a result of the greater deposition of hemicelluloses and lignin between the microfibrils (Plomion et al. 2001; Önnerud 2003). Such a modification in the way the cellulose network is deposited in the cell wall layers during CW formation suggests that a shift in microtubule orientation may occur during CW formation. The arrangement of microtubules in CW tracheids and particularly the helical thickening of the innermost surface of cell walls of artificially inclined stems of *Taxus cuspitada* have been revealed by immunofluorescence staining and CLSM during secondary wall formation (Furusawa et al. 1998). In this study, the cortical microtubules were found to be oriented at an angle of 45° to the tracheid axis. Artificial inclination has been shown to lead to an alteration in the pattern of alignment of the microtubules (Abe et al. 1995). While the direction of microtubules in NW tracheids changed from transverse to a steep Z-helix, oriented at about 5–10° to the tracheid axis, it became oblique in CW tracheids at an angle of 45° in a Z-helix. This corresponds to a clear relationship between microfibrils and microtubule orientation during cell wall formation. Similarly, superimposition of microtubules and helical thickenings has been observed in *T. cuspitada* (Uheara and Hogetsu 1993). Spokevicius et al. (2007) have identified a β-tubulin gene (*EgrTUBI*) that they suggest is associated with the orientation of MFA. However, the mechanism controlling the orientation is still unknown (Donaldson 2008).

In situ characterization of cellulose in Sitka spruce [*Picea sitchensis* (Bong.) Carr.] CW has been recently carried out (Altaner et al. 2007) in CLSM using three different cellulose-binding modules (CBMs) specific for crystalline cellulose or amorphous cellulose (Blake et al. 2006). While crystalline cellulose is abundant in all cell types, amorphous cellulose appears predominantly in ray cells and CW tracheids. However, the staining of CW may be due to the presence of the β-1,3-glucan since recognition of callose by the CBM has been reported Boraston et al. (2002). It is interesting that the extent of binding of CBMs may provide insights into the relative crystallinity index between tissues and cell wall layers. Thus, a higher proportion of paracrystalline cellulose has been found in CW. Estimates of the average cellulose crystallinity in CW have been made by

CP/MAS 13-NMR spectroscopy (Newman 2004). It was found that the cross-sectional dimension of crystalline domains is slightly smaller for CW than for OW (Marton et al. 1972; Tanaka et al. 1981). The mean values of cellulose crystallinity provided by solid state ^{13}C-NMR showed only a slight reduction for CW. These results suggest that the correlation between ring number and wood crystallinity can be ascribed mostly to variation in cellulose content rather than to cellulose crystallinity (Newman 2004).

Examination of cellulose using dual axis electron tomography showed more weak points due to kinks, creating dislocations along the length of cellulose microfibrils, within in CW (Xu et al. 2011). These dislocations were suggested as contributing to the reduced stiffness and tensile strength of CW.

Numerous observations and correlations to explain compressive growth or mechanical stresses in trees have been made, leading to the conclusion that the conjunction of higher lignin content plus larger MFAs in secondary walls are the main factors (Yamashita et al. 2007). Models have been proposed (Okuyama 1993; Yamamoto 1998) in which it is suggested that contraction during polymerization and crystallization of cellulose microfibrils generates tensile force. In such models, the matrix polymers, hemicelluloses and lignin generate the compressive force, although this is mostly due to the increased lignin deposition while MFA is the determinant for the prevailing force. However, the exact function of each of the polysaccharide constituent of the walls of tracheids from CW and their possible participation in the mechanical properties of wood in response to stresses are still largely unknown. It is likely that the development of molecular biological methods linking gene expression, wall polymer variation and mechanical stresses will provide further information about the physiological response of plants to mechanical stimuli.

3.3.2 Non-cellulosic Polysaccharides

Descriptions of the characteristic features of CW are usually based on those expressed by severe CW. In fact, no clear boundary exists between NW and CW, and there is instead a continuum from mild to severe CW (Singh and Donaldson 1999; Plomion et al. 2000; Yamashita et al. 2007).

As has been shown for TW (Sect. 3.2.4 above), it has long been known that the cell wall constitution of softwood compression wood differs markedly from that of normal wood in its carbohydrate content (Meier 1964; Yeh et al. 2006; Nanayakkara et al. 2009). Most of the chemical analyses of wood samples having high levels of CW or TW, as evaluated by colorimetric estimation, were carried out decades ago using wet chemistry techniques. Most of the results have come from averaging the composition of a number of different cell types. Such an analysis can be refined today at the level of a single cell type with material collected using the recently developed approach combining the laser capture microdissection (LCM) technique coupled to microanalysis (Angeles et al. 2006; Ruel et al. 2009). This

Table 3.8 Average composition (as a %) of normal wood and compression wood of conifer species (adapted from Timell 1982; Hon and Shiraishi 2001)

Constituent	Tracheids		Ray cells	
	Normal wood	Compression wood	Normal wood	Compression wood
Cellulose	42	30	35	35
Galactoglucomannan	20	9	9	11
Glucuronoarabinoxylan	8	7	11	10
1,3-Glucan	–	3	2	2
Galactan	t	10	t	t
Lignin	28	40	40	40

t = trace; – = not detected

enables an assessment of the cell specificity of wall modifications induced in reaction wood in which the changes in cell wall carbohydrate composition correspond to variations in the respective contribution of non-cellulosic polysaccharides and cellulose. The wall matrix polysaccharides of CW differ qualitatively and quantitatively from those of NW. Interestingly, a comprehensive and comparative evaluation of the morphological and chemical composition of juvenile wood, mature wood, and compression wood of loblolly pine (*Pinus taeda*), using X-ray diffraction, carbohydrate and lignin analysis and NMR spectroscopy, has shown that among these different morphological wood types, juvenile and mature compression wood always had similar variations in lignin, cellulose and hemicelluloses (Yeh et al. 2006). In particular, among the hemicelluloses, the contribution of galactoglucomannans in CW falls to 9–12 % compared to 15–20 % in NW (Table 3.8), while the proportion of glucuronoarabinoxylans shows no comparable variation.

Polysaccharide and lignin composition of CW and OW across 27 rings of a 35-year-old tree of loblolly pine (*P. taeda*) have been measured by micro analysis using transmittance near infrared (transmittance NIR) spectroscopy (Chen et al. 2007). (Transmittance NIR seems to be an interesting technique for predicting CW content as it uses a smaller of sample compared to the more traditional reflectance NIR.) A relatively good correlation was found to exist between individual sugars (particularly galactan) and lignin and lignin content and CW in the increment core analysed.

The most diagnostic characteristic of the non-cellulosic polysaccharides from CW is the presence of a significant proportion of β-1,4-galactan which is totally absent in NW. This hemicellulosic polysaccharide accounts for 9–11 % of the cell wall material. Also unusual is the presence in some species of a β-1,3-glucan of the laricinan type that may account for 3–5 % of the CW. This kind of polysaccharide is also absent from NW. Also noticeable in CW is the increased acetyl content. A recent study (Nanayakkara et al. 2009) analysed the carbohydrates in the cell walls of material collected from NW, from wood displaying only some features of CW (mild form of CW, MCW), from wood with pronounced features of CW (severe CW, SCW) and from opposite wood (OW). Interestingly, the variations in each of the cell wall carbohydrate could be directly related to the degree of CW

(as determined by microscopy). In particular, it appears that the content of galactose reflects the level of CW when a more than fourfold difference was observed between OW and SCW. From the analytical results it can be suggested that mannose content also reflects the degree of severity in CW with its level decreasing by a factor of about 2 between OW and CW. Variations of similar orders of magnitude have been observed in the carbohydrate composition of CW induced by wind and by mechanical bending in loblolly pine (*P. taeda*) (Yeh et al. 2005). However, in some species, the low galactose content cannot be used as a marker of CW as shown in the analysis of the composition of juniper (*Juniperus communis* L.) fibres (Hänninen et al. 2012).

A global investigation of compositional differences between NW and CW carried out with spin-echo magnetic resonance imaging (MRI) using gadolinium as a paramagnetic contrast agent showed that CW of southern yellow pine fixed higher amounts of gadolinium than NW (Eberhardt et al. 2009). This was ascribed to higher lignin and/or carboxylic acid residues in CW, as has been suggested earlier with the presence of polyuronide hemicelluloses in CW (Timell 1986).

3.3.2.1   β-1,4-Galactan

Among the non-cellulosic polysaccharides of CW as compared with NW, galactan is by far the most characteristic. The chemical structure of this pure galactan has been demonstrated to consist of a β-1,4-linked D-galactan chain (Bouveng and Meier 1959; Jiang and Timell 1972). Such a β-1,4-linked backbone of sugars in their pyranosidic conformation is a typical feature of hemicellulosic polymers, therefore the galactan from CW may be regarded as part of the hemicellulose family of plant polysaccharides. The average chain length of CW galactans has been estimated to be from 200 to 300 (Timell 1986) and up to 380 units (Nanayakkara 2007). The linear galactopyranosyl main chain has often been described as slightly branched (Meier 1964) at the C-6 position, carrying single terminal β-D-galacturonic acid residues or in some cases D-glucuronic acid (Jiang and Timell 1972). A few short side chains of β-1,4-D-galactose are also linked at position C-6 of the main chain. All these structural features of CW galactan differ from the galactan moiety of the arabinogalactan found in low amounts in the primary wall of conifers (Willför et al. 2002), which consists of a highly branched β-1,3-galactan backbone. Although the galactan from TW also consists of a β-1,4-linked galactopyranosyl main chain, its pattern of substitution is far more complex than that in CW, carrying β-1,4- and β-1,6- galactose side chains with α-L-rhamnopyranosyl residues themselves substituted with terminal α-D-galacturonic acid units. Terminal 4-*O*-methyl-β-D-glucuronic acid and α- and β-L-arabinofuranosyl residues have also been found (Kuo and Timell 1969) (Fig. 3.6).

The significance of galactan in the physiology of CW formation and in CW mechanical function is currently not understood. The most commonly proposed hypothesis is that galactan could participate in the strengthening of the secondary wall. A simple model describing the mechanism of longitudinal shrinkage has been

$$\rightarrow 4)\text{-}\beta\text{-}\text{D-Gal}p\text{-}(1\rightarrow 4) \overset{\ulcorner}{} \text{-}\beta\text{-}\text{D-Gal}p\text{-}(1\rightarrow 4)\text{-}\beta\text{-}\text{D-Gal}p \overset{\urcorner}{}\text{-}(1\rightarrow 4)\text{-}\beta\text{-}\text{D-Gal}p\text{-}(1\rightarrow 4)\text{-}\beta\text{-}\text{D-Gal}p\text{-}(\rightarrow$$

Structure with branches:

$\underset{\llcorner \qquad\qquad \lrcorner_n}{}$

                                                6   6
                                                ↑   ↑
                                                1   1
                                        $(\beta\text{-D-Gal}p)_m$ $\beta\text{-D-Gal}\Delta p$
                                                4
                                                ↑
                                                1
                                        $\beta\text{-D-Gal}p$
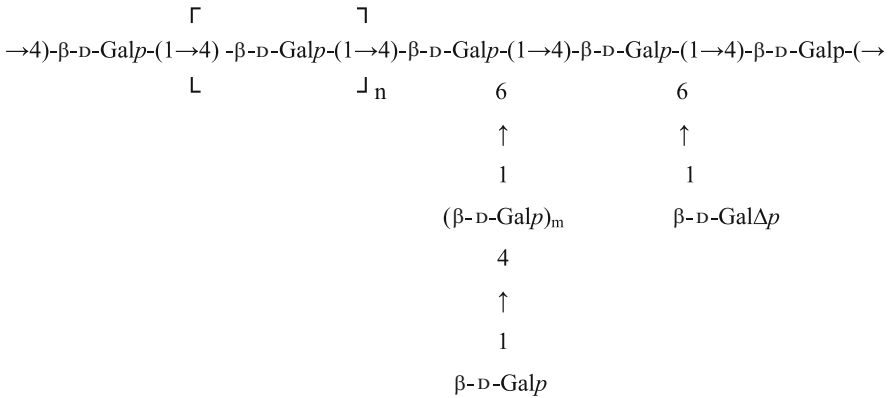
Fig. 3.6 General structure of CW galactan

proposed by Floyd (2005) in which a good fit between galactan-containing and cellulose-containing ratios seems to apply to samples containing obvious compression wood although the reason for this was not apparent. The modification of crystallinity and crystalline structure of bacterial cellulose during its synthesis in the presence of the β-1,4-linked polysaccharides, xyloglucan and glucomannan, has been studied by ^{13}C-NMR techniques (Hackney et al. 1994) which showed that the process of cellulose microfibril association was affected. However, the conformation of β-galactans (Yathindra and Rao 2003) in which the geometry of the glycosidic bond is influenced by the axial hydroxyl of D-galactopyranose differs somewhat from that in the xyloglucan and glucomannan, which are closer to the flat-ribbon conformation of cellulose, and prevents non-covalent interaction with cellulose. In spite of this, a role for galactan could be to influence wood mechanics by modifying the hierarchic cellulose microfibril aggregation in CW. The physiological importance of galactans in the formation and properties of CW is currently not explained. However, it is remarkable that the silencing of the 4-coumarate CoA ligase (*4CL*) gene in *P. radiata* induced an increase in the formation of CW that was accompanied by an increase in galactose content suggesting that elevated galactose and lignin levels are both integral to the gravitropic response in pine (Wagner et al. 2009).

In a series of ultrastructural studies Kim et al. (2012a), using immunochemical markers in TEM and FE-SEM observations, concluded there were some differences in the ultrastructural organization of hemicelluloses and lignin in CW tracheids and NW tracheids. A global view of cell wall polymers was obtained by Donaldson and Knox (2012) who conducted a study of the distribution of non-cellulosic polysaccharides in the NW and CW of *P. radiata* in relation to lignin deposition and cellulose microfibril orientation. They used monoclonal antibodies for detecting hemicellulosic polysaccharides combined with lignin autofluorescence and polarized light microscopy for crystalline cellulose, and colocalized the various

polysaccharides with lignifications. The increased lignifications in CW was associated with deposition of galactan and reduced amounts of xylan and mannan (sic, Donaldson and Knox 2012: should actually be glucomannans and galactoglucomannans) in the outer S_2 of the CW tracheids.

The monoclonal antibody LM5 developed by Jones et al. (1997) against a linear β-1,4-galactan has been used by Altaner et al. (2007) to probe the galactan in CW with immunofluorescence microscopy. A much stronger fluorescence was observed in the tracheids from CW than NW with the galactan exclusively located in the outer cell wall layers. Examination of a growth ring revealed that the incorporation of β-1,4-galactan was the first physiological reaction of a tree to mechanical stress and was therefore concluded to be an excellent marker for the severity of CW. Another immunolocalization study in *P. radiata* by Moller and Singh (2007) confirmed the close association of galactan with the highly lignified outer secondary cell wall layer of CW. In contrast, sub-cellular localization of β-1,4-galactan in *P. radiata* CW, using the same monoclonal antibody LM5 in confocal laser fluorescence and transmission electron microscopy, did not find galactan in NW of *P. radiata*, although it was evenly distributed in the lightly lignified S_2-layer of the secondary walls in CW (Mast et al. 2009), and only weakly present in the lignified S_1-region. Such discrepancy between the results was ascribed to the influence that the developmental stage of tracheids had on the binding ability of the antibody. It is therefore difficult to relate lignification and galactan deposition in CW formation, in spite of early evidence of covalent linkages between lignin and high galactose-containing structures isolated from CW in *Pinus densiflora* (Mukoyoshi et al. 1981).

Another factor that could modify the interaction between hemicelluloses and cellulose in CW is the increased degree of acetylation of hemicelluloses. Since the presence of the acetyl group at O-3 prevents the formation of an intramolecular hydrogen bond between OH at position-3 and the ring oxygen of the preceding sugar residue, the increased number of acetylated residues affects the cellulose-like conformation of arabinoglucuronoxylans and glucomannans of CW, lowering their capacity to hydrogen-bond strongly with cellulose (Jarvis 2005). This could modify the anisotropic arrangement of xylans and glucomannans relative to cellulose which has been suggested to be a determinant for the properties of the wood under mechanical stress (Stevanic and Salmén 2009).

3.3.2.2   β-1,3-Glucan

Another typical hemicellulose from CW is an acidic β-1,3-glucan, laricinan. It is a type of callose of low molecular weight with a degree of polymerization (DP) of 175–205 and was first identified in *Larix laricina* (Hoffman and Timell 1970). β-1,3-Glucans are peculiar kinds of hemicelluloses with a crystalline structure suggested to be a triple-stranded helix (Bluhm and Sarko 1977). The geometry of the β-1,3-linked glucosidic bond determines the conformation adopted by the chain and thus also the physical properties. Structurally, the CW glucan differs from other

β-glucans in carrying acidic substituents of glucuronic and galacturonic acids (Hoffman and Timell 1972).

The suggestion that β-1,3-glucans have a role in resistance to compressive stress has been made for callose in cotton hairs (Malby et al. 1979). More recently, some experimental evidence for a function of callose in the cell wall's capacity to resist different types of mechanical stresses was obtained from pollen tubes of *Solanunm chacoense* and *Lilium orientalis* (Parre and Geitman 2005). After reduction of the glucan level by partial enzymatic hydrolysis, the cellular stiffness was lowered and the viscoelasticity increased, as measured by the technique of microindentation resulting in local deformations. This technique had been previously applied to measuring longitudinal hardness and Young's modulus of spruce tracheid secondary walls (Wimmer et al. 1997), allowing direct comparison with ultrastructural and microchemical parameters for understanding intrinsic wood properties.

The acquisition of information about the distribution of polysaccharides has been hindered for a long time by the difficulty of staining polysaccharides selectively. However, β-1,3-glucan has been localized with aniline-blue staining in CW and has been found in association with the helical cavities of the tracheids (Waterkeyn et al. 1982) and between the lignified ribs of S_2. This localization towards the cell lumen was recently confirmed by the detection of callose in severe CW using a β-1,3-glucan monoclonal antibody (Altaner et al. 2007). It has been suggested that the swelling of the glucan on wetting may generate the longitudinal stress occurring in CW. The swelling and shrinking properties of CW as moisture content changes are directly related to its fibre structure and compositional differences from NW. It is interesting that a change in orientation of β-1,3-glucan occurs during hydration, such that the anisotropical chains become isotropically oriented (Waterkeyn et al. 1982; Stone and Clarke 1992).

3.3.2.3 α-1,5-Arabinan

This polysaccharide, which is typical of primary cell walls, has been detected by immunolabelling with a monoclonal antibody specific to (1→5)-α-L-arabinan (Willats et al. 1998) in the intercellular spaces of tracheids and in the walls of parenchymatic ray cells of severe CW (Altaner et al. 2007). Since this polysaccharide is normally located in the primary walls its appearance in the middle lamella area of CW led the authors to suggest an active control of cell separation during CW formation. The arabinan and the galactan of pine wood have been shown to be bonded to lignin through C-5 and C-6, respectively (Minor 1982).

3.3.2.4 Glucomannan and Xylan

It is interesting that the observed reduction in cellulose content of CW relative to NW is accompanied by a significant reduction in neutral glucomannans but not in acidic arabinoglucuronoxylans. The reduction in cellulose synthesis without

concomitant variation in glucuronoxylan is contrary to the statement that a reduction in xylan synthesis affects cellulose synthesis, as was concluded from a study of the secondary walls of the *Arabidopsis Fragile Fiber*8 (fra8). This mutant is defective in xylan synthesis, underlying the essential role of the glucuronosyl substituents (Zhong et al. 2005). This observation in a dicotyledonous plant does not seem to apply in the same manner to gymnosperms. In the CW of the latter it is the reduction in glucomannans that correlates with a reduction in cellulose synthesis. This underscores the importance of the special conformational interaction between cellulose and glucomannans which has been shown to establish a much stronger interaction with cellulose than xylans (Salmén and Fahlen 2006). The arrangement of these hemicelluloses parallel to cellulose and the fibre axis results in an anisotropic behaviour under mechanical stress (Stevanic and Salmén 2009).

3.3.3   *Lignin and Extractives*

3.3.3.1   **Biochemistry of Lignin(s)**

One of the most conspicuous traits observed in the biochemical analysis of CW is the higher lignin content, which reaches almost 40 % in Norway spruce [*P. abies* (L.) Karsten], while NW and OW contain just less than 30 % lignin (Tarmian and Azadfallah 2009). The involvement of lignin in the biomechanical functions of wood has long been known (Fukushima and Terashima 1991; Lange et al. 1995). Lignin content in the S_2-layer, more precisely in the external part of S_2 [S_2(L)], reaches a maximum in severe CW, while the first change accompanying CW formation is a reduction of lignin in the cell corners (Okuyama et al. 1998; Donaldson et al. 2004). Not only the amount of lignin but also its structure and composition in CW differ from that in NW. CW lignin is reported to be richer in *p*-hydroxyphenyl (H) units, up to 70 % (Gindl 2002), compared to the almost exclusively guaiacyl nature of NW lignin in softwoods (Timell 1986; Önnerud and Gellerstedt 2003; Yeh et al. 2005). Altered lignin composition can be determined by measuring the variation of the ratio of UV-absorbance of the cell walls at 280–260 nm (Musha and Goring 1975). The use of this technique on Norway spruce samples grown under compressive stress revealed an increasing proportion of *p*-hydroxyphenylpropane units in the stressed wood (Gindl 2002). Because the H units do not carry substituents at positions 3 and 5 of their aromatic ring, the possibilities of radical polymerization can give rise to more complex lignin structures (Önnerud 2003) as shown by the oligomeric structures from thioacidolysis containing H units. Thioacidolysis has shown that spruce CW yields more trimeric and fewer monomeric structures. Most of the lignin from spruce CW investigated by thioacidolysis has been determined to be of the non-condensed β-O-4 structural type (Önnerud 2003). However, the ratio of condensed to non-condensed linkages has been suggested to be related to the mechanical behaviour of the cell wall (Ruel et al. 1999, 2000). This has been confirmed by confocal Raman microscopy

(Gierlinger et al. 2006) and analytical pyrolysis (Alves et al. 2009), which showed a greater ratio of condensed to non-condensed lignin in CW than in NW, and suggests that the lignin of CW is more cross-linked than that of NW, and therefore stiffer. These findings are in agreement with the generally accepted higher condensation of CW lignin (Sakakibara 1980; Kutsuki and Higuchi 1981). According to the variations of some enzyme activity involved in lignin formation (Kutsuki and Higuchi 1981), the more condensed lignin in CW could be due to the increased activity of phenylalanine ammonia lyase (EC 4.3.1.5), caffeate 3-*O*-methyltransferase (EC 2.1.1), *p*-hydroxycinnamate: CoA ligase (EC 6.2.1.12) and cinnamyl alcohol dehydrogenase (EC 1.1.1) which should induce an increased supply of monomers and result in a "Zulaufverfahren" mode of polymerization yielding a bulk polymer containing large amounts of condensed lignin. The higher content of *p*-hydroxyphenyl propane units and the more condensed type of lignin in CW has been suggested as contributing to the comparatively high compressive strength of CW (Gindl 2002). In a recent approach combining enzymatic mild acidolysis lignins (EMAL) and the analytical methods of derivatization followed by reductive cleavage (DFRC)/31P NMR or thioacidolysis, Guerra et al. (2008) found that the total amount of guaiacyl units involved in non-condensed β-O-4 bonds was lower in CW than in NW from Southern pine (*Pinus palustris*). On the contrary, the amount of H units involved in non-condensed linkages was higher in CW than NW.

3.3.3.2 Molecular Biology of Lignin Formation

To understand the anatomical and chemical differences resulting from CW formation, the identity of genes differentially expressed in NW and CW has been investigated (Zhang and Chiang 1997; Plomion et al. 2001; Yamashita et al. 2008). Several genes expressed at a higher level in CW than in NW have been reported and suggested to be involved in CW formation. The gene PtaAGP4, which encodes a cell wall arabinogalactan-rich protein, has been found to be highly expressed in CW of loblolly pine (Zhang et al. 2000). In addition, several other genes with modified expression levels have been found in loblolly pine in cDNA libraries and through microarray analysis (Whetten et al. 2001). In maritime pine 26 proteins correlated with growth strain have been identified and their level of expression has been used as an indicator of reaction wood formation (Plomion et al. 2000). Using fluorescent differential display (FDD) Yamashita et al. (2008) screened for genes whose expression changed during CW formation in *Chamaecyparis obtusa*. From the 67 selected cDNA fragments tested, they found 24 having reproducible expression patterns, indicating that these fragments changed their expression during CW formation. In addition, the expression of a gene coding for XET has been reported to be higher in side wood next to CW (Allona et al. 1998).

Because of the variation in lignin content any changes in the level of genes encoding SAM-S and caffeic acid *O*-methyltransferase seem likely to be correlated with the degree of development of CW (Plomion et al. 2000; Yamashita

et al. 2008). Also, the differential expression of oxidase genes, such as laccase, in CW and NW of loblolly pine (Allona et al. 1998) and the expression of the proteins in Sitka spruce (McDougall 2000) point to the role of specific laccases linked to the lignification of CW. In the same way, in a study of expression profiling of the lignin biosynthetic pathway in Norway spruce using EST sequencing, Koutaniemi et al. (2007) reported potential peroxidase genes that could be specifically involved in the polymerization of monolignol in CW and that could have a more general stress-induced function. In a study of the regulation of lignin biosynthesis, Bedon et al. (2007) followed the expression of 13 spruce *R2R3-MYB* genes, implicated as regulators of lignin metabolism (Rogers and Campbell 2004), and five other cell wall related genes during the early phase of CW formation. They found that the transcript levels of six genes were upregulated during the induction of CW. Similarly, Yamashita et al. (2009) examined the relative abundance of transcripts among *C. obtusa* saplings with various degrees of CW development. They showed that some transcripts changed their abundance with increasing development of CW. In particular, laccase transcript increased in abundance gradually with increasing development of CW and, the abundance of the transcript of ascorbate oxidase decreased abruptly with the beginning of CW formation.

Comparative transcription analysis between developing CW and opposite wood using a combination of differently constructed DNA libraries and microarray analyses showed reprogramming of cell wall genes during CW formation in *Pinus pinaster*, Ait. (Villalobos et al. 2012). A total of 496 genes were identified which changes in expression during differentiation of CW: 331 were upregulated and 165 downregulated. In particular, the modification of a set of genes involved in S-adenosyl-methionine synthesis was demonstrated to be coordinated with increased demand for coniferyl alcohol for lignin synthesis in CW.

All these modifications in gene expression and regulation factors identified in compression as well as in tension wood formation illustrate the complexity of the mechanisms that are implemented by plants to adapt to changes and stresses in their environment.

3.3.3.3 Ultrastructural Distribution of Lignins

Typical morphological features of severe compression wood are the thicker secondary wall exhibiting helical cavities and a rounded shape of the transverse section of tracheids with open intercellular spaces. These criteria for CW are always consistent features even in less severe CW (Nanayakkara et al. 2009). All these aspects of morphology and microstructure of the tracheids in CW can be found in early reviews by Wardrop (1964) and Timell (1986). The absence of the S_3-layer is another typical trait of CW. At the ultrastructural scale, the variation in MFA orientation and the distribution of lignin have been extensively investigated, since they are thought to be related to the physical (Yamamoto 1998) and pulping properties of CW (Brändström 2004). Quantitatively, the distribution of the higher lignin content in CW is well documented, using lignin autofluorescence and by

microdensitometry of confocal fluorescence microscopy images (Donaldson et al. 1999; Singh and Donaldson 1999). The thickened secondary wall exhibits enhanced lignification in its outer S_2-region as shown by UV- and fluorescence microscopy. Differences in lignification within the S_2-layer in tracheids have been visualized with electron microscopy (Blanchette et al. 1994; Yoshizawa et al. 1999). Quantitative estimation of lignin concentrations by interference microscopy has revealed the differences in S_2- (26 %) and in S_2L-layers (46 %), and cell corner (57 %) regions in *P. radiata*. Another tentative approach for quantifying the severity of CW in relation to lignin in the secondary walls of *P. radiata* involved a combination of confocal fluorescence imaging and related spectral deconvolution (Donaldson et al. 2010). However, a clear value of CW content still remains a difficult property to measure.

Chemical imaging by confocal Raman microscopy underlined the highly ligni-fied outer S_2 layer in CW from *Pinus bungeana* and in addition showed that coniferyl alcohol and coniferyl aldehyde in NW and CW had opposite patterns to lignin distribution (Zhang et al. 2012). The highly typical structure of lignin with its increased proportion of *p*-hydroxyphenyl propane units and the consequence that it forms a highly condensed type of lignin is more difficult to identify and differen-tiate at the ultrastructural level. The identification and sub-cellular localization of H units by the microautoradiography method (Fukushima and Terashima 1991) has shown that the rate of formation of *p*-hydroxyphenylpropane units is higher in the outer S_2-layer, giving rise to a more condensed type of lignin. In situ detection of the main monomers constitutive of lignins may be achieved by immunochemical labelling (Ruel et al. 1994; Joseleau and Ruel 1997; Ruel 2003).

Taking advantage of the capacity of the immunological probes to distinguish condensed from non-condensed lignin epitopes Ruel et al. (1999, 2005) have demonstrated the unequal distribution of condensed and non-condensed sub-struc-tures in the tracheids of *P. abies* CW (Fig. 3.7). Higher proportions of condensed guaiacyl structures have been found in the inner part of the S_2-sublayer. In these investigations, the labelling with the antibody directed against H units showed that these units were differentially distributed in the middle lamella and the cell corners and gave a stronger labelling in the S_1-sublayer. The TEM investigation also revealed that the outer S_2-sublayer may be viewed as a clearly differentiated sublayer with respect to its anatomical organization and lignin composition. Another subunit, the 8-ring dibenzodioxin 5-5-O-4 sub-structure, a condensed motif in softwood, was localized immunologically by CLSM in Norway spruce and Scots pine (*Pinus sylvestris*) NW and CW (Kukkola et al. 2008). Here again, the labelling with the antibody (Kukkola et al. 2003, 2004) showed that the dibenzodioxocin epitope was essentially localized in the inner part of the cell wall in the S_3-region (Fig. 3.8).

Recently, Donaldson and Radotic (2013) have shown the interest of fluorescence lifetime imaging of lignin autofluorescence in normal and compression wood have demonstrated the usefulness of fluorescence lifetime imaging of lignin autofluorescence for characterizing in NW and CW, measuring significant changes
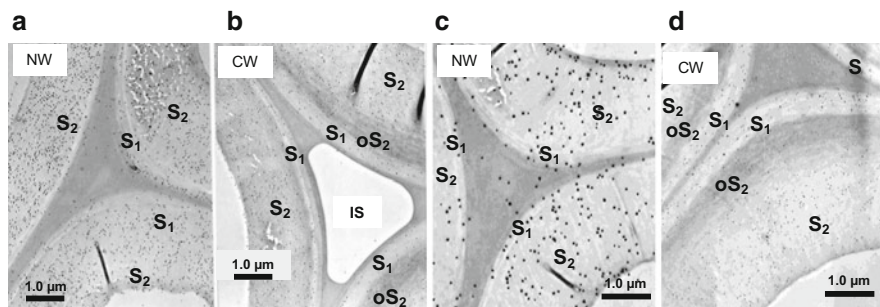
Fig. 3.7 Immunolabeling of non-condensed lignin epitopes in Norway spruce (*Picea abies*): (**a**) normal wood; (**b**) compression wood and condensed epitopes, (**c**) normal wood; (**d**) compression wood (Ruel and Joseleau unpublished)
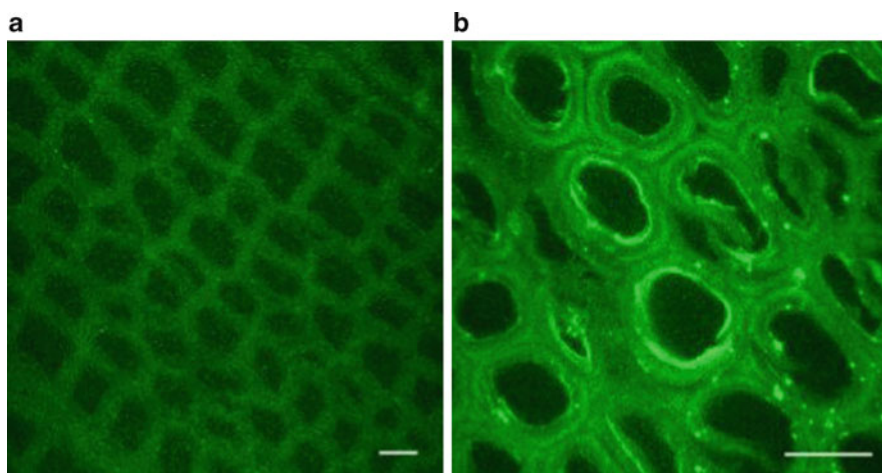
Fig. 3.8 Confocal fluorescence laser scanning microscope images of Norway spruce (*Picea abies*) CW thin sections (25 μm) stained with a primary rabbit antibody on dibenzodioxocin and a secondary anti-rabbit goat antibody labelled with Alexafluor 633. (**a**) Control sample lacking the primary antibody. (**b**) A xylem section treated with the two antibodies and showing the presence of the dibenzodioxocin lignin substructure especially in the innermost part of the tracheid cell walls. *Bars* represent 20 μm (unpublished photos by Kurt Fagerstedt)

in fluorescence lifetime resulting from changes to lignin composition as a result of compression wood formation.

3.3.3.4 Extractives

Very few studies have been done on the amount of wood extractives in CW. In Norway spruce it has been noted that while the amount of water soluble extractives

is at the same level in CW as in NW and OW, the CW shows lower acetone-soluble extractives than NW and OW (Tarmian and Azadfallah 2009). In general, only moderate variations of extractives were observed between NW and CW.

Given the intertwining between lignin and lignan biosyntheses it is not surprising that the expression of several genes common to both pathways be altered in CW. Thus Villalobos et al. (2012) demonstrated that during CW formation the coordinated modulation at transcriptional level of a gene set involved in S-adenosylmethionine synthesis and ammonium assimilation was increased for lignin and coniferyl alcohol-derived lignan synthesis.

3.3.4   Future Prospects

Even though we have gathered a lot of information during the recent decades on the macromolecular components of the CW, many of the physiological and biochemical mechanisms of CW formation still remain unclear. The latest transcriptomic and genomic information has opened up new ways of increasing our understanding of the complicated phenomena leading to changes in the cell wall structures when CW growth rings start to be formed. Even though the effects of plant hormones on CW formation were studied a long time ago, it seems that we are only beginning to understand the intricacies and details of plant hormone interplay in CW formation. Here, work with auxin mutants and ethylene receptor transformants in poplar has already indicated how these hormones may work in TW formation, and we have some indication that they may also take part in CW formation in gymnosperms (Cha 1999; Du and Yamamoto 2007), but it is suggested that other plant hormones and other substances may have a role, individually or in interaction. The interplay of plant hormones in RW formation is discussed in detail in Chap. 4 in this book.

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Chapter 4
The Molecular Mechanisms of Reaction Wood Induction

Kévin Tocquard, David Lopez, Mélanie Decourteix, Bernard Thibaut, Jean-Louis Julien, Philippe Label, Nathalie Leblanc-Fournier, and Patricia Roeckel-Drevet

Abstract Reaction wood originates from cambial activity which adjust cell division activity, proportion of fibres, cell wall structure and properties, so that the resulting growth event will be the appropriate response to endogenous and environmental stimuli.

When addressing the question of the induction of reaction wood formation, the physical parameters inducing reaction wood are first presented leading to discuss the importance of gravisensing versus proprioception (sensing of the local curvature of the stem) in this process.

Molecular candidates for the perception of cellular deformation that is hypothesized to occur in a gravistimulated stem are located at the CPMCW (cytoskeleton–plasma membrane–cell wall) continuum. These candidates would mediate intracellular signalling. Insights from global approaches (e.g. transcriptome and proteome analyses) performed on tilted trees suggest calcium, reactive oxygen species and phosphatidylinositol signalling in the gravitropism sensing network. It has been unambiguously shown that several of the aux/IAA gene family mediators of auxin signal transduction pathway change on induction of tension wood formation. Gibberellins and ethylene seem also to be involved in reaction wood formation. The role of these different plant hormones in upstream primary response to reaction wood sensing or alternatively in the transmission of the signal from the perception to the reaction wood forming cells is discussed.

K. Tocquard • D. Lopez • M. Decourteix • J.-L. Julien • P. Label • N. Leblanc-Fournier •
P. Roeckel-Drevet (✉)
Clermont Université – Université Blaise Pascal, INRA, UMR PIAF, BP 10448, 63000
Clermont-Ferrand, France

B. Thibaut
Laboratoire de Mécanique et Génie Civil (LMGC), CNRS, Université Montpellier 2,
Place E. Bataillon, cc 048, 34095 Montpellier cedex 5, France

4.1 Introduction

Reaction wood (RW) develops in stems and branches in response to the perception of endogeneous and environmental stimuli caused by a change in the natural position. As a result the stem or branch bends back towards its original position. It is generally stated that in most cases, in gymnosperms RW is formed on the lower side of branches and bent stems and is called compression wood (CW). In angiosperms it is formed on the upper side of branches and bent stems and is called tension wood (TW). Wood formed on the other side of branches and bent stems is called opposite wood (OW).

It was commonly believed until the end of the 1980s that RW was induced by the stress state of the new wood layer at cambium vicinity. The bottom of a branch should be in compression thus promoting CW in gymnosperms, the top being in tension should promote TW in angiosperms. For experimentation the challenge was to submit the external layer of a living stem to high tensile or compressive axial stress without any other signal such as gravity or light, and without too big a physiological stress due to the mechanical loading. Fournier et al. (1994) showed that the cross section grows while it is loaded. For each material point, the superposition of stress and strain begins from the time the material is differentiated. That comes from the obvious assumption that "a tissue cannot be loaded before it exists". The main consequence is that the new wood layer does not contribute at all to support the load of the existing trunk or branch and the resulting support stress should be zero at the stem periphery. A change of paradigm therefore had to be made: it is not the stress that induces RW formation but the RW formation that produces different stress levels (in tension or compression) in the new wood layer.

Very often, successive growth events are used by trees "to solve" some mechanical problem, in addition to building of the prescribed structure, in order to restore the posture of an inclined tree (Thibaut et al. 2001; Moulia et al. 2006), to search for the light, to change the tree architecture after death of a major axis, and so on. RW is a solution for a drastic and sudden change in the existing wooden structure of the tree. It is commonly used by all trees, particularly in the juvenile stage. RW is created very locally in answer to a global mechanical problem for the tree by creating a step change in the pre-stressing state of the new layer. According to modelling simulations, the curving efficiency of asymmetrical stressing of the axis using RW is nearly five times higher than the best solution using normal wood asymmetry alone (Almeras and Fournier 2009).

Solving the mechanical problems of a tree through growth is possible because of the flexibility in growth of the meristematic tissues in the length or ramification (primary growth) and the diameter (secondary growth) of each axis (trunks and branches). This structure needs to be mechanically sound and able to respond to most hazards faced by the tree. In the tree, each elementary growth event has to be precisely managed: action or dormancy, rapid or slow cell division, cell differentiation and cell wall formation. And each of these events has mechanical consequences. In addition, the new growth events that involve primary and/or secondary

growth seem to be coordinated at the whole tree level. RW originates from cambial activity (secondary meristem), which adjusts the number of cell division to modify the axis diameter and in particular its second moment of area, as well as the proportion of fibers and the cell wall thickness to modify the density and the mechanical properties of the new layer. Through cambial activity the microfibril angle (MFA) in the S2 layer of the cell wall is also adjusted to modify the axis flexibility both by changing the modulus of elasticity for a given tissue density and the maximum allowed strain before damage (more flexibility appears to be an adaptation to wind); this may also modify the pre-stressing state of the new wooden layer. Last but not least, cambial activity adjusts the chemistry of cell wall components to modify the pre-stressing state of the new wooden layer; this may be done in conjunction with the adjustment of MFA (these changes are discussed further in Chaps. 2, 3, 5 and 6).

Hence, the following questions are raised concerning the induction of RW through modulation of cambial activity. What are the different external or internal signals related to secondary growth in order to solve different mechanical requirements? Where are the perception sites for the new mechanical requirements for the tree? Could a signal get to particular cells in the cambium in order to manage new growth? If such a signal exists from the perceptive cells to the cambium, what about the conversion of the perception into messages transmitted to the secondary meristem? What is the process of "regulation"? How are these messages transcribed in the making of RW? Also most of the questions raised for RW formation could also be addressed to the regulation of primary growth since both primary and secondary growth must be coordinated at the whole tree level.

In this chapter, after reviewing different kinds of signals (gravity, light, mechanical strain) that can induce a mechanical reaction causing RW formation, we will focus on the molecular mechanisms that might be involved in the perception and response to gravity and other mechanical stimuli. Since it is quite clear that the signal perception gives way to synthesis of proteins guiding the production or translocation of various plant hormones, we will review their implication in the gravitropic or phototropic mechanical response inducing the making of RW. We will also discuss the insights provided by global approaches such as transcriptomic, proteomic and metabolomics, made possible by the sequencing and annotation of the genome of trees such as poplar and eucalyptus. In particular, these global approaches gave new information on genes involved in RW formation.

4.2   Perception and Signal Transduction

4.2.1   Physical Parameters Inducing RW

To maintain a branching architecture that is optimal for growth and reproduction, plant stems continuously control their posture to counterbalance environmental physical parameters such as gravity, wind and light, that shift their orientation

from the vertical (Moulia et al. 2006). In trees, this postural control has been mainly studied in response to gravity (Du and Yamamoto 2007). In the primary growing zone of stems, reorientation of woody plant organs involves local differential elongation growth between opposite sides of the stem. In stem parts undergoing radial growth, sectors of RW are produced that can be associated with eccentric cambial growth. In angiosperm woody species, TW is often characterized by fewer vessels and the formation of fibres with smaller diameter containing a gelatinous layer inside the S2 layer of secondary cell walls where cellulose microfibrils are aligned into a vertical orientation (Fig. 4.1 and Mellerowicz et al. 2008; see also Chap. 3) with a lower lignin content (Pilate et al. 2004). In conifers, CW is characterized by tracheids with a thicker secondary cell wall than in normal wood, with higher lignin content, intercellular spaces at cell corners, and a realignment of cellulose microfibrils in the S2 layer orientation with respect to the axis of the stem (Timell 1986). These differences in secondary cell wall biochemical composition and architecture of RW generate internal growth stresses in the stem (Chap. 5), and because of its unilateral formation in the stem, it induces a directional movement, bending the stem towards a favourable position.

As discussed recently by Felten and Sundberg (2013), many experiments were performed where branches or stems are tilted, bent into complicated shapes, grown on clinostats or centrifuges, to identify if a single stimulus is responsible for the induction of RW. For loop experiments on shoots or branches, the localization of RW suggested that its induction depends rather more on positional sensing (sensing of the local angle of the growing organ relative to the gravitational field) than sensing of mechanical stresses such as tensile or compressive stresses (Spurr and Hyvärinen 1954).

However, RW formation is not only induced by gravitational stimulus. TW has been reported to form in the vertical stems of rapidly growing poplar (*Populus*) trees (Telewski 2006). RW was also observed in branches and stems contributing to crowns reshaping after loss of apical dominance (Wilson and Archer 1977) and is part of the mechanism allowing up-righting of apricot tree stems in response to increased shoot and fruit load (Almeras et al. 2004). Plant exposure to wind spray or to repeated stem bending to mimic the wind triggers (1) transitory inclination of the stem, but with a duration of stem inclination much shorter than the presentation time necessary to observe the induction of RW (Jourez and Avella-Shaw 2003) and (2) mechanical signals such as stresses and strains. Exposition of poplar stems to repeated transitory bendings produced a flexure wood with anatomical similarities to TW (Pruyn 1997; Pruyn et al. 2000). In *Abies fraseri*, the morphology and function of wood developed after daily flexures (<20 s) were more closely related to CW than normal wood (Telewski 1989). CW was also detected in wind-treated *Pinus* (Berthier and Stokes 2005). One of the best examples demonstrating that RW is not exclusively induced in response to positional sensing came from analysis of the kinematics of stem straightening (for review, see Moulia et al. 2006; Moulia and Fournier 2009). The characterization of the spatio-temporal curvature field during stem straightening allowed the recognition of a biphasic pattern: an initial phase of spatially homogeneous up-curving due to gravitropic response and a second phase of stem decurving that propagates basipetally to finally reach a steady state where
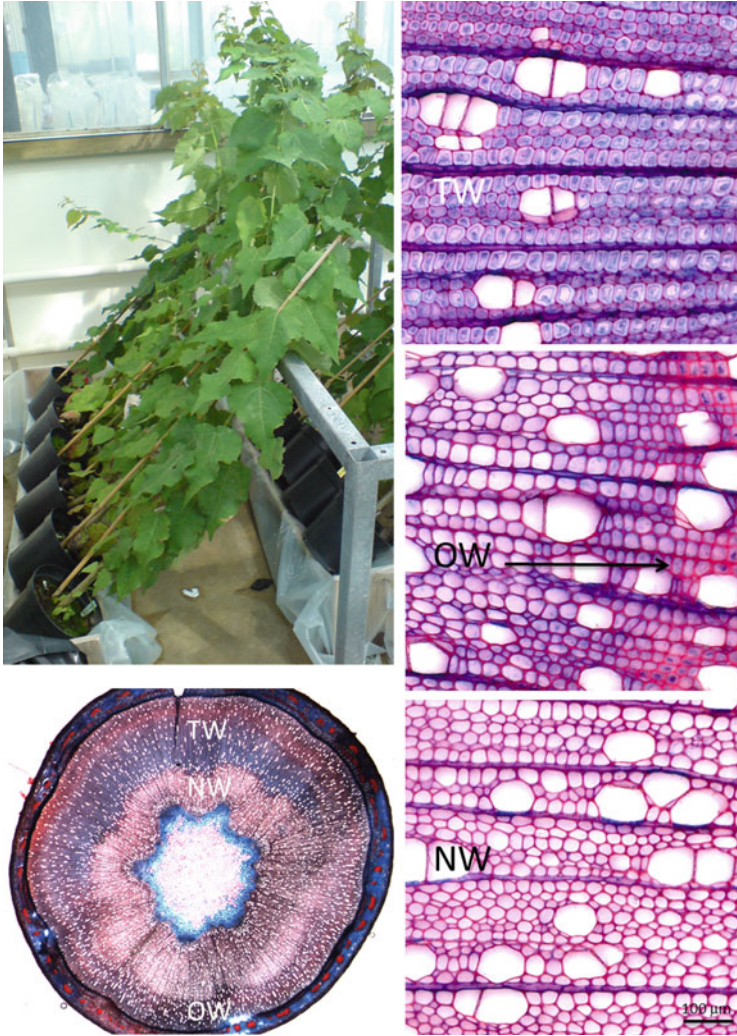
Fig. 4.1 Induction of TW by tilting of hybrid aspen trees (*Populus tremula × tremuloides*). After 17 days of tilting, a band of TW (TW) is seen on the upper side of the tilted stems. The mature normal wood (NW) produced before tilting, and the opposite wood (OW) produced at the lower side of the stems are shown at higher magnification on the right side of the figure. The sections were stained with safranin and alcian blue. Picture courtesy of Ewa Mellerowicz

the curvature is concentrated to the base of the growth zone. In some plants, this decurving phase occurred before the shoot apex had overshot the vertical, demonstrating that this phenomenon was not due to the perception of the inclination angle, and was called autotropism. This autotropic decurving has been observed in many plants (Moulia et al. 2006). Studying this process in poplars (*Populus nigra × Populus deltoides*), Coutand et al. (2007) observed that no part of the trunk overshot

the vertical during stem straightening. Indeed, during the initial phase of up-righting, arcs of RW were detected homogeneously all along the upper side of the trunk, whereas a second sector of RW was produced on the initially lower side in the most distal part of the trunk, contributing to this autotropic decurving. Recently, Bastien et al. (2013) studied the gravitropism kinematics of different organs from 11 angiosperms by time lapse photography, including both primary elongation zones and zones of secondary growth in which active bending is achieved through the production of TW. The biphasic pattern of tropic reactions described above was found to be generic whatever the type of the organ, and it was shown to lead to a steady state shape in which the apical part is straightened whereas the curvature is more concentrated at the base of the stem. However, inter- and intra-specific variability occurred in the steady states and in the transients: whereas some plant organs reached a steady state without overshooting the vertical, others exhibited oscillations around the vertical axis. Bastien et al. (2013) also demonstrated that the minimal dynamic model cannot involve only gravisensing but the simultaneous sensing of the local curvature, referred by the authors as proprioception. When the organ is tilted and straight, gravisensitive control is dominant and the organ bends up actively. However as curvature increases, the inclination angle decreases and proprioceptive control takes over and autotropic counter-bending is produced starting in the apical parts of the organs, allowing it to straighten and align with the gravity vector. These data also suggest that the different shapes observed along the straightening response reflected a different ratio between graviceptive and proprioceptive sensitivities.

Another physical parameter that can influence reorientation of the stem by RW formation is light interception. Because of their co-occurrence under natural conditions gravi- and phototropism are intrinsically linked (Correll and Kiss 2002). Remarkably, phototropism sensing converges to common molecular actors with gravitropism and notably auxin transport and perception (Hohm et al. 2013). Typically, stems and stem-like organs have positive phototropism and negative gravitropism. Additive or synergetic effects were reported (Kern and Sack 1999) making the identification of their relative contribution and their possible interactions problematic. To address this issue, gravity effects were experimentally reduced or abolished (e.g. microgravity, space flight) while applying directional light source to induce phototropism (Millar and Kiss 2013). To date, it remains technically difficult to alleviate or reduce gravity effects on trees. The few possibilities offered to researchers consist in the manipulation of gravity orientation by tilting potted trees in combination with directional light modifications. Matsuzaki et al. (2006) report phototropism in trees submitted to different gravitational stimulations as observed on mountain slopes. Basal parts of the trees did not show bending in response to tropisms, which was limited to upper parts of stems. The authors suggested that reorientation could be achieved by asymmetric radial growth due to the formation of RW as is the case for gravitropism and further proposed trees inclination on slopes depends on phototropism. The same team later proved that the mechanism involved in phototropism required differential xylem production (Matsuzaki et al. 2007). In a recent study, Collet et al. (2011) studied long-term (4 years) phototropic response of *Fagus sylvatica* and *Acer*

pseudoplatanus after canopy opening in natural conditions. Plants reacted by righting themselves towards the light source and this involved reorientation of the lignified parts of the stems through asymmetrical formation of RW. Herrera et al. (2010) noticed changes in the orientation of apical part of pine seedlings but not in the basal parts even after 22 days of light and gravi-stimulation. Although limited to primary growth, this work provided molecular data on the interaction of these two tropisms, scarce for tree models. Interestingly, photo- and gravitropic responses of potato plants were different depending on the time of the day suggesting they were also regulated by an endogenous circadian clock to some extent (Vinterhalter et al. 2012). Such complexity, far from being completely understood in herbaceous plants, still needs to be established in trees where secondary growth in reaction to phototropism and gravitropism is still a matter of exploration.

All these data converge on the induction of RW during plant postural control. Clearly the triggering of RW formation during tropic reaction not only is related to the sensing of the inclination of the stem versus gravity but also involves curvature sensing. TW has been shown to systematically be formed on the lower side of the branch when autotropism dominates gravitropism, allowing for curvature compensation (Coutand et al. 2007). Similar shifts in the location of RW along the tropic motion have also been described for CW in conifers (Sierra de Grado et al. 2008). But, how these diverse physical parameters (gravity, local curvature, light) are perceived by plant cells in order to induce RW remains unclear. Are all these physical parameters perceived by a common sensing mechanism or is there any crosstalk at a later stage during the signaling pathway?

4.2.2   Molecular Mechanisms Involved in the Perception of Mechanical Stimulation Leading to RW Formation

In case of a gravitational stimulus, the resulting physical forces can deform or move objects of specific mass inside the cell. Two hypotheses are currently favoured: (1) the amyloplast-sedimentation in specialized cells named statocytes and the perception of the direction of this sedimentation and (2) the weight of the protoplasm itself triggering mechanical deformation of subcellular structures such as membrane, cytoskeleton elements and cell wall (Baluška and Volkmann 2011).

The role of starch-filled amyloplast sedimentation during graviperception is well documented in *Arabidopsis* (Morita 2010). In young shoots, statocytes are localized in the endodermis layer surrounding vascular tissues. These cells are highly vacuolated and equipped with prominent F-actin bundlets (Morita et al. 2002). The studies of different mutants affected either in starch formation (*pgm*) or in intracellular components such as the vacuolar membrane (VM) or actin microfilaments (AFs) that both modify cytoplasm viscosity and activity showed that amyloplast dynamics are important during shoot gravisensing (for review, Hashiguchi et al. 2013). Recently, by using a centrifuge microscope to analyse gravitropic

mutants in *Arabidopsis*, Toyota et al. (2013) confirmed the importance of amyloplast movement perception during shoot gravisensing. In woody species, amyloplast localization in endoderm cells has been observed (Nakamura 2003) in the young shoots of Japanese flowering cherry tree (*Prunus spachiana*) and in young poplars (*Populus tremula* × *alba*) (Fig. 4.2a–d). However, a link between amyloplasts sedimentation and RW formation has not yet been demonstrated. Moreover, the endoderm is disrupted by secondary growth. In cross sections of older poplar stem, lugol-stained starch grains are observed in whole bark tissue as well as in the wood rays (Fig. 4.2e).

The cellular mechanism underlying curvature proprioception is unknown (Bastien et al. 2013). The sensing of cell deformation (strain sensing) or more precisely of the deformation of some cellular component is a good candidate (Wilson and Archer 1977; Bastien et al. 2013). Moreover the perception of the deformation of cellular components is suggested also through amyloplast sedimentation, in the gravitational pressure model (Baluška and Volkmann 2011), and through wind sensing (Moulia et al. 2011). A few molecular candidates have been identified as mechanoperceptors of this deformation. In trees, these are only putative. Results obtained from the herbaceous model plant *Arabidopsis thaliana* suggest molecular candidates at the CPMCW (cytoskeleton—plasma membrane—cell wall) continuum. They would be able to sense the mechanical signal from the plant cell wall and convert it into a molecular signal in the cell (Baluška et al. 2003; Telewski 2006).

The first molecular candidates are mechanosensitive (MS) ion channels. MS ion channels are membrane proteins that allow the transit of ions through cellular membranes. They open either directly by the force applied on the membrane or indirectly through links between the channels and/or both the cytoskeleton and cell wall components (Haswell et al. 2011). To date the involvement of MS ion channels in the perception of mechanical signals has yet to be clearly established. Nevertheless, several pieces of evidence show that ionic changes occurred after mechanical signals (Haley et al. 1995). In plants, MS ion channels (Cl^-, K^+ and Ca^{2+}) have been identified at plasma membranes using patch-clamp electrophysiology (Cosgrove and Hedrich 1991; Ding and Pickard 1993; Haswell et al. 2008). Currently three main groups of MS ion channels have been described in plants. First, the mechanosensitive channel of the small conductance (MscS) family from *Escherichia coli* which releases osmolytes from the cell (Booth and Blount 2012) has ten homologues (MSL1–10) in the *A. thaliana* genome (Pivetti et al. 2003). MscS homologues may release osmolytes in response to membrane tension and may be modulated by additional signals (Monshausen and Haswell 2013). The second Mid1-complementing activity (MCA) family is structurally unique to the plant kingdom. The MCA proteins are located in the plasma membrane and promote calcium influx upon mechanical stimulation (Nakagawa et al. 2007). Finally, the Piezo proteins are a class of MS cation channels which respond to mechanical stimuli (Coste et al. 2012; Kim et al. 2012). In *Arabidopsis*, there is a single Piezo protein (Coste et al. 2010; Kurusu et al. 2013; Monshausen and Haswell 2013) but it has yet to be characterized.
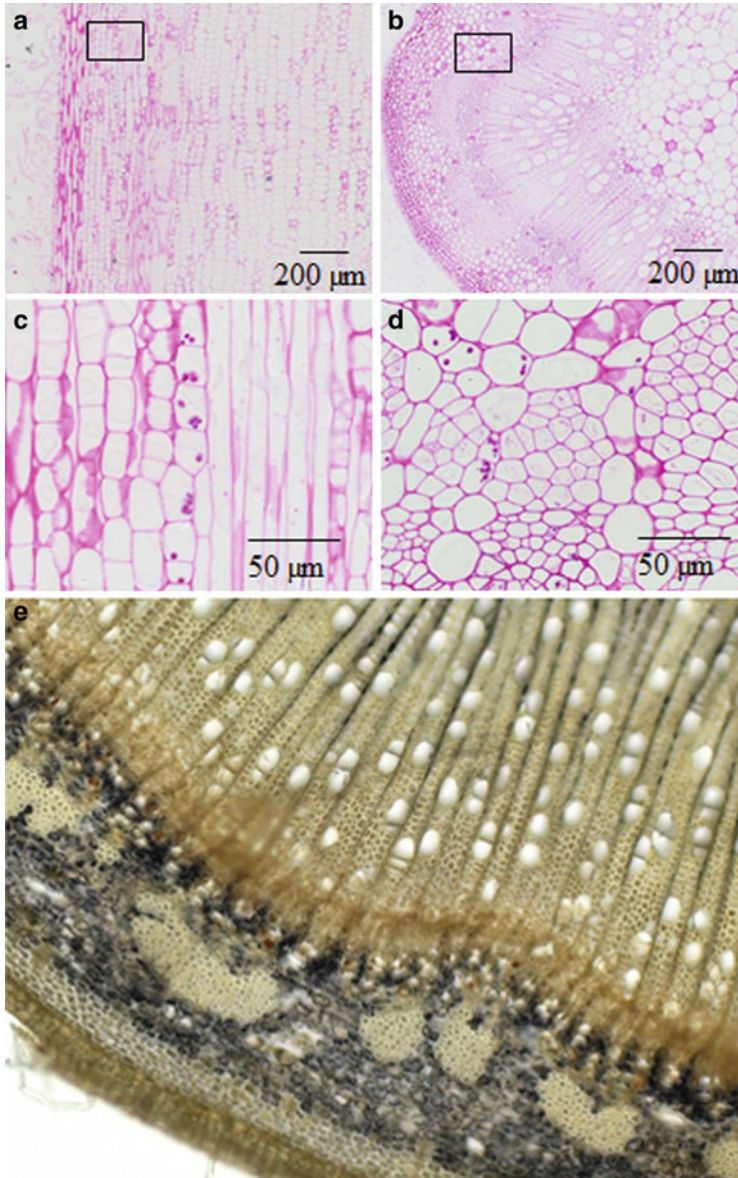
Fig. 4.2 Sections of poplar stems (*Populus tremula × alba*). Photographs (**a–d**) represent sections from the fourth bottom internode of a young plant with 20 developed internodes. Longitudinal sections (**a**, **c**) or transversal sections (**b**, **d**) were stained using Periodic acid/Schiff (PAS) reaction to detect starch and polysaccharides. Photographs (**c**) and (**d**) are, respectively, enlarged views of the photographs (**a**) and (**b**) (*black rectangle area*). Photographs courtesy of Wassim Azri. (**e**) This picture represents a cross section of a poplar stem, 40 cm under the apex. The tree height was 2.35 m and the diameter of the section is 5.3 mm. The freehand cut was stained by lugol. Photographs courtesy of Kevin Tocquard

Other molecular candidates are receptor-like kinases proteins (RLKs) that are a family of proteins with an extracellular domain, a single transmembrane region and an intracellular cytoplasmic kinase (Shiu and Bleecker 2001). Among RLKs, wall-associated kinases (WAKs) are the most well-studied potential cell wall status receptors (He et al. 1999; Verica and He 2002). They are of particular interest because WAKs extracellular domains are able to bind to the cell wall (He et al. 1996). Notably, Wagner and Kohorn (2001) showed that *At*WAKs covalently bind the cell wall pectins, in a calcium-induced conformation (Decreux and Messiaen 2005). Moreover, a reduction of *WAK* expression inhibited cell elongation and altered morphology (Lally et al. 2001; Wagner and Kohorn 2001), indicating an activity in growth control. Therefore, WAKs are interesting candidates as sensors of cell wall integrity. Another candidate among RLKs for sensing the cell wall integrity is the *Catharanthus roseus* RLK1-like subfamily (*Cr*RLK1L). The 17 members present an extracellular malectin-like domain (Lindner et al. 2012). Malectin proteins bind to glycoproteins in animal endoplasmic reticulum (Qin et al. 2012). The hypothesis that the malectin-like domain of CrRLK1L proteins binds cell wall polysaccharides or glycoproteins in plants has been proposed (Monshausen and Haswell 2013). In this subfamily THESEUS1 (THE1), a particular member with a plasma membrane location, could be a candidate for cell wall integrity sensing in *Arabidopsis*. THE1 was identified at a suppressor screen of cellulose-deficient mutant *cesA6* (Hématy et al. 2007). The *the1* mutant partially restored the hypocotyl elongation of the *cesA6*. However, mutations or over-expression of the *THE1* gene did not exhibit any effects in *Arabidopsis*. Consequently, THE1 was therefore proposed as a sensor of the cell wall status and modulator of cell elongation during perturbed cellulose synthesis.

Members of ArabinoGalactan proteins (AGPs) bind pectins and are also hypothesized to be cell wall integrity sensors. Indeed, AGPs are highly glycosylated proteins located in the cell wall (for more detail see Chap. 3). Some AGPs bind cell wall pectin (Serpe and Nothnagel 1995) and could be attached to the plasma membrane via GPI anchors which would mediate intracellular signaling (Humphrey et al. 2007).

4.2.3 Mechanical Signal Transduction: Secondary Messengers

Regarding secondary messengers, few data are available in trees and almost lacking in the context of gravitropic stimulation and/or stem bending. However, herbaceous plant model data can be a starting point for future studies on tree models. According to Toyota and Gilroy (2013), calcium is an important and ubiquitous cell secondary messenger. Its specific role as a secondary effector in MS signaling has been extensively investigated in *Arabidopsis* and *Nicotiana* (Knight et al. 1991, 1992; Haley et al. 1995; Plieth and Trewavas 2002; Toyota et al. 2008). Gravity

stimulation of *Arabidopsis* seedlings indicated a cytosolic Ca^{2+} concentration $[Ca^{2+}]_{cyt}$ increase with a first sharp increase followed by another less intense but longer signal (Plieth and Trewavas 2002). Wind also induced $[Ca^{2+}]_{cyt}$ increase in *Nicotiana* and *Arabidopsis* seedlings (Knight et al. 1992; Haley et al. 1995; Plieth and Trewavas 2002). Experiments conducted on trees support the involvement of Ca^{2+} in RW formation. By using a Ca^{2+} chelator (EGTA) or a calcium channel inhibitor ($LaCl_3$) which allowed modification of Ca^{2+} availability, CW formation was inhibited in *Taxodium distichum* gravistimulated stems (Du and Yamamoto 2003). The involvement of calcium in TW induction has also been suggested by indirect data obtained from several global approaches (see Sect. 4.4) notably through regulation of protein accumulation such as calreticulin, a Ca^{2+} storage protein (Azri et al. 2009). The overall results suggested Ca^{2+} ion as a second messenger in the early stages of mechanical signal transduction.

Other secondary messengers have been identified as important after mechanical/gravitational stimulation. Azri et al. (2009) suggested the involvement of reactive oxygen species (ROS) in poplar TW formation induced through the accumulation of glutathione-dehydroascorbate reductase (GSH-DHAR), glutathione S-transferase (GST) and thioredoxin *h* (Thr *h*) proteins. Azri et al. (2013) further showed an induction of the *Thr h* gene in response to gravitropic stimulus. With an immuno-chemistry approach, they co-located Thr *h* proteins with amyloplasts in stem endoderm cells, thus providing a coherent framework for graviperception. More evidence from herbaceous models demonstrates that an interplay of ROS and Ca^{2+} could mediate mechanosensing: ROS can stimulate $[Ca^{2+}]_{cyt}$, and an increase of $[Ca^{2+}]_{cyt}$ can also lead to a ROS production via NADPH oxidase (Mori and Schroeder 2004).

Molecular candidates to convert the transient ionic Ca^{2+} signal to long-term biochemical signal are mainly calmodulins (CaM) and Ca^{2+}-dependent protein kinases (CDPK). Calmodulins are Ca^{2+}-dependent regulation proteins linking calcium to MS. CDPK are cytosolic proteins with a kinase domain, an autoinhibitory domain, and a calmodulin-like domain that bind calcium ions (Hrabak et al. 2003). In poplar, the expression of calmodulin encoding genes is up-regulated as soon as 10 min after a transient stem bending (Martin et al. 2009). Although very likely, a role for these proteins in the Ca^{2+} signaling after mechanical stimulation still needs to be demonstrated.

Phosphatidylinositol signaling is another part of the gravitropism sensing network (Strohm et al. 2012). Membrane phospholipid Phosphatidylinositol 4,5-bisphosphate (PIP2) releases inositol 1,4,5 triphosphate (IP3 or InsP3) after phospholipase C (PLC) hydrolysis. InsP3 is accumulated during gravitropic response which later is repressed through PLC inhibition (Perera et al. 2001). InsP3, like Ca^{2+}, is a second messenger suggested as triggering intracellular calcium flow from vacuole (Allen et al. 1995).

Taken together, these results indicate that complex and interactive signaling pathways are involved after mechanical/gravitational stimulation. To date, no study has yet addressed these mechanisms in tree stems. Numerous questions remain to be resolved to understand the induction of RW. Why RW is produced in only one side

of the stems, whereas the physical parameters triggering RW production are potentially perceived on both sides of the stem. Are the putative receptors uniformly localized in cells? Furthermore, if the endodermis cells are considered as sensitive cells, how is the inductive signal transmitted to cambium cells or developing xylem cells to modify the secondary wall composition and architecture? What cells act as gravisensor in older stems? How and where does crosstalk between gravisensing and proprioception occur?

4.3   Signal Transmission to Reaction Wood-Forming Cells

4.3.1   Hormone Action and Reaction Wood

The anatomical and biochemical features of RW have been the subject of extensive studies, which are described and reviewed in Chaps. 2 and 3. However the physiological and molecular signals inducing the formation of RW remain poorly described. Several plant hormones have been implicated in the regulation of cambial cell proliferation activity and in wood cell differentiation (for review, see Elo et al. 2009; Nieminen et al. 2012; Ursache et al. 2013). Until recently, their role in RW formation was studied through the application of exogenous compounds, the hormones themselves, their antagonists or transport/perception inhibitors. These experiments proved to be very informative as a first step towards deciphering whether hormones have the potential to induce RW formation. However, the possibility to genetically transform poplar and the use of high throughput molecular technics are, and will continue to be, of great help in confirming and completing these observations.

4.3.1.1   Auxin

The application of auxin antagonists or transport inhibitors on both gymnosperm and angiosperm tree stems led to the assumption that auxin plays a role in RW formation. Increased amounts of auxin could induce CW formation in gymnosperms while a deficiency in auxin was required to form TW in angiosperm (for review, see Du and Yamamoto 2007; Felten and Sundberg 2013). However, such experiments do not prove the function of auxin under natural conditions because of the risk of uncontrolled modification of auxin homeostasis. To be validated, they need a better documentation of *in planta* auxin concentrations/amounts. Reports on the relationship between endogenous auxin levels and the formation of CW or TW are scarce and sometimes contradictory. For example, while Wilson et al. (1989) did not find a clear and conclusive correlation between the occurrence of CW and auxin concentrations, Funada et al. (1990) working on *Cryptomeria japonica* and Du et al. (2004) working on *Metasequoia glyptostroboides* found a higher amount

of endogenous IAA (indole-3-acetic acid) in the cambial region producing CW. More recently, Hellgren et al. (2004) conducted a kinetic survey of endogenous IAA distribution across the cambial region of Aspen (*P. tremula*) and Scots pine (*Pinus sylvestris*) trees after bent-stem gravistimulation. These experiments showed that RW could be formed even when the pattern of IAA distribution in the cambial region is unmodified. The authors concluded that modulation of the auxin concentration gradient across the cambial zone might not be the signal that maintains the cells in an RW developmental state.

Data found in the literature are contradictory and were obtained on different species with different techniques and at different time points after stimulation. It is therefore difficult to give a clear model of auxin role in RW formation. However, an involvement of auxin in the early steps of the induction process has not yet been ruled out. Actually, several reports showed that auxin signaling is responsive to gravistimulation. After 6 h of stem bending, the expression of two *AUX/IAA* genes in poplar (*P. tremula* × *tremuloides*) (Moyle et al. 2002) and one in *Eucalyptus* (Paux et al. 2005) was altered in TW compared to normal wood in unbent trees. In yellow poplar (*Liriodendron tulipifera*), a species that does not produce a typical G-layer, the expression of ARFs and AUX/IAA genes as well as other auxin-related genes is modified in TW compared to OW (Jin et al. 2011). Similar results were also obtained in poplar (*P. tremula* × *tremuloides*) after 3 weeks of TW induction by leaning the stem (Andersson-Gunnerås et al. 2006).

High throughput approaches have already helped in improving our knowledge of auxin function in TW formation. Coupling these with functional genomic approaches could help to gain a better understanding of the role of auxin in TW formation.

4.3.1.2 Ethylene

The gaseous hormone ethylene has long been known to be produced in response to diverse stresses including mechanical solicitations (for review, see Braam 2005; Telewski 2006; Du and Yamamoto 2007) such as bending and tilting. These stresses usually stimulated wood production by increasing cambial activity and sometimes led to the production of RW. Like auxin, ethylene involvement in RW formation was first investigated by measuring ethylene or its precursor (ACC: 1-aminocyclopropane-1-carboxylic acid), and by using application experiments. For example, in the vascular cambium of *Pinus contorta* Dougl. ssp. *latifolia* branches, endogenous ACC was detected in association with CW differentiation, but not with OW (Savidge et al. 1983). Applications of ethrel, an ethylene releasing compound, stimulated wood production and were able to modify anatomical features of xylem in gymnosperm and angiosperm trees (Du and Yamamoto 2007). In angiosperm, although some of these features, like fewer and smaller vessels, can be reminiscent of TW characteristics (Little and Savidge 1987; Du and Yamamoto 2007; Love et al. 2009), it has to be noted that there is no report of G-layer induction by ethylene treatments.

Molecular approaches have helped to gain new insights about regulation of TW formation by ethylene. In bent poplars (*P. tremula* × *tremuloides*), a clear induction of *PttACO1* (ACC oxidase the last enzyme in the ethylene biosynthesis pathway) expression and relative activity were observed in the TW-forming tissues (Andersson-Gunnerås et al. 2003). Therefore, PttACO1 may represent a major control of ethylene asymmetric production during TW formation. In poplars leant for 3 weeks, the expression of genes related to ethylene signaling was also modified in TW compared to OW (Andersson-Gunnerås et al. 2006). In *L. tulipifera*, such modifications were seen as soon as 6 h after bending of the stem (Jin et al. 2011). Love et al. (2009) combined the use of ethylene-insensitive trees, ethylene-overproducing trees, and the application of the ethylene-perception inhibitor MCP (1-methylcyclopropene) to explore ethylene physiological function in gravistimulated poplars. They showed that ethylene could be responsible for the stimulation of cambial cell divisions on the upper TW-forming side of leaning stems. In 2013, Vahala et al. identified 170 gene models encoding ERFs (ethylene response factors) in the *Populus trichocarpa* genome. Among these, 17 had a minimum of a twofold induction of expression in TW compared to normal wood. Over-expression of some of them in poplar resulted in anatomical or wall chemistry modifications that are reminiscent of TW features.

Ethylene and its signaling pathway seem therefore to control part of the molecular and physiological modifications underlying RW formation, especially the asymmetric increase in radial growth. However, it seems that the establishment of the full characteristics of RW involves ethylene in combination with yet unidentified other signaling factors (Love et al. 2009; Vahala et al. 2013; Felten and Sundberg 2013).

4.3.1.3 Gibberellins

Gibberellins (GAs) constitute another group of plant hormones known to promote secondary growth and xylem fibre length (Eriksson et al. 2000; Mauriat and Moritz 2009; Gou et al. 2011). Applications of exogenous GAs or GA inhibitors to tree stems can only provide indirect evidence for a role of GA in RW formation. In gymnosperms, the possibility of a role of GAs in CW formation has not yet been clearly demonstrated. However, experiments conducted on upright or tilted angiosperm trees helped to establish a correlation between GA and TW formation. For example, it has been shown that the application of GA to vertical stems of *Fraxinus mandshurica*, *Quercus mongolica*, *Kalopanax pictus* and *Populus sieboldii* induced the development of TW with typical G-fibres in the absence of gravistimulus (Funada et al. 2008). When tilted *Acacia mangium* seedlings were treated with GA their negative gravitropsim was stimulated. On the contrary, when they were treated with paclobutrazole or uniconazole-P, inhibitors of gibberellin biosynthesis, the gravistimulated upright movement of the acacia stems was inhibited and the formation of TW was suppressed (Nugroho et al. 2012, 2013).

No functional genomic experiment has yet proved that gibberellins could control RW formation. However, the use of a natural weeping mutant of *P. spachiana* brought some evidence for a role of GAs in TW formation. Exogenous application of GAs on branches of these Japanese cherry trees (*P. spachiana*) stimulated cambial growth and promoted TW formation on the upper side of branches resulting in an upright movement (Nakamura et al. 1994; Baba et al. 1995; Yoshida et al. 1999).

Together these results indicate that GAs seem to be involved in RW formation. However, more direct supporting evidence and a better understanding of the involved signaling factors is still needed to make a clearer conclusion.

As mentioned above, although sometimes quite indirect, an important amount of data indicates a role for auxin, ethylene and gibberellins in RW formation. This holds especially true for angiosperms since knowledge obtained on CW is less advanced. On the contrary, no such relation has been identified for cytokinins, abscissic acid or brassinosteroids. Most of the experiments designed to gain a better understanding of the role of plant hormones in TW formation focused on a single hormone, studied independently. It is, however, important to keep in mind that many hormones have been shown to interact with each other in a synergistic or inhibitory manner. For example, GA is known to stimulate polar auxin transport (Björklund et al. 2007) and IAA to promote ethylene biosynthesis (Abeles et al. 1992). Although the studies made so far have greatly improved our knowledge of RW biology, the use of high throughput molecular technics combined with functional genomics has started to, and should in the future, help to gain a deeper understanding of the processes underlying RW development. Moreover, hormones are currently mostly regarded as upstream primary responses to TW sensing (Felten and Sundberg 2013), but studies on hormone distribution and transport are still too scarce and contradictory to rule out the possibility of their involvement in the transmission of the signal from the perceptive to the RW-forming cells.

4.3.2 Other Candidates for Signal Transmission to Reaction Wood-Forming Cells

miRNAs are small non-coding RNA molecules (about 21 nucleotides) which cleave or degrade messenger RNA targets. In plants, they are involved in the regulation of a large number of physiological processes (Jones-Rhodes et al. 2006) through the targeting of cell metabolism, signal transduction and stress response mRNAs. Different authors (Griffiths-Jones et al. 2008; Lu et al. 2005, 2008; Zhang et al. 2010) have characterized mechanical stress-responsive miRNAs in *P. trichocarpa*, especially miRNA that were differentially regulated by bending. The predicted target genes encode transcription factors and proteins involved in various cellular processes. For example, the function of the target of miR1446 is a gibberellin response modulator-like protein and the target of miR160 is an auxin

responsive factor. Although a direct link between miRNAs and RW has never been proven, the above-mentioned data indicate that these small molecules could be good candidates to explore the molecular network controlling RW formation. To do so, further genome-wide identification of miRNAs using a different experimental design (inclination) is needed, as well as functional characterization of the identified miRNA and corresponding targets.

Recently, several authors reported intercellular signaling by miRNAs and showed that some can move from one cell to another or over long distances (for review, see Marín-González and Suárez-López 2012). Since signaling from the cells that perceive the RW-generating stimuli to the RW-forming cells may require long distance regulation of gene expression, it is tempting to consider miRNAs as good candidates for the signal transmission from perceptive cells to RW-forming cells.

4.4   Insights from Global Approaches

Despite the economic impact of RW occurrence in industrial process and its importance from a tree developmental point of view, the molecular mechanisms involved in the perception and response to the gravitational stimulus have not been extensively studied. Furthermore, very few studies have addressed this question by global approaches, which require the genome of the studied tree species to be sequenced and annotated.

Investigating the induction of RW is also a very complex question because RW is formed very locally in answer to a global mechanical problem for the tree. In addition most of the regulations used in RW formation (division rate, cell elongation, cell wall thickening, MFA setting) are also used for normal wood formation. Experimental setups have to take into account this point to specifically address the question of RW induction. Most of the studies on genes or proteins acting as regulators of RW making were done on inclining experiments. By inclining the whole tree system by an angle of around 30° and letting it grow afterwards (see Chap. 5) a pure, long-lasting RW formation is induced at the base of the stem (see Fig. 4.1 and Fig. 9.11). At inclination angles of this magnitude there is a strong perception of disequilibrium, secondary growth processes are very active and no new primary axillary growth is observed. This is in contrast to very inclined (nearly horizontal) trees, which use growth through axillary buds (i.e. primary growth) to create new vertical axes.

4.4.1   Transcriptome Analysis

Transcriptomics of RW is still in its infancy. Quite limited reports are available although studies have been conducted for about two decades. Regarding the vast majority of transcriptomics works on normal wood, the reader should refer to the

most recent review of Zhong and Ye (2013). Early work addressed gene expression during RW formation through target genes approaches, leading progressively to recent transcriptome-wide overviews. Tools for deciphering gene involvement in the control of RW formation are becoming increasingly available and although most recent gene expression measurement tools, such as RNAseq, are still under-used in this research field, hopefully this will change in the near future.

Most studies of angiosperm RW formation using transcriptomics have been conducted with poplar species and less frequently with *Eucalyptus grandis*, *Eucalyptus globulus* and *Eucalyptus nitens*. Other angiosperms species have been rarely studied with the exceptions of *A. thaliana* and the tulip tree (*Liriodendron* sp.). In gymnosperms, most studies have been conducted with *Picea taeda* although *Pinus pinaster*, *Pinus radiata*, *Picea abies*, *Picea glauca* and *Chamaecyparis obtusa* have also been examined. Target gene studies began in this research area with the reporting of the involvement of 4-coumarate:coenzyme A ligase (4CL) during CW formation (Zhang and Chiang 1997). Along with up-regulation of *4CL* transcripts, the corresponding enzyme activity was also increased and its impact on lignin composition was observed. Regulators of lignin biosynthesis have been targeted as well, namely MYB factors in *P. glauca* (Bedon et al. 2007).

Meanwhile, transcriptome profiling started with the pioneering work showing *Pinus taeda* transcripts down-regulated for genes involved in lignin biosynthesis (Allona et al. 1998) and later in *C. obtusa* (Yamashita et al. 2008). At the same time, a large diversity of responses was reported for carbohydrates enzymes, i.e. xyloglucan endo-transglycosylase (XET), in CW using 1,097 ESTs in a co-expression clustering study (Allona et al. 1998). AGPs were reported as key-players through cDNAs encoding six novel so-called cell wall-associated proteins in CW formation by the same approach (Zhang et al. 2000). These results were further developed with a set of 2,400 ESTs from a cDNA microarray where 33 out of 69 transcripts were differential in CW and related to monolignols biosynthesis (Whetten et al. 2001). Following this, studies on angiosperms provided additional information by deciphering the gelatinous layer (G-layer) deposition in the so-called G-fibres during TW formation. Fasciclin-like arabinogalactan (FLA) proteins and their corresponding transcripts have been intensively studied emphasizing their involvement as a hypothetical adhesion factor facilitating cellulose deposition in the G-layer during TW formation in *P. tremula* × *tremuloides* (Andersson-Gunnerås et al. 2003, 2006), *P. tremula* × *alba* (Lafarguette et al. 2004), *E. grandis* (Qiu et al. 2008), *E. nitens* and *A. thaliana* (MacMillan et al. 2010). Along with FLA studies, transcriptional mechanisms of saccharide metabolism and deposition were also functionally dissected, even down to the cell scale using microgenomic tools (Goué et al. 2008). Major works outlined the role of sucrose synthase (SuSy) in *P. tremula* × *alba* (Déjardin et al. 2004), cellulose synthase (CesA) in *E. globulus* (Paux et al. 2005), XET and xylo-glucan endo-transglycosylase/hydrolase (XTH) in *P. tremula* × *tremuloides*, *Populus alba* and *P. tremula* (Nishikubo et al. 2007) and XET in *L. tulipifera* (Jin et al. 2011).

With the improvement of sequencing facilities and transcriptome-wide studies, development of collections of ESTs related to wood formation transcriptomics were

set-up, some of them including data related to RW formation as for *P. tremula* × *tremuloides* (Sterky et al. 2004), but also including expression data in RW for several other poplar species (Sjodin et al. 2009), for *P. abies* (Koutaniemi et al. 2007), for *P. radiata* (Ramos et al. 2012) and for *P. pinaster* (Villalobos et al. 2012).

A striking point with timescale studies of RW formation is that very few reports deal with early molecular events of the process. In other words, most of the published works dealing with transcriptomics of RW formation, either gene-targeted or genome-wide, focus at developmental stages when RW is already histologically observable in the xylem. Precursor work in the field of the induction of RW does exist but is only gene-targeted at the moment. ZFP2 transcription factor was firstly reported in *Juglans regia* (Leblanc-Fournier et al. 2008) and *P. tremula* × *alba* (Martin et al. 2009). This ZFP2 is coined "mechano-sensitive" and addresses the xylem cell response to mechanical stress at a very early stage in a timely and structured manner in the transduction pathway to TW formation in trees, along with TCH2 and TCH4 as reported in *P. tremula* × *alba* from quantitative PCR studies (Martin et al. 2010). TCH4, reported to encode an XET in *A. thaliana* (Xu et al. 1995), draws attention to enzymatic-oriented cellulose modifications in the cell wall. The field of early RW induction, at the cell level and before any macroscopic tissue organization can be observed in the stem, is hopefully a must in any forthcoming experiments.

4.4.2 Techniques for Proteome Measurement

Proteomics is a powerful molecular tool for describing proteomes at the organelle, cell, organ or tissue levels and for showing the modifications of protein expression in response to environmental changes (Abbasi and Komatsu 2004). Proteomics completes the large-scale analysis of the transcriptome. On many occasions, the level of mRNA is not correlated with protein expression level. One transcript may be translated into more than one protein because of alternative splicing or alternative post-translational modifications. In addition, post-translational modifications such as phosphorylation and glycosylation may modify protein activities and subcellular localization (Yan et al. 2005).

Although attempts have been made at identifying proteins whose abundance, localization, and/or post-transcriptional modifications are altered by gravistimulation, most studies were conducted on *A. thaliana* seedlings and tended to unravel the mechanisms that control root gravitropism (for review, see Harrison et al. 2008). As for the understanding of the response of tree shoots to gravity, both gymnosperm and angiosperm species should be considered separately since the structure and properties of CW are different from those of TW. Although studies have been conducted to elucidate wood formation in trees, few of them have addressed the problem at the proteomic level, and even less focussed on RW genesis.

Among the first global attempts to unravel xylogenesis in trees, two-dimensional (2D) electrophoresis has been used to characterize xylem maritime pine proteins (Costa et al. 1999) or seasonal changes in protein expression in wood-forming tissues of poplar (Minsbrugge et al. 2000). The first description of the proteome of maritime pine wood-forming tissue (identification of 175 proteins) was provided by Gion et al. (2005). The variations in the proteome of differentiating xylem collected from the top to the bottom of adult maritime pine (*P. pinaster*) trees have provided a list of candidate genes for wood properties (Paiva et al. 2007). Using a large-scale approach, regeneration of the secondary vascular system in poplar was studied after peeling of the bark and sampling by scraping regenerating tissues (Juan et al. 2006). A dynamic view of the changes occurring during the juvenile wood formation in the proteome of *E. grandis* has been provided using xylem tissues from 3- and 6-year-old trees (Celedon et al. 2007). More recently, a focussed analysis of plasma membrane proteomes from different tissues isolated from 3 to 4 m high poplar trees identified 108 proteins that were specifically expressed in the xylem (Nilsson et al. 2010). The authors proposed a schematic model for wood formation, integrating proteins expressed in the xylem such as cellulose-synthesizing complex, receptors, glucan sythase, AGPs, and enzymes of lignin biosynthesis. In particular the thorough investigation of cellulose synthase complexes in differentiating *Populus* xylem has been realized using complementary approaches including laser microdissection, immunolocalization along with proteomic analysis (Song et al. 2010).

With the aim of understanding TW induction or formation, proteomic analyses have been conducted on *Poplar* and *Eucalyptus*. These species are used as models in forest genetics and woody plant studies because they grow rapidly, they can be genetically transformed and the size of their genome is relatively small (5 to 6×10^8 bp) (Plomion et al. 2001). As for CW, different pine species, Sitka spruce (*Picea sitchensis*) and Japanese cypress (*C. obtusa*) have been used because of their economic and ecological interest. To investigate differentially expressed proteins in response to gravity, most studies report 2D polyacrylamide gel electrophoresis (PAGE) patterns and include identification of proteins by matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF), mass spectrometry (MS) or by liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS). One study using Multidimensional Protein Identification Technology (MudPIT) reported on the proteome of *Populus* developing xylem (Kalluri et al. 2009). The proteins were extracted from subcellular fractions of xylem stems, enzymatically digested and the resulting peptides were analysed using LC-MS/MS. However, this study was not specifically addressing RW induction/formation. In the next paragraphs, proteomic studies on TW induction/formation are first presented, followed by data on CW. Depending on the studies, the gravistimulation design, the organs and tissues used for protein extraction have been very different. In addition, studies on TW formation after bending using constraining strings are also presented. Because of the diversity of experimental designs, the synthesis of the results remains problematic.

4.4.3 Measured Changes in Proteome

Azri et al. (2009) studied young poplars (14–20 internodes) inclined at 35° from the
vertical axis. Whole internodes from the basal and apical regions of vertical and
gravistimulated stems were collected. The purpose of this experimental procedure
was to allow analysis of the differential expression caused by gravistimulation
between regions showing different motors for stem reorientation. The apical region
responds to inclination by differential growth due to elongation of primary tissues
while reorientation is due to asymmetrical formation of RW at the base. After
45 min of gravistimulation, the stem showed no reorientation. After 1 week, RW
was observed at the base of the stem. Differential protein expression was reported
between inclined or non-inclined conditions and also between the regions of the
stem. Among 300 protein spots, 40 % showed significant changes after inclination.
Sixty protein spots whose staining intensity was altered by gravistimulation were
identified by mass spectrometry. These 60 proteins fell into a large range of
functional categories. Interestingly, the patterns of expression of these selected
proteins differed strongly between the conditions tested (apical and basal regions,
45 min and 1 week of inclination). At 45 min and 1 week, respectively, three and
four proteins were similarly regulated by gravistimulation between the top and the
basal regions. These observations suggested that different metabolisms and signal-
ing pathways were involved in each region of the stem following a short (45 min) or
a long (1 week) exposure to gravistimulation. At 45 min, before any visible
reorientation of the stem, some of the proteins regulated by gravistimulation may
be involved in graviperception. At the top of the stem (where reorientation will later
occur through differential elongation of primary tissues), the results suggested the
implication of ROS (regulation of oxidative stress-responsive enzymes). The reg-
ulation of actin and tubulin subunits, or microtubule-binding proteins showed the
importance of cell wall—plasma membrane—cytoskeleton structural continuum
for graviperception. Several proteins suggested some signaling via the
endomembrane system and that calcium and phosphoinositides might act as cellular
messengers (calreticulin, phosphatidyl inositol transfer protein SEC14). At the base
of the stem (where orientation will later occur through the formation of RW), the
most noticeable enzymes that were differentially expressed by gravistimulation
were involved in lignin biosynthesis (phenylcoumaran benzylic ether reductase,
S-adenosylmethionine synthase). However, members of the S-adenosyl-L-methio-
nine-synthase gene family, which serve as universal methyl-group donors, are
potentially involved in lignin as well as in ethylene biosynthesis pathways.

In *Eucalyptus gunnii*, proteins were extracted from xylem tissue harvested from
a crooked tree. Two-dimensional gel electrophoresis images from normal and TW
were compared showing that 12 proteins out of 140 proteins analysed were differ-
entially expressed (Plomion et al. 2003). However, none of these proteins were
identified.

A different approach was carried out by Kaku et al. (2009) who focused on the
proteome of the G-layer in poplar TW. Leaning stems and branches from field-
grown poplars were used as sources for isolation of G-layers from TW. Among the

proteins separated by 2D gel electrophoresis, 108 were identified. Most abundant were lignin synthesis-related proteins although the G layer did not contain lignin itself. Cytoskeleton proteins, methionine synthesis-related proteins and cell wall-related proteins were also identified. Lignification in TW is still a matter of debate. Andersson-Gunnerås et al. (2006) using a global analysis reported a decrease in monolignol biosynthesis in TW compared with normal wood. However, on-going lignification was observed during G layer deposition in the compound middle lamella, S1 and S2 layers in poplar TW (Yoshinaga et al. 2012). An assay based on protein cleavage isotope dilution mass spectroscopy (PC-IDMS) has been developed for quantification of proteins regulating monolignol biosynthesis in *P. trichocarpa* (Shuford et al. 2012) and could potentially bring valuable data to decipher lignification in RW.

In conifers, CW is formed in response to gravitropic stimulus or environmental disturbances such as prevailing winds, and "pushes" the stems toward a vertical orientation. In the same way as for the TW studies, proteomic analyses of CW formation concerned either developing CW or inclined stems where no CW had been formed yet.

A comparative protein-based approach to identify proteins specifically expressed in CW was conducted with branches of Sitka spruce (*P. sitchensis*) (McDougall 2000). The developing xylem was sampled from the compression and non-compression sides of the branches. The comparison of polypeptides patterns by SDS-PAGE led to the identification of a laccase-type polyphenol oxidase that was over-expressed in compression tissues. This enzyme is thought to be involved in lignin biosynthesis.

On a larger scale, the identification of CW responsive proteins has been conducted with a 22-year-old crooked maritime pine (*P. pinaster* Ait.) (Plomion et al. 2000). Wood samples were mechanically and chemically characterized by measuring growth strains and lignin and cellulose contents, respectively. Of the 137 spots studied, 19 % were associated with growth strain effect. The results indicated the importance of ethylene in CW formation. The implication of 1-aminocyclopropane-1-carboxylate (ACC) oxidase which catalyses the final reaction of the ethylene biosynthetic pathway in CW formation has also been suggested by Yuan et al. (2010). These authors examined PtACO1 and *PtACO1*-like (encoding putative ACC oxidases) transcript levels by quantitative PCR in loblolly pine seedling stems that were bent to a 90° angle using constraining strings. They observed an increase in these transcripts levels starting at 30 min and peaking at 3 h after bending. *PtACO1*-like transcripts were higher in CW than in opposite wood (OW). Besides, Plomion et al. (2000) have found that lignin biosynthesis was also affected during CW formation and that enzymes involved in Krebs cycle, sucrose and starch metabolism were up-regulated.

In another study, sampling of compression and OW was done with 16-year-old maritime pines bent to a 15° angle by tying their trunks to neighbouring trees for 2 years (Gion et al. 2005). Other types of wood were also analysed (juvenile and mature woods, early and late woods). The clustering of 215 proteins identified over the six types of wood was presented. It appeared that 20 % of the identified proteins

exhibited distinctive expression patterns between CW and OW. Profilin, actin and nucleoside diphosphate kinase, 40S ribosomal S12 proteins were under-expressed in CW.

LC-MS analysis of Golgi-enriched membrane fraction from developing *P. radiata* CW has been done following in-solution digestion with trypsin (Mast et al. 2010). CW was sampled from 6-year-old trees in late summer to maximize the identification of proteins involved in secondary cell wall formation. As expected most proteins detected were involved not only in cell wall synthesis (i.e. cellulose synthase, laccase, phenyl alanine ammonia-lyase) but also in hormone biosynthesis and signaling (i.e. auxin-induced proteins, ACC synthase) and stress and defence response. Within this last putative functional category, numerous receptors were found (CC-NBS-LRR protein, NBS/LRR, TIR/NBS, TIR/NBS/LRR disease resistance protein).

Gravitropism is not the only process that determines stem orientation; phototropism is also an important factor that can lead to RW production. The interaction between these two processes has not been extensively studied in trees. Herrera et al. (2010) have presented a proteomic analysis of inclined pine seedlings submitted to an orthogonal light source. However, the apices were collected instead of the basal part of the stem where secondary growth takes place. Thus this study mainly identified differentially expressed proteins in the primary response to stem tilting.

Proteomic studies have been realized with different organs and tissues (stem, branches, whole internodes, xylem, G layer) from seedlings to trees aged from 2 month-old to 22 year-old. In some cases the plants were inclined or mechanically bent and the proteome was analysed after varying times depending on the study (from 45 min to days or weeks). In other cases, aged plants showing RW were used. The problem is to discriminate between overlapping events such as induction, signal transduction (first events following stimulation) in the stimulated cells which are not clearly identified yet, reorientation of cambial cell programming, and differentiation of newly formed cells in the developing xylem. In addition, perception of the gravitropic stimulus and response probably occur in different cells. The proteomic approach has been executed either on whole internodes or on xylem tissue which may not contain the perceptive cells. Among the proteins listings published, large functional categories appear such as primary metabolism, cytoskeleton organization and biogenesis, cell wall synthesis, hormone biosynthesis and signaling. However, the role of most proteins is still hypothetical. Focused studies are needed to evaluate the role of the proteins brought forward by global proteomic analyses.

4.4.4 Metabolomics and RW Formation

Metabolomics is a global approach used in biology for systematic metabolite quantification, a metabolite being any intermediate or product of the metabolism,

e.g. amino acids, carbohydrates, hormones, and many more. Although several studies targeted some metabolites related to carbon and secondary metabolism that potentially play a role in RW formation (Yeh et al. 2005, 2006; Shi and Li 2012), "without *a priori*" approaches have been marginally exploited in this field. To date, only Andersson-Gunnerås et al. (2006) have published work using this technique. A combination of metabolomics and transcriptomics recently gave precious insights on the *Arabidopsis* gravitropism and phototropism interplay (Millar and Kiss 2013). Andersson-Gunnerås et al. (2006) used a similar approach to gain access to the G-layer formation in poplar TW induced after 11 days bending. Conclusions from biochemical measurements follow predictions coherent with gene expression showing in TW: an increased activity of cellulose synthesis and pectin degradation-related genes while those involved in lignin biosynthesis are decreased. An advantage of their transcriptomic approach consists in fine identification of differentially expressed genes from multigenic families, thus refining the implication of gene candidates individually. Based on their expression and metabolic profiles, they propose extensive relational models for carbon metabolism and lignin biosynthesis in TW. Despite the importance of this work that provides a coherent framework based on quantitative and qualitative data on TW chemistry and gene expression, the earlier steps remain a matter of discovery.

4.5 Concluding Remarks

The formation of RW allows woody structures to adapt their position in response to gravitational and mechanical stimulation and/or a change in the light environment. The deciphering of the molecular mechanisms underlying this particular growth response is complex. It requires at the very least, tree models with sequenced genomes, which allow global approaches such as transcriptomics and proteomics. Functional genomics which aims to elucidate the function of proteins encoded by candidate genes is limited by a scarcity of mutants and the long generation times of forest trees. In the face of these complex challenges, Wyatt et al. (2010) presented *A. thaliana* as a model for a molecular and genetic analysis of the mechanisms of TW formation. In addition, RW formation having many external or internal causal agents (gravity, light, interactions of both stimuli, inherent patterning mechanisms), it is difficult to set up an experimental design that addresses the impact of one particular stimulus in trees. Most studies utilized inclined trees in greenhouse conditions, although both phototropic and gravitropic reactions occurred in such conditions. Signalisation pathways leading to tree stature adjustments are different whether starting from a light or a gravi-mechanical stimulus. For a short period of time, dark or isotropic light conditions could be used to gain insight into gravitropic signalisation pathway leading to RW formation. Moreover the issues around staking also need to be considered since different molecular pathways may be induced if stem deformation is allowed or not.

Hypotheses about perception of gravistimulation were previously defined through studies using *A. thaliana* mutants. However, in trees the question about the role of amyloplasts remains since starch is present in high level and everywhere in old woody structures. In trees, the tissue/cell that perceives gravistimulation is not clearly identified. As for the early events of signal transduction, one has to emphasize that very few studies were done at the very beginning of induction of RW, before any macroscopic observation of RW formation. Mechano-receptors involved in RW induction have still to be characterized. Global approaches suggest the role of calcium and ROS as second messengers and some signaling via the endomembrane system and phosphoinositides. Although components of the signaling network have been identified through global analyses, the way they relate to one another in space and time is still unknown. Wyatt and Kiss (2013) speak about a "more or less amorphous gray cloud" when relating to the understanding of the signaling network. More precision could come from microdissection of chosen tissues or cells prior to molecular investigations. Typically, the early events of signal transduction are supposed to lead to hormonal response (i.e. synthesis, degradation, redistribution, reallocation, compartmentalization, and so on) that will finally provoke the growth and cell differentiation response. The involvement of ethylene gibberellin and auxin has been discussed but more studies are needed in order to decipher hormone signaling crosstalk in RW induction at the cell level, and also at the organ and whole plant level. For example, Azri et al. (2009) suggested that different signaling pathways occurred at the top and the base of a tilted poplar stems.

In conclusion, global approaches reveal the complexity of the RW induction both on a temporal scale and as a function of the location in the tree. Therefore, although the transcriptional network, the organization of protein synthesis and the subsequent hormonal response at the whole tree level is still unknown, the beginning of an understanding of how trees manipulate RW formation to solve their mechanical requirements is emerging.

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Chapter 5
Biomechanical Action and Biological Functions

Meriem Fournier, Tancrède Alméras, Bruno Clair, and Joseph Gril

Abstract The main biological function of reaction wood is to act as "muscle" for trees, enabling them to control their posture. The key property to achieve this function is the development of high mechanical stress during the formation of reaction wood cells, called "maturation strains". Actually, reaction wood formation is basically the asymmetric formation of wood around the tree circumference, with higher maturation strains on the side where reaction wood is formed than on the opposite side. This asymmetry enables stems to bend upward or to compensate for the downward bending induced by gravity. At the cross section level, the performance in this biological function is linked not only to the magnitude of this asymmetry but also to an effect of the cross-sectional size (diameter) of the stem. Eccentric growth and variations in wood mechanical stiffness are second order effects that can modify this performance. Differences in maturation strains between reaction and non-reaction woods are related to their specific cell wall structure and composition. The swelling of the cell wall matrix during maturation and the effect of microfibril angle explain the differences in maturation strains between normal and compression wood. However, this mechanism fails in explaining the high maturation shrinkage of tension wood, and several hypotheses at the molecular levels are still under debate. How trees perceive their gravitational disequilibrium is also an open question for physiologists. Integrative biomechanical modelling (from the polymer level to the cell wall, cross section and whole tree levels) enables defining key variables that explain the performance of reaction wood as a system that insures the stem motricity. Maturation strains can be precisely measured only

M. Fournier • T. Alméras (✉) • J. Gril
LABEX ARBRE, Laboratoire d'Etude des Ressources Forêt Bois (LERFoB), UMR 1092, AgroParisTech INRA, 14 rue Girardet, 54000 Nancy, France
e-mail: t_almeras@hotmail.com; jgril@lmgc.univ-montp2.fr

B. Clair
Laboratoire "Sciences des Bois de Guyane" UMR "Ecologie des Forêts de Guyane" (EcoFoG), CNRS, UMR EcoFoG, Campus Agronomique, BP 701, 97387 Kourou, French Guiana
e-mail: clair@lmgc.univ-montp2.fr

in recently formed wood at the tree surface, but their changes during the whole tree life can also be estimated by retrospective dendrochronological analysis through structural markers of reaction wood. Lastly, wood in living trees ensures general storage, defence, vascular and skeletal functions, that ask general questions about synergies and trade-offs as the structural characteristics of reaction wood can affect all these functions.

5.1 Introduction

5.1.1 General Questions About the Biological Functions of Reaction Wood

Wood is made of different cell elements and the spatial and temporal organization of this heterogeneous structure allows it to perform several functions. For example, in temperate climates wood structure and functions differ within the growing season, so that earlywood and latewood are structurally and functionally different. Other examples are that due to the ageing process of heartwood formation, only the peripheral wood, i.e. the sapwood, is involved in sap transport and wound tissues are formed by cambial growth in reaction to injuries. Reaction wood is another kind of specialized wood tissue.

Wood anatomists were the first scientists who defined reaction wood, so that reaction wood structural properties are usually better known than its biological functions. Nevertheless, the IAWA definition (IAWA 1964) pointed out not only how reaction wood can be recognized from distinctive anatomical characteristics, but also how reaction wood is linked to tree morphology (reaction wood is "wood with distinctive anatomical and physical characteristics, formed typically in parts of leaning or crooked stems and in branches"). In addition, the IAWA definition also mentions the function of reaction wood as wood "that tends to restore the original position of the branch or stem when it has been disturbed".

This assumed biological function of reaction wood poses several questions to different scientific disciplines:

- From a mechanical and physical point of view, how can reaction wood restore the position of a rigid woody stem? In particular how can wood formation produce the necessary mechanical energy and stress to bend growing stems?
- Wood technologists know that reaction wood is not just a wood pathological reaction or a peculiar characteristic of crooked stems and branches. Indeed reaction wood can also be observed in straight and vertical trunks. Does it confirm of contradict the assumed function of position restoration?
- Can physiology explain how trees perceive signals that trigger reaction wood formation? How is reaction wood formation genetically and biochemically controlled?

Lastly, wood trait analysis across wide environmental gradients is currently of great interest for plant ecology (Chave et al. 2009), in order to understand how plant distributions and growth are driven by environmental factors. How are wood biological functions that are linked to plant ecological strategies affected by reaction wood structure? To explore such a question, we need to define how all the relevant functional properties of wood differ between non-reaction[1] and reaction wood.

5.1.2 Plant Movements in Woody Systems

By analogy with the function of bones in animals, it can be said that wood stiffness and strength provides trees with an efficient "skeletal" system (Moulia et al. 2006). However, this skeletal function is not the only mechanical function of wood fibres. The other biophysical function of wood is to provide stems with the ability of performing movement, i.e. a "motor" system (Darwin and Darwin 1880; Wilson 1984; Moulia et al. 2006; Moulia and Fournier 2009; Martone et al. 2010). Although usually much less often considered than the vascular and skeletal functions, this motricity function is of major importance to the biology of woody plants. Plant stems, and in particular woody stems, seem static at the usual observation timescale of humans, but they are not, and plant movements have been studied at least since Darwin's works on this issue (Darwin and Darwin 1880). Stem reorientation movements are common, and they are necessary for plants to adapt to their environment, for example to avoid shade and maximize light interception, avoid obstacles or recover from mechanical perturbations. Figure 5.1 (from Alméras et al. 2009) illustrates movements observed in experiments where gravitropism is stimulated by tilting (the aim of such studies is to induce reaction wood in order to compare plant behaviour or analyse reaction wood physiology). Sinnott (1952) made a series of experiments with *Pinus strobus* and observed that tying of vertical shoot axes and lateral branches provoked significant bending movements, which tend to restore the initial position and was associated with compression wood formation. This seminal work on reaction wood induction demonstrated that reaction wood is not a simple response to gravity or mechanical stimuli, but is associated with the more complex regulatory function of posture restoration. Under natural conditions, Collet et al. (2011) observed on advanced regeneration of beech (*Fagus silvatica* L., age: 10–31 years old, initial basal diameter 0.9–2.4 cm) that a gap opening stimulates radial growth associated with great righting movements (Fig. 5.2): between 2004 and 2008 the global tilt angle of the already lignified trunks changed from 58° to 76° (mean values on 31 trees, 90° is the vertical). All these studies illustrate significant changes of tree shape that

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[1] Note that in Chap. 2 the term "non-reaction" wood was used in preference to "normal" wood because "opposite" wood can also have different properties from "normal" wood.
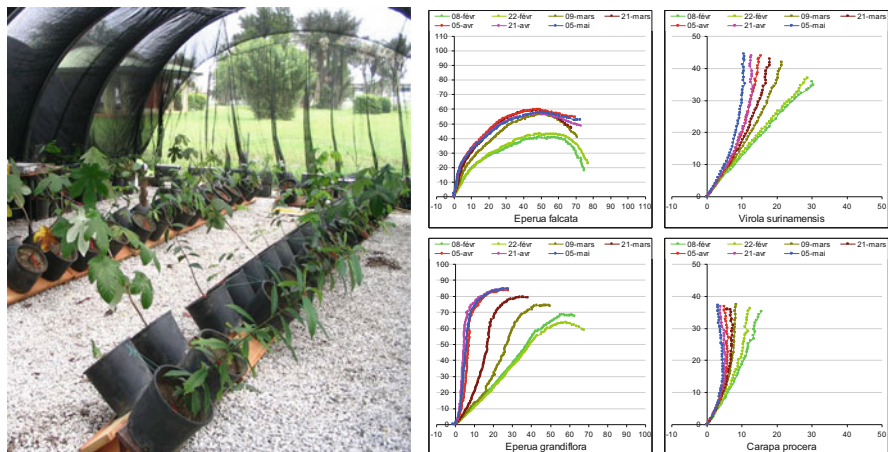
Fig. 5.1 Righting movements of young trees of different tropical species during a period of 3 months. Described in Alméras et al. (2009)
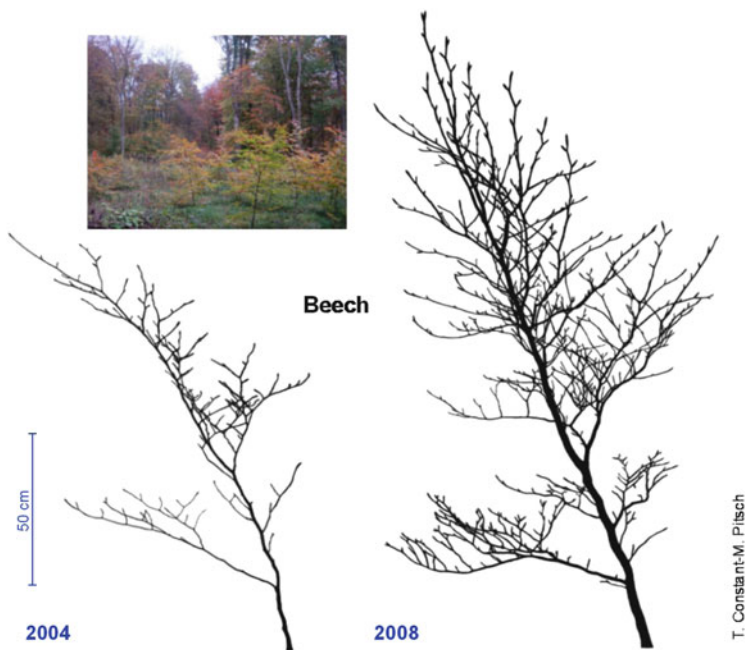

Fig. 5.2 Righting movements of a beech sapling during 4 years of growth. Described in Collet et al. (2011) (pictures T. Constant and M. Pitsch)

involve curvatures of the still lignified stem. This means that stem orientation is not only initially controlled by the direction of primary growth or branching pattern, but also mainly by later movements provoked by cambial activity and wood formation.

Moreover, because plants are slender structures growing in the field of gravity, motricity is necessary even in the absence of visible movement. Indeed, to maintain the orientation of a woody stem while it grows, it is necessary to compensate for the effect of the increasing weight by actively generating counteracting forces within the stem (Alméras and Fournier 2009). This function has been termed the "posture control" and is more general than the function of stem reorientation. Its necessity can be understood through an analogy with terrestrial vertebrates, in which an active muscular system is necessary not only to perform movements but also to stay immobile because continuous corrections are necessary to compensate for small mechanical perturbations and keep a stable equilibrium position (Moulia et al. 2006). Both stem reorientation and posture control are achieved by the active generation of mechanical stress during the development and maturation of wood fibres or tracheids. Reaction wood, as a tissue, is functionally specialized to provide this posture control.

5.1.3 Reaction Wood Is the Motor System of Posture Control in Woody Plants

Generally speaking, stem reorientation and posture control requires the generation of motion from bending forces. The basic mechanism for achieving this is similar in most plant stems including fungi, herbaceous and woody plants. It involves differential tissue expansion or shrinkage between the two sides of the stem, which generates a bending moment and thereby induces a change in stem curvature (Wilson 1984; Hejnowicz 1997). This mechanism will be set out in more detail in the following section.

As recently pointed out by Moulia and Fournier (2009), the efficiency of this mechanism results from the balance between the motor process itself (which provides the mechanical energy for straining the active tissue) and the mechanical resistance of surrounding tissues. In herbaceous stems, the motor process is based on changes in cell turgor pressure, adjusting the swelling and shrinkage of the active tissue. The magnitude of stress generated by turgor pressure changes is sufficient to bend herbaceous stems because their tissues have low stiffness and therefore offer weak resistance to bending. Woody stems are, however, much stiffer, and the magnitude of stress required to efficiently bend them is much larger than for herbaceous stems. To achieve stresses of large magnitude, they use a specific motor process, not based on turgor (which is anyway lost when the cells die) but rather on the development of stresses directly in the cell walls at the end of wood formation. Therefore, the characteristic property associated with the reaction wood motor function is the ability to generate large mechanical stresses during the last

stage of cell development (cellular maturation), and this is achieved by specific transformations of the fibre walls. At the cell wall level, the process generating this stress is not yet completely understood, but current hypotheses and evidence will be discussed below.

The concepts and mechanisms involved in this issue have a general value beyond the question of "reaction wood" itself, because (1) the motor function of wood is also to some extent achieved by non-specialized (normal) wood cells, (2) all maturing cells develop strains in trees (e.g. Archer 1987b; Boyd 1972), or in monocots (Huang et al. 2002) and (3) conversely, specialized fibres found in tension wood are also found in other tissues than wood [e.g. in phloem (Gorshkova et al. 2010; Salnikov et al. 2008)] and in organs other than stems, such as roots (Fisher 2008; Schreiber et al. 2010) or tendrils (Bowling and Vaughn 2009).

5.1.4 Linking Reaction Wood Structure and Function

The structural characteristics of reaction woods have been introduced in Chaps. 2 and 3. At the cellular level, compression wood is not very different from normal wood and tension wood is usually defined by the presence of a gelatinous layer. Ultrastructure and chemical composition are the major features that define reaction wood. However, the distinction is not completely dichotomous because variations between non-reaction and reaction wood suggest a continuum in structure between compression wood, non-reaction wood and tension wood, with only the gelatinous layer in some tension woods as a truly unique characteristic (Mellerowicz and Gorshkova 2011). The general trend along this gradient is a decreasing microfibril angle, decreasing lignin content, and increasing cellulose content and crystallinity. Then, although gelatinous fibres are found in less than 40 % of botanical genera or families of angiosperms, tension wood—as wood defined along this gradient and associated with the reaction wood function—can be found in all angiosperm species. Such a continuum in structure can be a source of confusion. This is because although reaction wood can probably be found in any growing tree, it has been described mainly under extreme conditions (crooked stems or branches), so that many biologists consider reaction wood as an abnormal and scarce phenomenon.

Actually, the characteristic property associated with the posture control function is the ability to generate large mechanical bending stresses during cell development and maturation. The biomechanical analysis developed in the next section aims at understanding how stress is generated in any kind of wood, normal or reaction wood, and then, how transformation of the fibre or tracheid walls explains the stress variations that induce bending at the cross section level, and then the posture control at the whole organism level.

5.2 How the "Tree Muscle" Works: The Biomechanical Point of View

5.2.1 Evidence of Strain Generation in Maturing Cells

Growth stresses have been studied since the beginning of the twentieth century, with several syntheses (Dinwoodie 1966; Archer 1987a; Kubler 1987) of the pioneer works. Studies on this phenomena resulted from tree fellers and sawmillers observing surprising cracks and warp when cutting, sawing or machining green wood. Forest and wood researchers were asked to prevent such problems through silviculture or technological solutions and they tried to understand how mechanical energy could be stored during tree growth before being released by cutting. Synthesizing all the observations, Boyd (1950) concludes that this mechanical energy, namely growth stresses, originates close to the periphery of the tree during secondary wall formation. Actually, only a phenomenon that occurs immediately after cellular expansion can explain mechanical stresses in the youngest wood located just beside the cambium. Moreover, as stresses are always present and high (order of magnitude 10 MPa along the grain), the phenomenon must be closely regulated by wood formation with no requirement for an external source of energy. Actually, as concluded by Munch (1937–1938, cited by Archer 1987b; Dinwoodie 1966), such stresses could only be generated by "chemical forces" involved in the formation of the secondary cell wall and not by gravity or other external forces.

Later Archer and Byrnes (1974) described mechanically and mathematically how wood in growing trees becomes stressed just after its differentiation: during the maturation process, wood tends to strain, with a longitudinal shrinkage of about 0.1 % and a transverse swelling of about 0.2 % in the normal wood of both gymnosperms and angiosperms. As this new wood is "glued" onto the older stiff core of wood, longitudinal and tangential strains are prevented so that wood is in a state of longitudinal tensile stress and tangential compressive stress.

5.2.2 How to Bend a Growing Stem by Generating Maturation Strains in Differentiating Peripheral Wood?

Historically, growth stresses have been described as homogeneous tensions generated continuously around the growing cross section, so that the older internal core is compressed by the younger peripheral wood (Kubler 1959; Archer 1987a). For foresters interested in sorting or breeding trees with low peripheral stresses with the aim of increasing industrial wood quality, the question was how to determine which trees develop high levels of stress, in order to describe the ecological or silvicultural

situations leading to a high risk of felling cracks or timber splitting and distortion during sawing.

Observations of stresses at the stem periphery in different angiosperm tree populations emphasize the fact that high tensile stress values associated with high longitudinal maturation shrinkage and high risk of felling cracks are scarcely homogeneous, but concentrated in small angular sectors of tension wood. In gymnosperms, although high tensile stress values are not observed (so that the occurrence of felling cracks is generally low), an asymmetry of stresses is also observed as longitudinal maturation swelling in compression wood is opposed to maturation shrinkage in opposite wood (Archer 1987a; Fournier et al. 1994b). Then reaction wood is mechanically described as an active guy rope (for non-reaction or tension wood producing tensile stress) or a forestay (for compression wood producing compressive stress) that can bend the stem. This mechanical system can be modelled using the theoretical background developed by Archer and Byrnes (1974), Fournier et al. (1991), or Fournier et al. (1994a). Such mechanical models give the scaling laws of the motricity function from the cell wall to the whole tree (Fourcaud et al. 2003; Fourcaud and Lac 2003; Alméras and Fournier 2009; Coutand et al. 2011).

A first step is to describe how maturation strains generated in the differentiating cells act at the cross section level to provoke local curvatures through asymmetry of growth and/or cell wall properties. The basic but general equation of reaction wood action (Alméras and Fournier 2009) from the tissue to the cross section level expresses the elementary change in stem curvature dC_R (see Moulia and Fournier 2009), in terms of the cross-sectional diameter (D), growth (dD), and a dimensionless efficiency e that is a function of the asymmetry of maturation strains between the reaction wood and wood on the opposite side of the tree and different form factors (asymmetry of growth, and heterogeneity of wood stiffness between the core and the periphery):

$$dC_R = 4e\,\frac{dD}{D^2}.$$

As the rate of bending with radial growth dC_R/dD scales as $1/D^2$, righting movement kinetics change a lot with the tree size, as noticed by Boyd (1973) who mentioned that compression wood can bend only small trees. Moreover, the model can be applied to the analysis of experimental observations of tree righting movements (Coutand et al. 2007), in order to compare efficiencies between species (Alméras et al. 2009) and genotypes (Sierra-De-Grado et al. 2008) in tilting experiments, or individual responses in the natural environment (Collet et al. 2011), because it allows the separation of size and growth effects from wood properties and shape factors.

In addition, different components of the efficiency parameter e can be analysed including asymmetry of wood maturation strains, growth eccentricity and wood stiffness heterogeneity. The assumption of a sinusoidal variation around the cross

section circumference of ring width, longitudinal maturation strain α and wood modulus of elasticity (MOE) E (Alméras and Fournier 2009) leads to:

$$e_r = \Delta\alpha \cdot f \cdot \frac{\overline{E}}{E_{section}},$$

where:

- $\Delta\alpha$ is the difference in maturation strain between the two opposed sides of the stem (along the bending axis).
- $\frac{\overline{E}}{E_{section}}$ is the ratio between the mean MOE of the new ring, and that of the stem section inside the new ring.
- The effect of circumferential variations in ring width and stiffness is accounted for by a form factor f:

$$f = 1 + \frac{3}{4}k_E \cdot k_O + (k_E + k_O) \cdot \frac{2\overline{\alpha}}{\Delta\alpha},$$

where $\overline{\alpha}$ is the mean maturation strain (around the circumference), k_E and k_O (between -1 and 1) are the relative circumferential variations of, respectively, the MOE and the tree ring width (see Alméras and Fournier 2009 for details).

If the section is homogeneous and concentric, the form factor is 1 and e is the difference in maturation strain between the lower and the upper side $\Delta\alpha$, as assumed by previous models (Coutand et al. 2007; Fournier et al. 2006). The models show that the effects of eccentricity or stiffness variations can be positive (synergic effects) or negative. For example, in conifers, because the MOE of compression wood is lower, this variation has a negative effect on the righting movement (compared to the theoretical case of no variation of E between normal or reaction wood Alméras et al. 2005b).

In order to calibrate the model from springback strains (i.e. strains measured at the stem periphery after removing the self-weight), Huang et al. (2010) modified slightly the expression of the efficiency e assuming that the force rather than each component (MOE and radial growth) varies sinusoidally. This assumption of sinusoidal variations provides an easy way to derive analytical formulas, but could be far from the real circumferential variation in wood properties. More general models (Alméras et al. 2005b; Coutand et al. 2011) allow simulation of the real pattern of reaction wood formation including variable width.

5.2.3   Is Reaction Wood the Main Way to Produce a "Tree Muscle"?

Growth eccentricity is a complementary but second order effect often associated with reaction wood formation (Alméras et al. 2005b). Reaction wood is usually but not necessarily associated with faster growth (see synthesis on this subject in Schweingruber 2007, pp. 131–132). For example, Wang et al. (2009) and Tsai et al. (2012) described faster growth in opposite wood of branches. Nevertheless, in extreme conditions of eccentric growth such as buttresses that are known to act as tension members but usually not made of reaction wood (Schweingruber 2007, p. 132; Ter Steege et al. 1997; Fisher 1982), the posture control system can be induced by growth asymmetry alone without a change in wood properties (see Fig. 5.3). Eccentric growth without a clear modification of wood structure has also been mentioned by Fisher and Marler (2006) in *Cycas micronesica*. Similarly, in some species, stilt roots (which is an extreme case of eccentric growth consisting of new, external organs) plays a role in posture control (Leopold and Jaffe 2000) with no absolute requirement for reaction wood, although reaction wood is often present (Fisher 1982) to provide an additional driving force. In summary, in the most common tree species, the main driving force of bending arising from the maturation of new cells during radial growth is the asymmetry of wood properties between opposite wood and reaction wood (Fig. 5.3, case A or B).

As pointed out by Alméras et al. (2005b) and Huang et al. (2010), the asymmetry of the MOE increases the efficiency of tension wood (which is stiffer due to a high crystalline cellulose content with a low microfibril angle) but decreases the efficiency of compression wood (that is less stiff than normal wood because of its high microfibril angle). Moreover, the radial gradient of wood stiffness (Lachenbruch et al. 2011) increases the reaction curvature if the central wood is less stiff (as is the case for the juvenile wood of softwoods) but lowers the efficiency of reaction wood if the central core is stiffer. However, all these effects are of second order so long as the stiffness is within the usual range of variation for wood but things need to be reconsidered in peculiar cases such as hollow stems or plants with very soft cores (Alméras et al. 2009).

Lastly, taking into account the viscoelastic properties of wood, Coutand et al. (2011) demonstrated that creep could significantly catalyse the upward bending of stems even though the value of maturation strain (i.e. the quantitative difference between opposite and tension wood) remained the most influent parameter.

As a conclusion, reaction wood formation means from a functional point of view that wood of different maturation strains is created on each side of a tree and this is how they generate active bending movements to control the stems erect habit. Although some light compressive stress has been reported in opposite wood (Clair et al. 2006), the formation of both tension and compression wood (Fig. 5.3, A + B) that would be the most efficient system for stem redirection has not been observed in trees. For a given gradient of wood properties from reaction to opposite
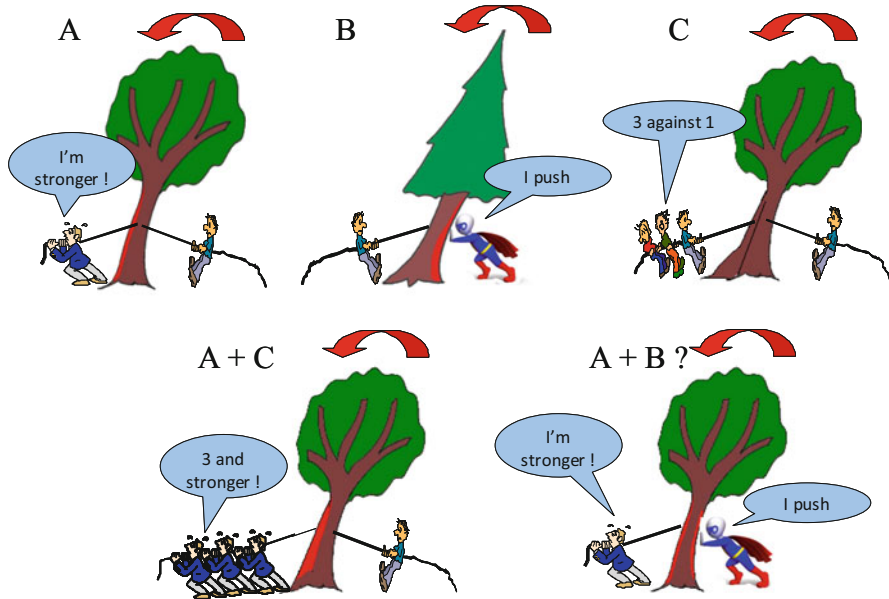
Fig. 5.3 The different ways to create a bending moment in a growing tree cross section: (*A*) tension wood in angiosperms (higher tension than opposite "normal" wood); (*B*) compression wood in gymnosperms (compression opposed to the "normal" tension in opposite wood); (*C*) tensile buttresses in angiosperms, or increasing growth with the same wood quality (i.e. the same tensile stress) so that the tensile force is greater; (*A* + *C*) is the commonly (but not systematically) observed situation of faster growth in reaction wood; (*A* + *B*) would be the never observed combination of tension and compression wood in the same tree

wood, the smaller the cross section and the faster the radial growth, the more efficient the reactive bending. Synergic effects linked with eccentric growth are also sometimes found.

5.2.4 How to Induce High Mechanical Strains in Differentiating and Maturing Cell Walls?

The question of maturation stress generation at the microscopic level—why and how this "spontaneous tendency to shrink" appears—has long been a matter of discussion. The swelling of the wood matrix substance during lignification has been proposed as the primary cause of maturation stress generation, together with lateral connexion established between microfibrils before lignin polymerization (Boyd 1950, 1972). According to the mechanism proposed by Boyd, the MFA controls the anisotropy of the resulting stress: swelling dominates for compression wood (large MFA), while in normal or tension wood (low MFA) the shortening along the microfibrils directly results in axial shrinkage. However, this mechanism fails to

explain the large tensile stress found in tension wood, and especially in G-layer tension wood, with little or no lignin present in the secondary wall. As an alternative, the hypothesis of shrinkage of cellulose microfibrils was proposed (Bamber 1987, 2001). Results obtained using micro-mechanical models showed that to account for the observed relation between microfibril angle and released maturation strains in conifer woods, a combination of both assumptions was necessary (Okuyama et al. 1994; Yamamoto 1998; Alméras et al. 2005a). However, the proposed mechanisms remain purely hypothetical, and no evidence of such behaviour has yet been provided at the molecular level.

The recent revival of interest in the question of the generation of maturation strains, linked to the acquisition of new knowledge about chemical composition, physical structure and mechanical state of the G-layer (see Chap. 3 for more detail), has generated new hypotheses (Goswami et al. 2008; Mellerowicz et al. 2008b). However, to date no convincing model has been provided and successfully tested (Mellerowicz and Gorshkova 2011). Because of the apparent paradox between the axial stiffness of the G-layer and longitudinal maturation strains of tension wood, some authors suggested that maturation stress must be supported not by the G-layer, but by adjacent layers of the tension wood fibre (Münch 1938; Goswami et al. 2008). This idea arose because the G-layer was often observed partly detached from adjacent layers, and therefore must be loosely connected and not able to transmit the stress to the surrounding tissue. Further observations showed that this detachment was a preparation artefact (Clair et al. 2005a). Based on a number of observations (Clair and Thibaut 2001; Clair et al. 2003, 2005b), it is clear that the G-layer is indeed submitted to axial tensile stress and transverse compressive stress as a result of maturation. Moreover, experimental evidence was recently provided that the cellulose microfibrils of the G-layer are put in tension during their maturation (Clair et al. 2011). This does not solve, however, the question of the primary cause of maturation stress. What mechanism generated tension in the microfibrils? A hypothetical effect of the daily variations in water tension in the cell lumen was suggested (Okuyama et al. 1995), but it was later proved that this external factor is not involved (Alméras et al. 2006). Therefore, the cause must be internal, directly related to a process occurring within the cell wall during or after its formation. It could be either a modification of the cellulose structure after its deposition or a transfer of stress between the matrix and the microfibrils, as is observed during wood drying (Abe and Yamamoto 2005, 2006; Clair et al. 2008). Recently, it was shown that the G-layer, like gels, is characterized by a large amount of water-filled meso-pores, having a mean size of 7 nm (Clair et al. 2008). Moreover, dimensional changes of microfibril aggregates due to variations in water content in the matrix have been observed (Lee et al. 2010). Therefore, changes in water content during maturation could be involved in the appearance of swelling or shrinkage strains in the matrix, depending on the osmotic concentration, the ion concentration and the valence (monovalent or divalent) of the cations (van Ieperen 2007). Figure 5.4 shows mechanisms that could produce longitudinal tension within a G-layer. The preferred mechanism (d) is the only one able to produce lateral compression also and is very similar to the one suggested by Boyd with lignin swelling within a
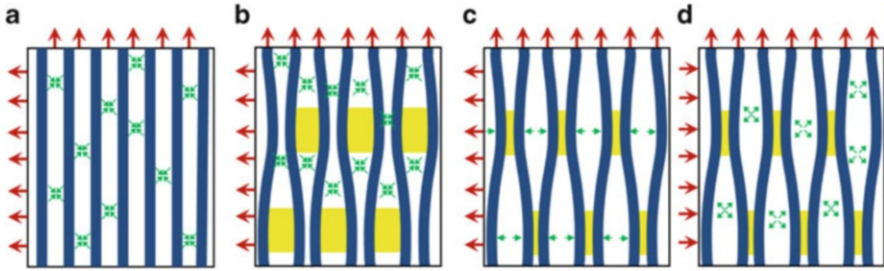
Fig. 5.4 Possible mechanisms of stress generation within a G layer: (**a**) matrix shrinkage only; (**b**) matrix shrinkage associated with the presence of stiff zones between microfibrils; (**c**) active creation of bridges between microfibrils; (**d**) matrix swelling between bridged microfibrils. Arrays of *red arrows* at boundary indicate the stress direction (outward: tension; inward: compression); *green arrows* within the domain indicate movements (horizontal: lateral movement of the microfibrils; inclined: matrix swelling or shrinkage)

trellis-like microfibrillar network (except that the swelling cannot be attributed to lignin in the G-layer). More studies are needed to explore these hypotheses and to predict their mechanical effect. Moreover, generic models need to be able to describe not only G-layer tension wood but also all types of tension wood characterized by high crystalline cellulose content.

5.2.5 Adjusting the Response to the Stem Requirements: How Do Trees Perceive Posture and React to Control It?

Gravi-perception, mecano-sensing and gravitropism are very general physiological processes in plants and a comprehensive synthesis of these topics goes far beyond the scope of this chapter. The reader could refer to recent review papers such as Moulia and Fournier (2009) or Moulia et al. (2006), or general books such as Wojtaszek (2011) or Gilroy and Masson (2008). However, the function of reaction wood cannot be described without some insight into how the reaction is biologically induced and controlled. As suggested by the terminology "tension" or "compression" wood, reaction wood is usually associated with mechanical stress. Compression or tension stress can be measured in recently differentiated compression or tension wood (see next section) but such stress is a biomechanical response and not a stimulus. The stimuli perceived by the wood remains an open question for molecular and cellular physiology, involving complex interactions between gravisensors such as statoliths, mechanosensitive channels, and photo-receptors. At a macroscopic level, dose response laws can be tested, such as Sachs' "sine law". This law states that the local response rate is proportional to the sine of the difference between the current position and a theoretical equilibrium position, called the gravitropic set-point angle, taking into account a time lag (reaction time). The first issue is how to choose the relevant response variable. In any plant

system (coleptils, hypocotils) usually studied by physiologists, observed lean is a confusing variable as the response basically involves bending and curvature rather than just angles of lean (Moulia and Fournier 2009). Secondary growth induces further artefacts. Firstly, the response in terms of change in curvature is physically linked to the stem's initial radius and internal wood stiffness (see previous analysis in Sect. 5.2.2). Therefore, the slower reaction of bigger stems (or of basal parts of stems compared to distal parts closed to the apex) is not the result of a slower perception or physiological response, but is because bigger stems are stiffer and therefore less easy to bend. Secondly, compared to movement based on hydraulic pressure, the formation of secondary wall involves the mobilization of carbon assimilates with several constraints on the availability of resources, and slow characteristic times. In addition the similar responses of trees grown in very different environments demonstrate that stressed trees (e.g. drought or low-light conditions) have developed a much more efficient response because they need to compensate for their slower growth. Generally speaking, the assessment of stimulus–response curves cannot be based directly on the observation of righting movements, as variations of movements involve not only the gravitropic response but also variations of diameter and tree ring width. Lastly, the apparent lack of response in a leaning stem that maintains its position does not mean that there is no response and no mechanical work, because some sort of response is always necessary to counteract the gravitational bending due to growth in mass of the tree. Understanding how the posture control system reacts and acclimates to gravitational stimuli from the observation of reaction wood occurrence based on structural (chemical, ultrastructure of cell walls, anatomical) analysis should be more accurate than the direct observations of curvature changes provided that the link with the functional efficiency of reaction wood in the posture control is demonstrated through (1) strong relationships between maturation strains and the structural characteristics observed (see Sect. 5.2.4) and (2) a careful spatio-temporal analysis (because the structure is generally only observed retrospectively at the end of the process whereas the stimuli change with time during the movement). Actually, interpretations of experiments where different stimuli are applied (e.g. different initial inclinations) are easier to discuss when they are based on a fixed angle as done by Yamashita et al. (2007) who demonstrated on *Cryptomeria japonica* that the response increases with the tilt angle, up to a saturation level (30° of lean). However, up to now and even after a careful preliminary analysis eliminating the previously described artefacts, no unified theory based on physiological and mechanistic knowledge is able to explain the reaction wood distributions observed in Sinnott's loop experiments (Sinnott 1952) or in other seminal works (Archer and Wilson 1973). Actually, Archer and Wilson observed shifts in the location of compression wood from one side of the stem to the other. This distribution means that an opposite curvature is generated after the first reaction. This is obviously a necessity to ensure posture control as reaction wood formed all on the same side of the stem will lead to an upward curved stem not a vertical and straight one. But, as observed by Wilson and Archer and pointed out by Coutand et al. (2007), the puzzling question remains to explain physiologically the

"autotropic" perception as the shift develops before the stem has passed the vertical, increasing the performance of the shape regulation and avoiding oscillating systems. Recently, Bastien et al. (2013) demonstrated that the proprioceptive sensing of curvature changes is as important as gravisensing to understand gravitropic movements. Furthermore, light is well known to influence gravitropic responses with many poorly understood underlying physiological processes (Iino 2006).

5.3 Practical Assessment of the Functional Performance of the Posture Control

As developed in the previous section, reaction wood is the main motor of posture control, and posture control results from multi-scale processes. We have discussed reaction wood at the stem cross section level in detail in Sect. 5.2.2 and to investigate reaction wood influence at the stem level requires summing curvature changes and cross sections along the whole stem as done by Coutand et al. (2011) or Fourcaud et al. (2003). Maturation strains in reaction wood, because they are different from opposite wood, are the main relevant property at the tissue level to assess the reaction functional performance but, as previously shown, other characteristics at a more macroscopic level (tree size, reaction wood distribution, growth rate) have interactive effects. Moreover, maturation strains are explainable by cell wall structure and chemical composition (Sect. 5.2.4). Table 5.1 summarizes the different scales with relevant variables linked to reaction wood formation, at each level. At the whole tree level, the problem is how to assess the global performance of control as a growth strategy, involving kinetics and spatial patterns of reaction wood formation during the whole life and in the whole tree, and analysing how the shape control by reaction wood formation impacts tree ecological fitness by modifying stem buckling or breakage risk or canopy light capture efficiency.

5.3.1 How to Measure or Estimate Maturation Strains

Although the importance of multi-scale analysis must be emphasized, maturation strains (and more accurately the difference of maturation strains from reaction to opposite wood) is probably in many cases the most significant component of the reaction efficiency. Alméras et al. (2005b) analysed statistically the contribution of different factors on the reaction efficiency of a diversity of tree species (11 angiosperms and 3 conifers) and found that the isolated effect of maturation strains explains 40–110 % of the righting curvature (values greater than 100 % are possible as the eccentricity of growth or stiffness variations can have a negative effect, see Sect. 5.2.3). However, other studies that estimated "e" from curvature observations (Coutand et al. 2007; Alméras et al. 2009; Collet et al. 2011) on seedlings found

Table 5.1 Assessment of the performance of the posture control function due to reaction wood formation: summary of the relevant variables at the successive organization levels from the cell wall to the whole tree

Level	Performance	Performance components (explicative variables)	Material
Cell wall	Cell wall maturation strain α_{wall}	MFA, cellulose and lignin content, cellulose crystallinity Mesoporosity of the G-layer? Organization of the cell wall (links between matrix and microfibrils)? Type of amorphous polysaccharides?	Cell wall from embedded or frozen sections Wood powder for chemical analyses
Wood (tissue)	Tissue maturation strain "α"	α_{wall} Area of tension wood fibres or compression wood tracheids	Microscopic sections
Maturing wood in a cross section	Efficiency "e" (see Sect. 5.2.3) linked to reaction wood formation	Circumferential variations of α Eccentricity of growth Radial variations of modulus of elasticity	Tree ring or peripheral new layer of cells
Growing cross section	Reaction curvature induced by reaction wood formation dC_{matur} during growth	Efficiency "e" Section size (diameter) Radial growth rate (dR/dt) Wood viscoelasticity	Stem cross section
Growing and loaded cross section and tree stem	Balance between reaction curvature dC_{matur} and gravitational curvature dC_{weight}	dC_{matur} and dC_{weight} function of lean, stiffness and viscoelasticity, weight increase (dW/dt) Spatial integration of curvatures along the stem	Successive cross sections along the stem, other organs inducing self-loading (branches, leaves...)
Whole life of a whole tree	Spatial and temporal distribution of reaction wood as a global strategy that impact long-term stem buckling and breakage risk and canopy light capture efficiency	Trajectories and cartography of reaction wood formation versus gravitational loads and other disturbances (see Sect. 5.3.3)	Wood retrospective analysis (discs and chronological series of tree rings) at different heights, adding biomass and morphological data (tree architecture analysis)

very high values of "e" of 10^{-2} when the highest values of maturation strains generally measured (see below) are several times lower, suggesting that in small stems, other factors play a greater role.

Fournier et al. ([1994a](), [b]()) and Yoshida and Okuyama ([2002]()) compared different methods used to measure maturation strains at the stem periphery. The most direct method (Fig. 5.5a) consists of gluing a strain gauge or a small extensometer to the wood surface (after debarking) and then measuring the released strain after drilling two groves designed to isolate the tissue located under the gauges from the mechanical influence of surrounding wood, so that the initially impeded maturation strains are entirely released. The technique requires a lot of care to measure strains properly (order of magnitude of strains are 1–10 μm for a length of 10 mm) when conducted in the field on wet wood (see Jullien and Gril [2008]() for a numerical analysis of the method). A widely used and cheaper technique initially developed by Archer ([1987a](), [b]()) consists in measuring the variation of distance between two pin targets (distant between 45 and 50 mm) induced by the drilling of a central hole (Fig. 5.5b). The higher the initial stress, the higher the variation of length. Therefore, the measurement estimates the maturation strain, although an accurate quantitative interpretation requires a more intensive analysis because the measurement depends not only on the initial strain but also on the sensor geometry (relative hole size versus distance between pin targets) and wood elastic anisotropy. As this method measures length variations of 10–500 μm, extensometers cheaper than electrical strain gauges can be used.

Whatever the measurement method, some bias can occur because the measured released strain is not always equal be the actual maturation strains (i.e. strains generated during the maturation of the last peripheral wood cells and impeded during growth). Actually, released strains measure all the mechanical strains supported by peripheral wood since its formation. They provide a reliable estimate of maturation strains only if maturation strains are very high compared to other loads that may have acted on peripheral wood since its formation. Such assumptions are generally adequate for big stems, but may be wrong in many specific cases. For example, in very thin (not stiff) and tilted organs such as branches, even in very new and peripheral wood, a quite low variation of weight induces a strain of the same order of magnitude as the maturation strains (and especially in some fruit trees, where fruit loads are an important mechanical component, see, e.g., Alméras et al. [2004]() for an example from apricot trees). Notice that in leaning stems, it is of great importance to measure maturation strains in the natural tilted position without displacing the stem, as suppressing the whole weight induces high non-reversible strains that are not the opposite of the strains provoked by gravity in the growing tree. Actually, these gravitational strains in the youngest wood located at the stem surface are due to the weight increment added during the last millimetres of radial growth, which is generally much lower than the whole weight. Maturation strains will also differ significantly from released strains in very slowly growing organs where the peripheral wood could have undergone a quite complex and long mechanical history. For example, in buttressed trunks (Fig. 5.6), we measured compressive values of released strains far from the ridges and such released strains just expressed the fact that only the ridges are growing, so that the other parts of the trunk have been progressively compressed by the growing ridges.
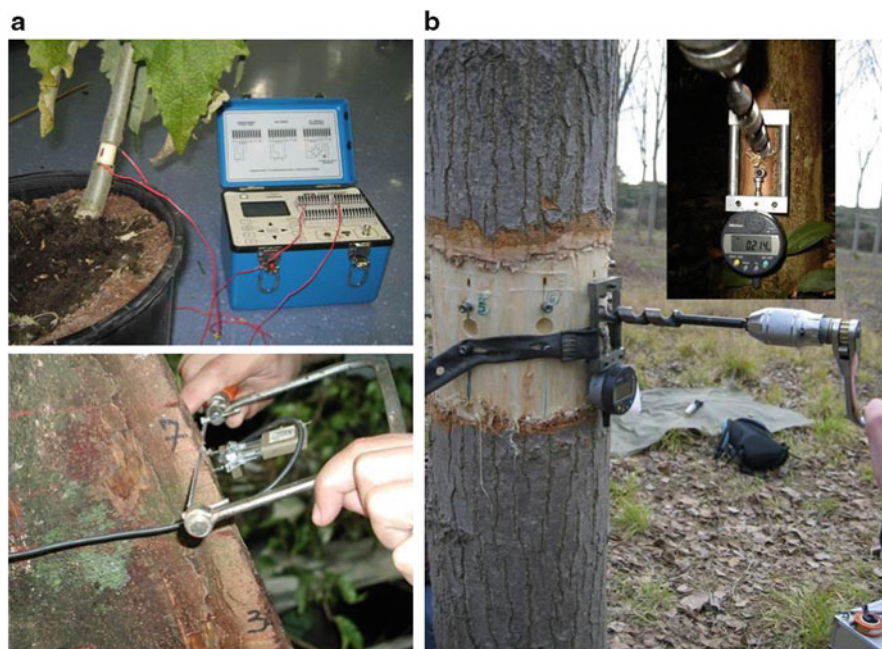
Fig. 5.5 Two different methods to estimate maturation strains: (**a**) the two grooves method using strain gauges or strain sensors. In this method, the measured strain is very localized and gives quite directly an estimation of the initially impeded maturation strain (if grooves are deep enough and not too far from the gauges, see Jullien and Gril 2008). (**b**) The single hole method. This method disturbs the bi-dimensional field of stress so that the measurement is an indirect indicator of the initial maturation strains (but depends also on the hole geometry, wood anisotropy, etc.). See Fournier et al. (1994b), for more details

5.3.2 Retrospective Analysis of Posture Control History Through Structure Analysis

Peripheral released strains are a good proxy of maturation strains because of the hypothesis that maturation strains are completely impeded during the growth process and are the only significant and long-term (rather than short-term such as induced by the wind) stress process in recently formed wood. Therefore, they can be only measured in the youngest peripheral wood. To estimate maturation strains in older wood, dendrochronological approaches must be developed, using quantitative and robust relationships between maturation strains and structural or physical wood characteristics, and using retrospective mapping of the variables chosen as proxies for maturation strains assessment (e.g. Dassot et al. 2012). Although many studies have established good relationships between maturation strains and (1) MFA, chemical composition or other cell wall characteristics or (2) drying shrinkage or other physical characteristics (e.g. Bailleres et al. 1995; Clair et al. 2003; Fang et al. 2008, see also Sect. 5.2.4), they haven't been developed, probably because the mapping of such characteristics for a large number of tree rings, cross sections and
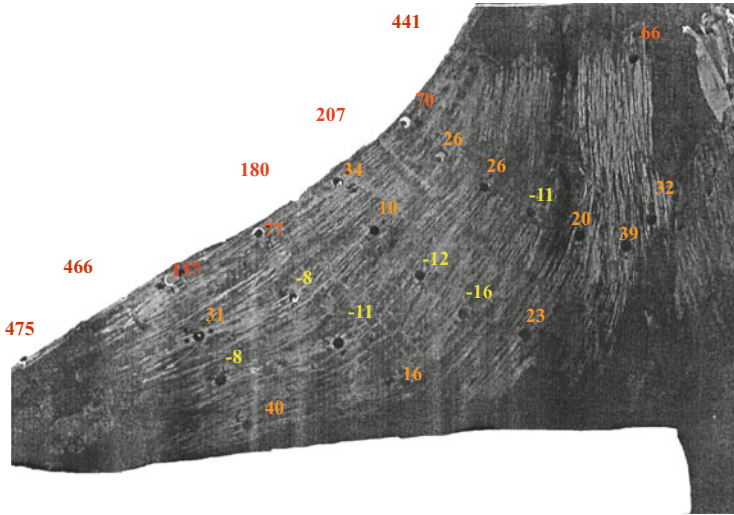
Fig. 5.6 Map of released strains (figures indicate the crude measurements in micrometres made by the single hole method and CIRAD's sensor) in a buttress trunk (*Ragala sanguinolenta*, Sapotaceae). Compressive values do not mean that compression wood is formed, but that only the ridges are growing significantly (M. Fournier, unpublished data)

trees requires expertise in high throughput metrologies (e.g. Silviscan for MFA, Evans and Ilic 2001). Up to now retrospective analyses have relied on staining methods (e.g. Grzeskowiak et al. 1996; Badia et al. 2005; Barbacci et al. 2008; Dassot et al. 2012). Such methods give binary information (presence/absence of reaction wood) and fail to estimate the "severity" of reaction wood (see Dogu and Grabner 2010), i.e. the continuous variations of structural maturation strains in both types of wood. Although such retrospective works are infrequent and rarely discussed by dendrochronologists (see Duncker and Spiecker 2008, or Stoffel and Perret 2006 for a more in-depth discussion about the potential of reaction wood for dendrochronological methods), they are usually successful in linking reaction wood formation to environmental events such as (1) wind events (e.g. Zielonka et al. 2010), (2) snow fall (Casteller et al. 2011), (3) rainfall on conifers growing on slopes (Furukawa et al. 1988), (4) ice storm damage (Hook et al. 2011), (5) high thinning (e.g. Washusen et al. 2005), (6) establishment and competition periods in fast growing species (Badia et al. 2006) and (7) apical bud death of mature trees (Loup et al. 1991) or young shoots (Delavault 1994)

5.4 Consequences of Reaction Wood on Wood General Biological Functions

In the previous section, we emphasized the specific function of reaction wood as a motor of posture control. As stated in introduction, wood is a multifunctional tissue made of specialized cells or sub-tissue, and the tissue or cell design adapted to one specialized function should therefore impact other functional properties. The aim of this paragraph is to study how other functional properties are modified in reaction wood, in order to discuss trade-offs between different wood functions. The term "functional properties" here refers to properties of the wood in the living tree that characterize its ability to perform a biological function. This excludes a number of properties that are of interest for the use of reaction wood as a product but not directly involved during the tree life, such as drying shrinkage or dry mechanical properties (these are presented in more detail in Chaps. 6 and 8).

5.4.1 Wood as a Multifunctional Plant Tissue: Vascular, Skeletal, Defence, Storage and Motor System

The transport of sap from the roots to the leaves and the mechanical strength and stiffness that allows the erect-against-gravity tree habit are the main wood functions usually considered by plant ecologists (e.g. Chave et al. 2009). Water transport and support are functions performed by dead elements such as vessels and tracheids in the living organism, and their performance and safety against embolism are related to biophysical laws and properties. Vessels or earlywood tracheids in the sapwood form a vascular system that has the primary hydraulic function of sap transport. Fibres or latewood tracheids are specialized in mechanical functions. They greatly improve the stiffness and strength of the stem, providing safe mechanical support for the foliage, which is necessary for the tree to grow in height and extend its crown. Additionally, living elements of wood (ray cells and axial parenchyma) perform physiological functions such as defence against pathogens, and the transport and storage of nutrients. All usual wood cell types can be found in reaction wood tissues: fibres or tracheids, rays, and, in the case of angiosperms, vessels and axial parenchyma. Table 5.2 summarizes the wood functions for each specialized cell or tissue.

5.4.2 "Skeletal" Properties: Mechanical Stiffness and Strength

The two main wood mechanical properties related to the skeletal function of wood are stiffness and strength in the fibre direction. These properties are a function of the

Table 5.2 The multifunctionality of wood, which is composed of specialized cells and tissues

Function	Specialized tissues	Specialized cells
"Skeleton" = mechanical strength and stiffness	Latewood (in temperate climates)	Fibres of tracheids
"Muscle" = movement	Reaction wood	Reaction wood fibres
Sap transport	Sapwood Earlywood (in temperate climates)	Vessels or tracheids
Storage of nutrients	No specialized tissue	Parenchyma cells
Defence against biotic attacks (insects, fungi, etc.)	No specialized tissue	Resin ducts

amount of water bound to its walls. In the living tree, where some free water is always present in the vascular system, the walls remain water-saturated regardless of the amount of free water. This implies that the mechanical properties of green wood do not depend on the amount of water in the conduits. However, they differ from the mechanical properties of wood in the hygroscopic domain, i.e. for water contents lower than the fibre saturation point (at approximately 30 % moisture content). Properties in the hygroscopic domain will be specifically discussed in Chap. 6. In the present chapter we will always implicitly refer to reaction wood properties measured in the green or water-saturated state, which are those directly relevant to its biological functions. Although shear and even transverse properties are obviously very important for the skeletal function (e.g. Mattheck and Kubler 1995), it is usually assumed that the most relevant mechanical properties are those measured in tension/compression/bending in the fibre direction. Therefore, structural parameters controlling the growth stress and the motor function, such as MFA, lignin content and composition, mesoporosity in the case of G-layers, all have an effect on other properties with stiffness being the most obvious one, and so indirectly impact the other functions of the tissue.

The stiffness of wood, usually quantified by the MOE, is a measure of the amount it bends or distorts as a function of the load imposed on it. Actually, the whole stem stiffness against gravitational forces combines the wood stiffness (MOE), the cross section diameter and the position of the centre of mass (linked to the stem length). A high wood stiffness (MOE) is thus a necessary condition for stems to maintain their self-standing habit despite their high slenderness (length/diameter ratio). The micro-mechanical design of wood makes it very efficient for this function, mainly because of its cellular structure and the ultrastructure of its secondary walls, that can be described as a polymeric matrix reinforced with oriented microfibrils made of stiff crystalline cellulose. The MOE in the fibre direction of reaction woods often differs from that of normal woods, the general trend being that tension wood is stiffer than normal wood and compression wood less stiff. The stiffness of compression wood has been extensively documented (Timell 1986) and due to the high MFA and low cellulose content of compression wood, it is always significantly lower than that of normal wood of the same species.

Data about tension wood are less abundant in the literature but clearly show the opposite trend. For example, in a study of eleven tropical angiosperm species where tension wood and normal wood were identified based on the values of residual maturation strains (Alméras et al. 2005b), the MOE of tension wood was found to be 10–30 % higher than in normal wood for six species but two species had a slightly lower MOE for tension wood (−10 % and −17 %). Three other tropical species, as well as the poplar tree (*Populus* spp.) examined in the study, exhibited a larger difference with tension wood being approximately 50 % stiffer than normal wood. Coutand et al. (2004) who examined small specimens of poplar tension wood found them to be three times stiffer than opposite wood. In chestnut (*Castanea* spp.) (Clair et al. 2003), the MOE of wood was found to be correlated with the residual maturation strain (indicative of the presence of tension wood), tension wood being approximately 50 % stiffer than opposite wood. This work also demonstrated that variations in stiffness were correlated with the proportion of fibres having a gelatinous layer, characteristic of the tension wood fibres for this species.

Strength is a distinct property, expressing the ability of the material to support mechanical loads without breaking. A large strength is necessary to withstand external loads (such as wind, snow, falling trees or animals) without structural damages. Wood strength can be described by a critical stress (MOR) or a critical strain, at a given stage of failure or at the elastic limit (i.e. the point where the relationship between stress and strain is no longer linear). There is usually a significant correlation between the MOE and the MOR at the elastic or failure limit because both are influenced by wood density. Therefore, because tension wood is usually stronger than other woods, its formation as a "muscle" for the motricity function has a beneficial effect on the performance of the skeleton function of the rest of the wood in a tree, whereas in conifers, because compression wood is generally weaker, trees must manage a trade-off between the "muscle" function and the skeletal function of the wood.

However, such a quick theoretical analysis of trade-offs and synergies can lead to the wrong conclusions. Basically, the skeleton performance at the relevant tree level involves not only wood stiffness and strength but also geometry and load, and therefore, for a given amount of biomass, making a thicker stem with less dense weaker wood is much more efficient for the skeletal function (see Larjavaara and Muller-Landau 2010 for more discussion on this topic). Moreover, even at the tissue level, MOE and MOR are not the only criteria that define the skeletal function. Critical strains at the failure or elasticity limit, in different loading modes (compression, shear, etc.), that are independent of MOE and wood density could be candidate additional criteria. To date they have been scarcely used, probably due to the lack of extensive databases on these properties. As the critical strain at failure for compression wood submitted to compressive loads is very high (due to its high MFA and low cellulose content), this should be a positive trait for the skeletal function.

5.4.3 Vascular Properties: Hydraulic Conductivity and Vulnerability to Embolism

Two physical properties are mainly used to quantify the functional hydraulic properties of wood: conductivity and vulnerability to embolism. In wood, water transport is monitored by gradients in negative pressure, i.e. water tension (Tyree and Zimmermann 2002). The conductivity expresses the relation between the water flow in wood and the pressure gradient. A large conductivity allows provision of water to the foliage while minimizing water tension in the conduits. Excessive water tension increases the probability that an embolism will occur. Because an embolized conduit can no longer contribute to water conduction, wood conductivity decreases when water tension becomes larger. Plant vascular systems have varying vulnerability to embolisms and safety against embolisms is directly related to the plant's ability to resist drought and maintain photosynthesis in conditions of large evaporative demand. Unfortunately, there are few studies on the vascular functional properties of reaction woods.

Concerning tension wood, Gartner et al. (2003) examined hydraulic conductivity and vulnerability to embolisms of stem segments of *Quercus ilex* seedlings that had previously been left inclined to induce the production of tension wood. They could not find any difference between the controls and the inclined stems. We did a similar experiment on seedlings of six tropical species (T. Alméras and S. Patiño, unpublished data) and also did not find any significant difference in hydraulic conductivity between controls and previously inclined stems, for any of these species. Note, however, that these two sets of experiments were performed on stem segments containing both tension wood and normal wood, so that normal wood possibly masked the specific properties of tension wood or compensated for them. It is generally considered that tension wood has fewer and smaller vessels than normal wood (Dadswell and Wardrop 1955). In an anatomical study of 21 tropical species, Ruelle et al. (2006) found that vessel frequency was systematically lower in tension wood than in opposite wood, but did not find a systematic pattern for vessel size, except for species with normally large vessels which generally had smaller vessels in tension wood than in opposite wood.

Studies on compression wood hydraulics clearly show that it has reduced conductivity. In Douglas-fir branches, Spicer and Gartner (1998) found that the lower halves, containing compression wood, had a 30 % reduction in conductivity compared to the upper halves. This lower conductivity is probably related to the lower lumen diameter of compression wood (Spicer and Gartner 1998). In a later study, they found that conductivity was 50 % lower in compression wood than in normal wood, but could not find any consequences on the water potential at the whole-plant level (Spicer and Gartner 2002). Working on Norway spruce, Rosner et al. (2007) found that the amount of compression wood in stem segments was negatively correlated with its conductivity, but not with its vulnerability to embolisms. On the same species, Mayr and Cochard (2003) found that compression wood conductivity is 79 % lower than that of opposite wood, and that its

vulnerability to embolisms was slightly higher. Further examination of Norway spruce compression wood (Mayr et al. 2006) revealed that in the compression wood tissue, the first-formed tracheids of an annual ring, called "light bands", have a primary hydraulic function and partly compensate for the very low conductivity of pure compression wood.

From the above studies, it is clear that reaction woods generally have lower hydraulic performance (especially for gymnosperms) than non-reaction wood, but because other wood parts partly compensate for this, the presence of reaction wood has only a minor influence on the hydraulics at the whole-plant level.

5.5 Conclusions on the Ecological Significance of Reaction Wood

Reaction wood impacts tree ecology in different ways: first, it has indirect effects because it modifies other wood traits that are linked to tree physiological functioning (see Sect. 5.4), it changes the pre-stress system in wood which is designed to prevent the tree from breaking (Mattheck and Kubler 1995), and finally it is the main motor of posture control (see Sect. 5.2).

Section 5.4 reviewed the variations of mechanical and hydraulic properties in reaction wood and concluded that it slightly decreases hydraulic performance and can increase or decrease the skeletal performance. To our knowledge, no one has yet studied the possible impacts of reaction wood on wood storage function (and anyway the storage function is not yet to date quantified as a tissue traits in the same way as hydraulic and mechanical functions). Moreover, as illustrated in Table 5.1 for the "muscle" function, each function is performed at the tree level, and therefore a relevant analysis of performance or safety must be done not only at the scale of tissues and cells but also at larger scales. Although a comprehensive multi-scale analysis of all wood functions is beyond the scope of this chapter, one must keep in mind that trade-off at the tissue level can be compensated for at the organism level, and that ecologically the relevant level is the population. So, for example, a large area of sapwood can compensate for low conductivity or a low MOE, and a high water flow is not required if the leaf area is small. Similarly a low MOE is easily offset by a thicker stem and high stiffness is not necessary to support low weights. Generally speaking, discussing the organism performance should include discussion of mechanical loading or disturbance (not only the intrinsic performance of the involved tissue). Actually, at the level of the whole organism, the performances of wood functions (hydraulics, skeletal, motricity, etc.) are very dependent on the stem size and shape. This is especially relevant to the posture control function where the wood properties (maturation strains) cannot be analysed independently of weight disturbance or cross section diameter and radial growth.

There are also important interactions between the skeletal and the "muscle" functions. Controlling the posture by bending stems from peripheral wood requires

counteracting the stem stiffness, which is a basic skeletal property. Therefore, as shown in Table 5.1, although the main tissue trait involved in the motor function is maturation strain, the MOE and the stem diameter are part of the "muscle" performance. Moreover, maturation strain and stiffness or strength share common anatomical determinants at the cell wall level, such as the microfibril angle.

Reaction wood could also be expected to have an impact on strength at the stem level and not only at the tissue level because it modifies the pre-stressing system. Actually, as wood is less strong in compression, this pre-stressing system that puts the periphery under tension is also believed to prevent the tree from breakage (Mattheck and Kubler 1995; Bonser and Ennos 1998). Therefore, the high peripheral tensions of tension wood should be an advantage in resisting bending forces (wind and gravity) in the living tree. However, no one has observed a significant relationship between the level of peripheral stresses and mortality rate, and moreover, Huang et al. (2002) report a reverse distribution of stresses (periphery under compression) in coconut that is known for its slenderness and its strength along windy coasts. Thus, even if high peripheral tensions are an advantage in the living tree, such a trait is not under strong selective pressure.

The use of wood properties from large databases has had great success in plant trait analysis, which aims at explaining plant strategies in different environments from plant strategy axes defined by reduced sets of independent traits (e.g. Chave et al. 2009). Wood density, which is the construction cost of the tissue per unit of volume, is often found to be a good proxy to the first order in predicting variations of the mechanical properties expressed as stiffness (MOE) or as critical stress at failure (MOR). Wood density (a measure of wood porosity: the ratio of cell wall volume to cell volume) is primarily responsible for the large variations of mechanical properties observed between tree species. However, as mechanical properties depend also on cell wall properties, large variations of mechanical properties can also be observed independently of wood density. For example, compression wood, although denser, has a significantly lower MOE than normal wood. Therefore, although not studied yet by ecologists, genetic and environmental variations of reaction wood occurrence could upset the validity of wood density as a general proxy for wood traits. Moreover, as already mentioned, the relevant level for discussing fitness and functional performances is the whole tree, so that discussing ecological performances through wood or cell wall traits requires an integrative biological view. For biophysical functions such as conductivity, skeletal support, motricity or posture control, biomechanical models are useful for integrating tissue and cell properties within the larger structure, taking into account mechanical loads and tree geometry. Therefore, in-depth and complete ecological studies are required, taking into account simultaneously this integrative biological view, more realistic biophysical views than simple compression-tension bending or maximal conductivity, and the construction costs versus benefits of the different wood tissues.

A last but not least question is to know whether posture control by reaction wood formation is a key ecological process. As shown in Sect. 5.3.2, dendroecological approaches use very severe reaction wood as an efficient marker (associated with

other traumatic reactions) of extreme events and disturbances such as storms. Another point of view is that reaction wood formation is not traumatic but very common and usual in "normal life" as gravitropic movements are a strong requirement to build a long living and gigantic but very slender erect structure such as a tree stem. Therefore, the traits involved in this process, including reaction wood presence and properties, should be studied as part of general plant strategies more or less expressed according to genotypes and conditions of stress, competition or disturbance (and not only in extreme conditions of disturbance). Gravitropism is widely studied by plant scientists (e.g. the book of Gilroy and Masson 2008), but although it is quite easy to demonstrate that without reaction wood, no tree could grow and stand up in the long term (Alméras and Fournier 2009; Fournier et al. 2006), plant ecologists who are unfamiliar with plant biomechanics are reluctant to recognize that posture control is as important a function in trees as hydraulic or skeletal functioning. There are two main reasons for this. First, current ecological studies are based on large databases and there are no equivalent large technological databases for maturation strains as there are for MOE, MOR or wood density. Then, looking only at maturation strains or reaction wood occurrence is not very informative because, as already discussed, the posture control function involves several interacting variables, and only a systemic view (rather than a single plant trait approach) can help to determine the exact effect on plant performance versus constraints. Secondly, posture control is a dynamic process that is not easy to observe or follow. For example, when under perfect control the plant reacts to gravitational constraints but always remains perfectly vertical and straight! Because a small tree with a thin cross section bends more easily the gravitropic process is probably more important in the first ontogenetic stages, with major long-term consequences (Dassot et al.; 2012). Long-term observations in permanent plot studies, for example of survival probabilities of young trees as a function of the performance of their posture control function are necessary for a better integration of reaction wood studies in tree ecology.

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Chapter 6
Physical and Mechanical Properties of Reaction Wood

Bruno Clair and Bernard Thibaut

Abstract Reaction wood produces very peculiar maturation stresses at the tree periphery, i.e. compressive stress or very high tensile stress, for compression and tension wood, respectively, as compared to moderately high tensile stress for normal wood. This means that both its mechanical state and its mechanical and physical properties differ from normal wood.

Compression wood shows big differences from normal wood in conifers, for all physical and mechanical properties: higher density and axial crushing strength (MOR) but lower modulus of elasticity (MOE), far higher axial (longitudinal) shrinkage but lower radial and tangential shrinkage, sometimes even lower than the axial shrinkage.

For tension wood things are less simple and can vary a lot from hardwood species to species. Globally there are no systematic differences in density and transverse shrinkage; MOE tends to be a little higher while MOR is slightly lower. However, axial shrinkage is much higher for tension wood with a gelatinous layer (G layer) than normal wood due to the specific gel-like organization of matrix in the G layer. For tension wood without a G layer (which is rather frequent) axial shrinkage is around two times higher than in normal wood. This paradoxical shrinkage is thought to originate from the release of maturation stresses during drying.

Overall the very high tensile stress and stored elastic energy in tension wood lead to problems in wood processing (end splitting and board warping), which is far less the case for compression wood. But due to the large difference in properties relative

B. Clair (✉)
CNRS, UMR Ecologie des Forêts de Guyane (EcoFoG), Campus Agronomique, BP 701, 97387, Kourou, French Guiana, France

Laboratoire de Mécanique et Génie Civil (LMGC), CNRS, Université Montpellier 2, Place E. Bataillon, CC048, 34095 Montpellier Cedex 5, France
e-mail: bruno.clair@univ-montp2.fr

B. Thibaut
Laboratoire de Mécanique et Génie Civil (LMGC), CNRS, Université Montpellier 2, Place E. Bataillon, CC048, 34095 Montpellier Cedex 5, France

to normal wood, compression wood occurrence is always a big problem for the in service behaviour of timber, which is seldom the case for tension wood.

6.1   Introduction

The technological properties of reaction wood (RW) have been extensively studied because they generally reduce the quality of wood products and therefore have important commercial consequences. The presence of reaction wood may affect timber at two levels: firstly at the tree or plank level when distortion arises as the heterogeneous stress field across the wood is modified by crosscutting and sawing. This manifests itself when the tree is felled as log-end cracks or later during sawing as distortion and cracks in the wood. The presence of reaction wood at the periphery of the stem tends to increase the stress gradient in the case of tension wood (TW) and decrease it in the case of compression wood (CW). It results, in some cases, an increase in log-end cracks when TW, but not when compression wood is present (Jullien and Gril 2003).

The second effect of reaction wood on wood products is linked to the differences in its structure and chemical composition compared with those of normal wood (NW) (see Chaps. 2 and 3). These differences affect its density, its mechanical behaviour, its behaviour with moisture change, and other properties such as colour and texture.

This chapter will focus on the properties of reaction wood as material, including the effect of the release of stress, but not the structural effects linked to heterogeneity in the tree. The latter will be discussed in detail in Chaps. 8 and 9.

6.2   Density

Density has long been understood to be the main factor affecting the mechanical properties of wood. Simply speaking, the denser the wood the stronger and stiffer it is. When properties are compared between species, this factor is of primary importance compared to other structural parameters such as microfibril angle (MFA), which is the second most influential parameter, or chemical composition. Thus, because of density differences related to species, oak (*Quercus* spp.) will always be stiffer than poplar (*Populus* spp.) whatever the MFA. Density is also linked to physical properties such as swelling and shrinkage of wood, although the relationship is not as direct as in the case of strength properties. Furthermore, density is the easiest parameter to measure in wood, so it is generally used to estimate its quality and potential uses.

However, although density is the first order factor affecting properties when comparing species or trees, the relationship becomes less clear when comparing properties in a single tree and especially when studying reaction wood, where structural changes become of greater importance in influencing wood behaviour.

Thus, compression wood, although denser than normal wood, is less stiff. In hardwoods, the relationship between density and stiffness is also disturbed, with large changes in stiffness occurring without concomitant changes in density.[1]

It is important not to confuse wood density and cell wall density. The term wood density refers to macroscopic measurement, and depends on the amount of cell wall compared to void volume (fibre and vessel lumina, for example) and can thus show considerable variation from species to species, whereas the term cell wall density refers to the cell wall itself and thus depends only on the chemical composition of the cell wall. Cell wall density is very stable among species.

6.2.1 Density of Compression Wood

Compression wood is almost always denser than normal wood. Timell (1986) cites numerous publications which all confirm this tendency. In more than 75 % of the studies described by Timell, a ratio of density of CW/NW of 1.1–1.8 was found with some extreme cases showing up to 2.2. In 16 % of the studies, the ratio was between 1 and 1.1. These rarer cases he attributes to the accidental inclusion of compression wood in the normal wood sample.

The specific gravity of the cell wall in compression wood can be computed assuming the densities of lignin, cellulose, and hemicellulose, respectively, as 1.4, 1.58, and 1.50. Since compression wood is richer in lignin (around 40 %) than normal wood (around 30 %), cell wall density is lower in compression wood than normal wood (respectively, around 1.48 and 1.50 $g\,cm^{-3}$). The high increase in macroscopic density is linked to the fact that the cell wall is much thicker in compression wood than in normal wood. This agrees with another observation by Timell (1986) that the ratio in density is even higher in species where normal wood is less dense.

6.2.2 Density of Tension Wood

General trends are more difficult to find in tension wood since it is much more variable than compression wood in terms of structure and occurrence. Several studies have compared tension wood and normal wood without considering the severity of the tension wood and found that tension wood has a higher density than normal wood (Chow 1946, 1956; Arganbright et al. 1970; Jourez et al. 2001a;

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[1] Density is defined as the ratio between the mass of a material and its volume. For wood, it has been traditional to use what is called basic density, which is the ratio between oven-dry mass and saturated volume. The latter measurement is chosen because it is easy to determine in practice, since volume measurement is generally made by immersing the wood in water and applying Archimedes' principle. The comparison in density between normal wood and reaction wood is, however, little affected by the definition used.

Coutand et al. 2004). Lowell and Krahmer (1993) observed no significant effect of leaning of tree on wood density. Ruelle et al. (2007a) studying ten tropical species reported that three of them showed no significant difference in density between normal wood and tension wood while in the five trees that showed a significant difference, two had a lower density in tension wood. However the differences were small except in the case of *Virola surinamensis* (Rolander) Warb. where density was lower in tension wood, and *Qualea rosea* and *Ocotea guyanensis* where density was higher in tension wood. Similarly, McLean et al. (2012) found on six tropical species only *O. guyanensis* with tension wood significantly denser than opposite wood, whereas on three of them (*Sextonia rubra*, *Virola michelii*, *Tachigali melinoni*) the trend was reversed and no trend was found for the two other species.

Some other studies measured the change in density for a gradual change in severity of tension wood. Severity was expressed as the percentage of gelatinous layers in the wall (which is of course only applicable to species which have a G-layer) or was measured using release growth-strain measurement. Where the amount of G-layer was measured, a positive correlation was sometimes found between density and tension wood fibre percentage (Kroll et al. 1992; Washusen et al. 2001; Clair et al. 2003c) although Arganbright et al. (1970) showed no correlation between density and percentage of gelatinous fibres. When density was related to growth-strain measurements, no general tendency was found. Fang et al. (2008a) showed a significant but weak positive correlation in poplar, as did Yang and Ilic (2003) in *Eucalypts globulus* Labill. Boyd (1980) showed a significant positive within-tree relationship between growth strain and density in 10 of 17 trees (16 *Eucalyptus regnans* and one *Eucalyptus obliqua*) studied (mostly the trees undergoing crown-reorientation), but no significant among-tree relationships were found between growth strain and density. Chafe (1990) found a highly significant positive relationship between growth strain and density for *E. regnans*, but not for *E. nitens*. Baillères (1994) found no relationship between density and residual maturation strain when all tree ages were considered but showed that the relationships became significant when analysis was done intra-tree for young trees. In older trees, density reached a maximum and the presence of tension wood did not increase it significantly.

The high variability described above results from more complex factors affecting density in hardwoods. Because angiosperms have specialized tissues, the density does not depend only on cell wall thickness as in compression wood tracheids, but also on the proportion of tissues and especially the number and size of vessels. Numerous studies have found that there is a lower proportion of vessels having a smaller diameter in tension wood (Jourez et al. 2001b on poplar; Ruelle et al. 2006 on 21 tropical species). Density is also affected by wall thickness, which is generally assumed to be greater in tension wood with gelatinous fibres. Despite the probability that most studies have overestimated this measurement since the G-layer appears in a swollen state in microscope sections (Clair et al. 2005c), a recent study on poplar showed that G-fibres have a thicker wall than normal fibres where they have not been affected by sectioning (Fang et al. 2007, 2008b). When growth stresses increase, an increase in G-layer thickness is observed but

compensated for by a decrease in thickness of the S_2 layer so that the wall thickness of G-fibres is similar to that of normal fibres in poplar (Fang et al. 2007). For species without a G-layer, fibre wall thickness is sometimes significantly thinner in tension wood (Ruelle et al. 2006) and could explain observations of lower density of tension wood in some species.

Following the same reasoning as for compression wood, the density of the cell wall in tension wood can be estimated. Whether or not a G-layer is present, tension wood is always reported to be less rich in lignin and more rich in cellulose than normal wood. Cell wall density would therefore be higher than in normal wood. However, in the peculiar case of the gelatinous layer, even if it is known that lignin is essentially absent, its density is probably not higher than the lignified wall. In fact, the high transverse shrinkage around 20 % (Fang et al. 2007) during drying is a proof of its high water content. Allowing for a 20 % void in the G-layer its density can be estimated at about 1.25–1.27 g cm^{-1}, much lower than that of the normal wood or compression wood cell wall. This low density is linked directly to its high mesoporosity (pore size around 6–10 nm) as observed in chestnut (*Castanea sativa*) or louro vermelho (*S. rubra*) (Clair et al. 2008; Chang et al. 2009a). This low density contributes to confusion about the relationship between wood density and tension wood since the amount of G-layer increases with increasing growth stresses (Fang et al. 2008b).

6.3 Mechanical Behaviour of Reaction Wood

An analysis of the literature shows that there are three main groups of workers interested in the mechanical behaviour of reaction wood. The larger group is made up of wood scientists who study wood as a raw material for commercial use. This means that they study wood properties in the conditions of use, i.e. dry, below the fibre saturation point. The other group comprises scientists working in the field of biomechanics who study wood in order to understand its function in tree. In this work, experiments are mainly carried out on wood in the green or wet state. A third group of workers study the change in the properties of the wood during drying to obtain information about the origin of the properties of reaction wood (Clair et al. 2003c, 2006a; Yamamoto et al. 2009).

As discussed it in the previous chapter, mechanical behaviour is largely affected by density. In order to identify the contribution of density to wood properties, mechanical properties such as modulus of elasticity (MOE) or strength can be expressed by their specific modulus or specific strength by dividing the studied properties by the density of the samples. This makes it possible to identify the contribution of ultrastructure to the particular property.

Fig. 6.1 Relationship between MFA and longitudinal modulus of elasticity (MOE) and relationship between MOE and density/MFA for three eucalyptus species between 15 and 33 years of age (from Yang and Evans 2003)
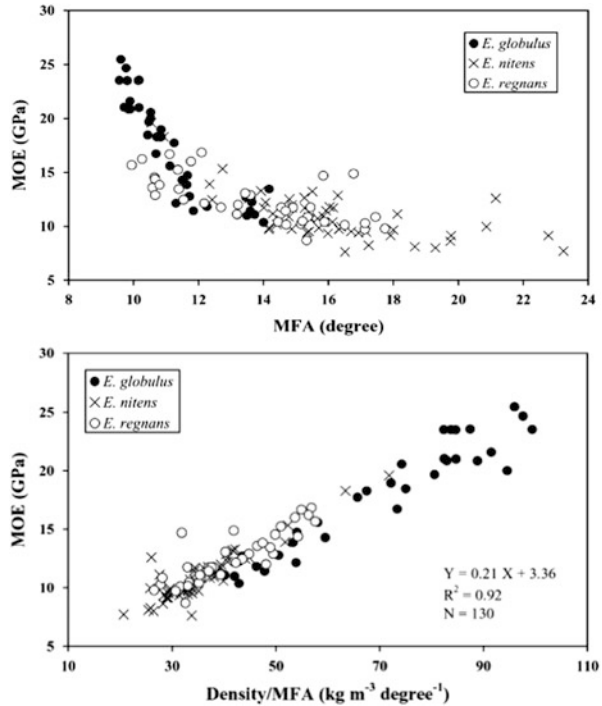

6.3.1   Visco-Elastic Properties

6.3.1.1   Longitudinal

Properties at the Macroscopic Level

Numerous investigations, both from measurement and modelling, have shown that there is a good relationship between elastic properties in the longitudinal direction and MFA in the secondary wall (Fig. 6.1) (Cave 1969; Mark 1973; Salmén and Ruvo 1985; Koponen et al. 1989, 1991; Astley et al. 1998; Harrington et al. 1998; Reiterer et al. 1999; Salmén 2004). Recent research has become more focused on second-order properties arising from properties and organization of other cell wall components (Salmén and Burgert 2009). Reaction wood is known to be character-ized by a great change in MFA, which is higher than in normal wood in compres-sion wood and lower in tension wood. Thus, during a test along the longitudinal direction, load will be applied essentially on the microfibrils rather than the matrix in tension wood. The matrix will be more affected when the MFA increases as in compression wood. As cellulose microfibrils are much stiffer than the matrix materials, the stiffness will be higher when MFA is low, as in tension wood and lower in compression wood where the MFA is large. A concomitant parameter is

Fig. 6.2 Relationship between E'/ρ and tan δ measured on strips; *filled symbols*: compression wood; *open symbols*: normal wood; *triangle*: spruce; *square*: maritime pine; *rhombus*: Scots pine (from Brémaud et al. 2013, Ono and Norimoto 1983)
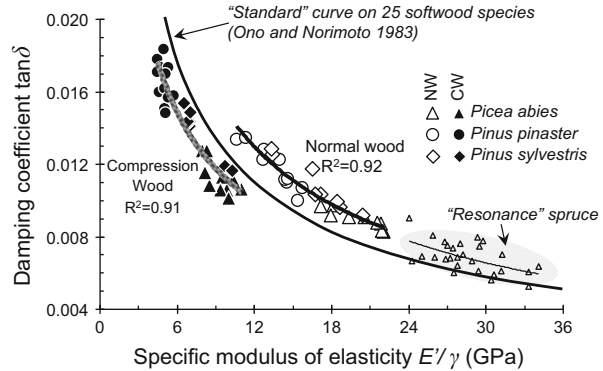
the amount of matrix compared to cellulose. Compression wood is known to be more lignified and contain less cellulose compared to normal wood and tension wood to be more cellulosic. This characteristic is directly related to the difference in reaction wood properties, but as shown by Gindl (2002) the effect of increased lignin content on the Young's modulus in compression wood is generally not discernible because of the dominating influence of MFA. However, concerning visco-elasticity, it has been shown that the changes in MFA cannot explain the complementary reduction in acoustical damping found in compression wood as compared to normal wood (Fig. 6.2) (Brémaud et al. 2013).

All investigators agree that the MOE of compression wood is much lower than that of either opposite or normal wood. This finding does not depend on the nature of the force applied, whether compression, tension, or static bending (Timell 1986), and agrees well with expectations based on the fact that the MFA in compression wood is large compared to that in normal wood (Reiterer et al. 1999). For example, Burgert and Jungnikl (2004) using wet thin strips of spruce wood found values of around 1,000 MPa for compression wood, 3,250 MPa for opposite wood, and an intermediary value of 1,750 MPa for lateral wood [and, respectively, around 2,000, 3,000, and 2,500 in Yew (*Taxus* spp.)]. They confirmed the relationship with MFA but were unable to find a relationship with density. Timell (1986) reported that the increase of MOE is modest after drying, and is the same for compression wood as for normal wood.

The situation with regard to the elastic behaviour of tension wood is less clear-cut than for compression wood. Because the MFA in tension wood is always lower than that in normal wood (Clair et al. 2006b), modelling would predict an increase in stiffness. However, studies have produced contradictory results even within a genus. For example, in poplar, some authors found tension wood to be much stiffer than normal wood (Fang et al. 2008a) up to two to three times (Coutand et al. 2004; Dinh et al. 2008 using three and four points bending, respectively), whereas in another study it was found that the difference was significant in only one of the three hybrids studied (Alméras et al. 2005 using the dynamic flexion test). Boyd (1980) using tensile testing compared MOE to growth strain measured in the tree

and found a significant positive within-tree relationships in only 9 of 17 eucalyptus trees studied (mostly trees actively reorientating their crowns) but no significant among-tree relationship between growth strain and MOE. Similarly, Baillères (1994) reported a poor relationship between MOE and growth strain when all trees were taken into account but noticed that the relationship became significant within each tree.

Chafe (1990) found that even within the tree no relationships were noticeable. The same observation was made by Yang and Ilic (2003) who reported that, unexpectedly, some of the specimens that had marked tension wood with high density and thick cell walls had lower MOE values than other normal wood specimens. This work was carried out using wet wood samples. Similarly, Clair et al. (2003c) observed a higher dispersion of MOE in chestnut tension wood than in normal wood, indicating that some tension wood samples have a higher MOE and some a lower MOE compared to normal wood. Yamamoto et al. (2009) showed that when comparing the relationship of growth strain and MOE of oak wood (*Quercus acutissima*) the difference was small in the wet state and became greater in dry wood.

Ruelle et al. (2007a, 2011), Alméras et al. (2005), and McLean et al. (2012) reported that in most species, tension wood is stiffer than normal wood. Ruelle et al. (2007a) studied ten tropical species and found that longitudinal MOE and specific MOE in the dry condition were higher in tension wood of eight trees (between 16 and 54 % higher, as specific MOE), except in *Cecropia sciadophylla* Mart. and *V. surinamensis* (Rolander) Warb in which specific MOE was very high both in tension and in opposite wood (the difference was statistically significant for seven trees). Alméras et al. (2005), working on 11 tropical species and 3 poplar hybrids (wet wood) noticed that trees with the highest MOE for normal wood had higher MOE for tension wood, whereas most of the trees with low MOE (*Populus* I-MC, *Populus* I-214, *Jacaranda copaia*, *Simarouba amara*) had similar MOE values for normal and tension wood. Among trees where differences were significant, five had a higher MOE for tension wood than for normal wood and 2 had a lower MOE in tension wood (Fig. 6.3).

Concerning the viscosity of tension wood along the fibres, McLean et al. (2012) reported a higher damping in wet tension wood compared to normal wood only in species producing a G-layer, whereas for non-G-layer tension wood the difference in tan δ was non-significant or reversed. After drying the difference remained significant only for two of the three G-layer producing species.

Properties at the Fibre and Cell Wall Level

Burgert et al. (2002, 2003, 2005a, b) carried out several studies comparing the properties of isolated fibres. Compression wood always had a much lower MOE compared to other studied wood types (Fig. 6.4). When comparing compression wood from different species Burgert et al. (2004) found that elastic behaviour differed more significantly at the tissue than at the fibre level, raising questions about the contribution of fibre arrangement to wood behaviour.
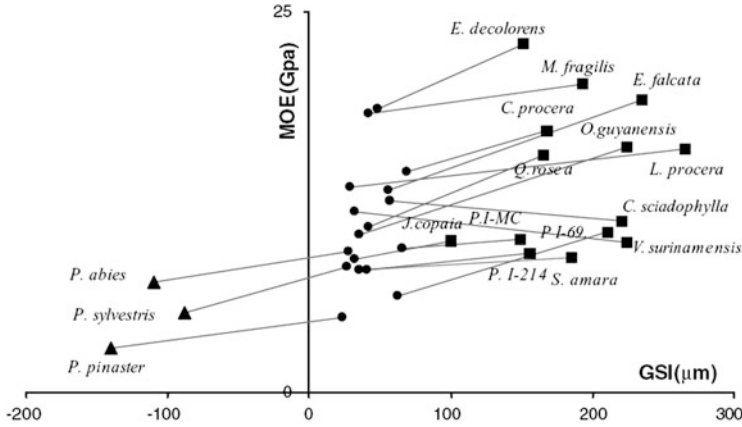
Fig. 6.3 Modulus of elasticity of reaction wood and normal wood versus growth strain indicator for 17 trees. *Triangles*: compression wood, *circles*: normal wood, *squares*: tension wood. Lines join the normal and reaction wood from the same tree (from Alméras et al. 2005)
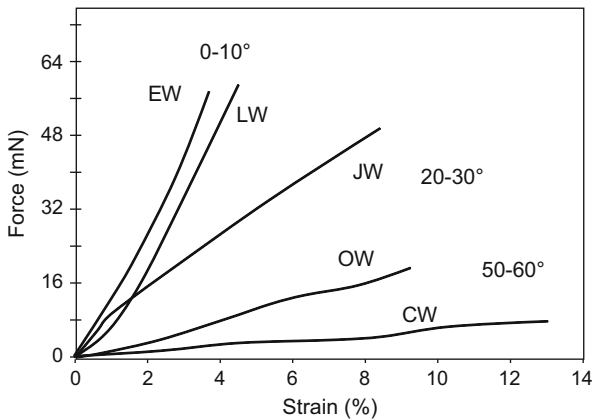
Fig. 6.4 Load strain diagram of mechanically isolated fibres. *EW* early wood, *LW* latewood, *JW* juvenile wood, *OW* opposite wood, *CW* compression wood. Corresponding range of MFA is given for each group of wood type (from Burgert et al. 2002)

Clair et al. (2003c) and Yamamoto et al. (2005, 2009) investigated the contribution of the gelatinous layer to the MOE. The first of these studies was performed on chestnut wood and showed a significant correlation between longitudinal Young's modulus and the percentage of fibres with a G-layer (G-fibres) both in the wet and dry condition, irrespective of the thickness of the G-layer. Similar results were found for maple (*Acer* spp.) wood (Yamamoto et al. 2005). Later, Yamamoto et al. carried out a similar study on kunugi oak wood (*Q. acutissima*) and found an even better correlation when they considered the area ratio of G-layer

Fig. 6.5 Mean ratio and standard deviation of modulus of elasticity from wet to dry as a function of the proportion of G-fibres in the samples. *Numbers* indicate the number of specimen for each class (from Clair 2001)

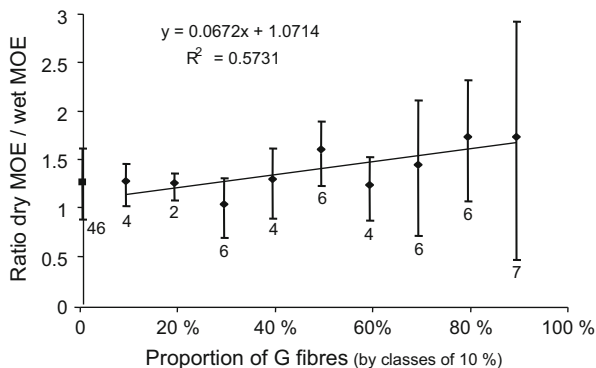**Table 6.1** Modulus of elasticity of G-fibres and non-G-fibres and for G-layers and other layers computed from a mixture model (Chestnut data: Clair et al. 2003c; Maple data: Yamamoto et al. 2005; Oak data: Yamamoto et al. 2009)

	Wet MOE (GPa)	Dry MOE (GPa)	Increase during drying
Non-G-fibres (chestnut)	14.5	15.1 (air-dry)	4.14 %
G-fibres (chestnut)	21.6	31.3 (air-dry)	44.91 %
Non-G-fibres (maple)	8.50		
G-fibres (maple)	16.24		
Cell wall in non-G-fibre (maple)	13.13		
Non-G-layer in G-fibre (maple)	16.28		
G-layer in G-fibre (maple)	28.27		
Non-G-fibres (oak tree 1)	14.79	17.70 (oven-dry)	19.68 %
G-fibres (oak tree 1)	15.98	39.16 (oven-dry)	145.06 %
Non-G-fibres (oak tree 2)	22.79	29.52 (oven-dry)	29.53 %
G-fibres (oak tree 2)	25.02	41.64 (oven-dry)	66.43 %
Non-G-layer (oak tree 1)	19.49	23.79 (oven-dry)	22.06 %
G-layer (oak tree 1)	27.88	84.92 (oven-dry)	204.59 %
Non-G-layer (oak tree 2)	24.86	32.24 (oven-dry)	29.69 %
G-layer (oak tree 2)	38.10	68.73 (oven-dry)	80.39 %

to the cell-wall area. In the chestnut and oak studies the difference was even higher in the dry state but with a higher dispersion when there was a high proportion of G-layer fibres (Fig. 6.5) (dry MOEs were not measured in the maple study). The increase of dispersion was explained by Clair et al. (2003c) as a possible consequence of some delamination between S_2 and G-layers during drying but this hypothesis was later refuted by a study showing that delamination is a sample preparation artefact and does not occur in the core of a sample even after drying (Clair et al. 2005b). These studies proposed a simple mixture model to determine the MOE of separated components. Results are summarized in Table 6.1.

There is a greater increase in rigidity from the saturated to the air-dry states for tension wood than for normal wood (Fig. 6.6). This increase of rigidity can be
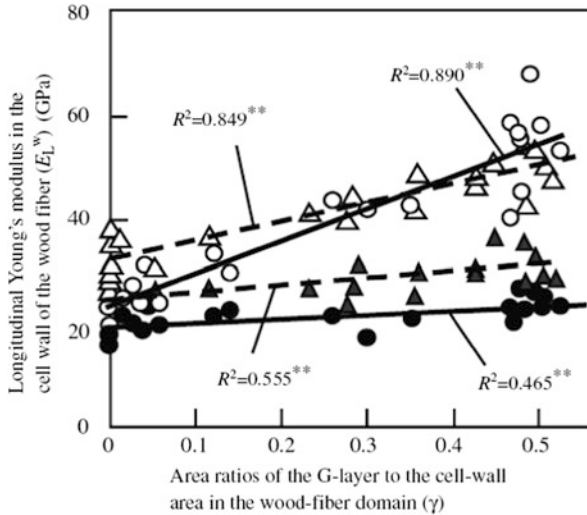
Fig. 6.6 Longitudinal Young's modulus in the cell wall of wood fibres versus the area ratio of the G-layer to the cell wall in the wood fibre domain. *Circles* and *triangles* represent two different oak trees. *Filled symbols*: wet condition, *open symbols*: dry condition (from Yamamoto et al. 2009)

attributed to the G-layer which increased by between 80 and 200 %. This stiffness increase is attributed by the authors to the xerogelation of the matrix, which loses its mesoporosity as shown by Clair et al. (2008) and allows stiff contact between microfibrils.

Experiments on mechanical properties at the level of the cell wall layers need the use of specialized microscopic techniques such as nano-indentation or atomic force microscopy. Few studies on reaction wood have been made using these techniques. Using nano-indentation, Gindl et al. (2004) reported a lower MOE in compression wood compared with any other wood type in Norway spruce (Picea abies (L.) H. Karst.) and commented on the good relationship of MOE with MFA. Konnerth and Gindl (2006) confirmed these observations in another study in which they observed mild compression wood. Regarding tension wood, only one reference shows mapping of elastic properties by atomic force modulation microscopy (Clair et al. 2003a). The G-layer, observed in the dry condition, appeared stiffer than other layers (Fig. 6.7). Authors describing both nano-indentation and atomic force modulation microscopy studies noted the limitations of the methods and pointed out that elastic moduli of wood cell walls determined by these techniques have to be interpreted with caution.

6.3.1.2 Transverse Elastic Properties

Timell (1986) reported no studies of transverse elastic properties of reaction wood and only one measurement has been reported by Placet et al. (2007) for spruce
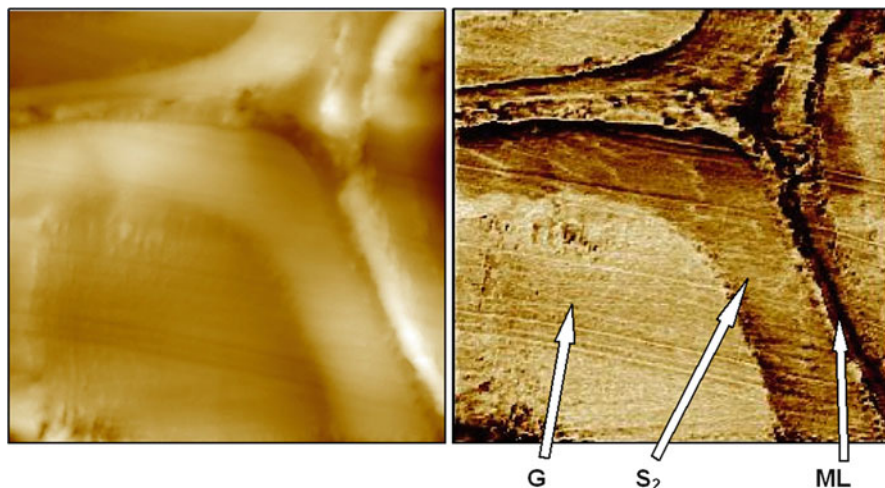
Fig. 6.7 Images obtained with atomic force modulation microscopy. *Left*: topographic image of oak tension wood cell wall. *Right*: corresponding image of elasticity. A *lighter colour* indicates higher rigidity than darker ones. G: G-layer, S₂ layer, ML: middle lamella (from Clair et al. 2003a)

compression wood in the wet condition where the storage modulus was found to be almost fivefold higher compared to normal wood in the tangential direction (CW: 447 MPa, NW: 84 MPa).

Passard and Perré (2005) used the cantilever bending test on wet oak wood and found that regardless of the direction, wet tension wood was less stiff than normal wood. In the radial direction they found a mean of 687 MPa for normal wood and a mean of 607 MPa for tension wood. Similarly, in the tangential direction, the modulus was found to be around 398 MPa in normal wood and 354 MPa in tension wood. Later, using a dynamic test at 1 Hz, Placet et al. (2007) found a greater difference between the wood types in similar conditions (tangential OW: 242 MPa, tangential TW: 146 MPa, radial OW: 680 MPa, radial TW: 495 MPa). Similar results were obtained on dry wood when Dinh et al. (2008) did a four point static bending test on millimetric samples of dry poplar and reported that differences in Young's modulus between normal and opposite wood and tension wood were low. Otherwise, MOE is lower in tension wood in both radial and tangential directions, from $E = 400$ MPa (TW) to 500 MPa (NW) in the tangential direction and from $E = 1{,}200$ MPa (TW) to 1,500 MPa (NW) in the radial direction. They explained this by the lack of cohesion of the G-layer with the rest of the wall, but following results from the study on the detachment of the G-layer (Clair et al. 2005b), an alternative explanation could be the very low transverse modulus in the G-layer resulting from its low MFA and the absence of lignin. In these cases, the part of the tension wood fibre wall contributing to the mechanical properties would be thinner than in normal wood fibre walls ($S_1 + S_2$ layers only rather than $S_1 + S_2 + S_3$ layers).

6.3.1.3 Shear Modulus

Very little work has been carried out into this question. Timell (1986) cited only two studies where shear modulus was found to be larger for compression wood than normal wood by a factor 2. No data were found concerning tension wood.

6.3.2 Strength Properties

6.3.2.1 Strength in Compression Wood

In the studies reported by Timell (1986), compression wood was generally stronger in compressive strength than normal wood, i.e. it can sustain a higher maximum load before failure. However, when taking into account the higher density of compression wood, compression wood is surprisingly similar to normal wood, being sometimes slightly stronger, sometimes slightly weaker. Gindl et al. (2001) showed that the compression and bending strength of compression wood increase proportionally with increasing density as is the case in normal wood. Gindl (2002) showed that compressive strength of compression wood was not negatively affected by the high MFA and suggested that the high lignin content in compression wood increases the resistance of the cell walls to compression failure.

During drying, strength properties generally increase, but compression wood generally improves less than does normal wood. Another strength property is the stress at proportional limit denoting the maximum stress at which load and strain are still proportional. For this property, compression wood appears weaker than normal wood.

Compression wood, like normal wood also has a higher tensile than compressive strength although results have been contradictory with regard to comparisons between the two wood types with compression wood being generally found to be weaker than normal wood although sometimes it has been reported to be stronger (Timell 1986).

Recently, Reiterer et al. (1999) testing 200 μm thin sections, showed that strain at maximum stress before rupture was higher in compression wood and was governed by MFA. The data for stress at the proportional limit in tension shows compression wood to be much weaker than normal wood.

Gindl and Teischinger (2003) found shear strength of compression wood to be significantly higher than expected on the basis of its density compared to normal wood. They found that the specific shear strength was 23.2 MPa cm^3 g^{-1} for compression wood compared to 17.7 MPa cm^3 g^{-1} for normal wood.
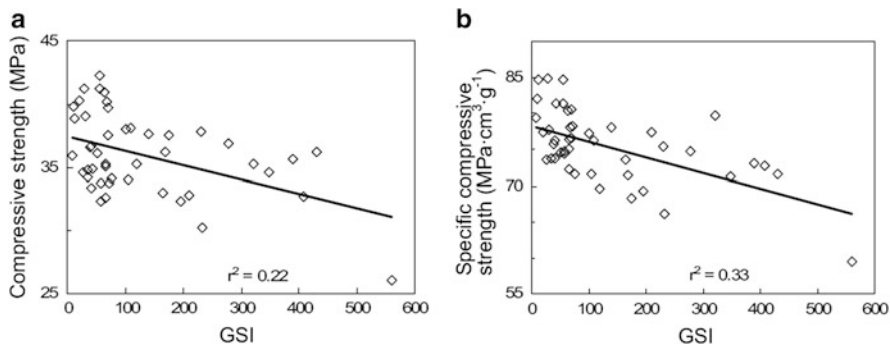
Fig. 6.8 Relationships of (**a**) compressive strength (MPa) and (**b**) specific compressive strength (MPa cm^3 g^{-1}) with growth stress indicator (GSI, μm) (from Fang et al. 2008a)

6.3.2.2   Strength in Tension Wood

Clarke (1937) reported that beech (*Fagus* spp.) tension wood was weaker under compression than normal wood and observed that tension wood failed by buckling, whereas normal wood had a shear type failure. He also reported that, under tension, a wide range of variation was found both in normal wood and tension wood, with tension wood on average stronger than normal wood. Similarly, Dadswell and Wardrop (1955) reported little difference in tensile strength between tension wood and normal wood. Ruelle et al. (2007a) reported that among ten species, only one showed a significant difference between tension and opposite wood for flexure and compressive strength. For the other species, no predominant tendency was found; sometimes tension wood was more resistant, at other time, opposite wood was more resistant. Fang et al. (2008a) reported a significant decrease of compressive strength from normal wood to tension wood in poplar (Fig. 6.8a). These results are understandable when plotting the specific compressive strength (Fig. 6.8b) since the decrease of compressive strength is accompanied with an increase of density from normal wood to tension wood in the samples.

The lower compressive strength in tension wood could be explained by the lower lignin content especially in the G-layer, allowing easy buckling of the cell wall giving rise to the formation of slip planes and minute compression failures as observed by Wardrop and Dadswell (1948, 1955).

6.4   Consequences of Internal Stresses on Wood Properties

6.4.1   *Boiling and Hygrothermal Recovery*

The internal stresses accumulated during the life of the tree are partially released through strain during sample or board preparation. However, some strains still

remain in the wood. These locked in strains will be released with time or with hygrothermal treatment, so-called hygrothermal recovery (HTR) (Kubler 1987). Little research has been carried out on the HTR of reaction wood. No data have been found by the authors on compression wood and just two publications refer to tension wood both on chestnut wood. Gril et al. (1993) found a clear difference of HTR in the tangential direction between tension wood and normal wood and also with opposite wood (NW = 0.55 %, OW = 0.3 %, TW = 0.85 %). The ratios between wood types were as follows, TW/NW = 1.5 and OW/NW = 0.5. In another study dealing with the contribution of internal stress to drying shrinkage (Clair 2012) it was shown that hygrothermal swelling stain along the tangential direction was clearly higher in tension than normal wood (NW = 0.15 ± 0.15; TW = 0.58 ± 0.26). Similarly, along the longitudinal direction, normal wood slightly swells (NW = 0.58 ± 0.26), whereas tension wood significantly shrinks (TW = −0.14 ± 0.05). This clear difference between wood types is directly connected to the stress level that can be expected in the tree.

In a study on the effect of boiling on *Zelkova serrata* tension wood, Abe and Yamamoto (2007) showed that longitudinal shrinkage on drying is less after boiling compared to unboiled samples. This could result from the initial release of strain by thermal treatment. The effect was higher when the amount of G-layer was greater in the sample. It can be deduced from this study that boiling affects the longitudinal Young's modulus, which is lower than in unboiled green wood.

6.4.2 Behaviour During Solvent Exchange

Kubler (1987) observed that solvent seems to trigger HTR without heat. Recently Chang et al. (2009b, 2012) made a similar observation of strain following various solvent treatments. The observed strain along the longitudinal direction allows a clear separation of wood types between normal wood and tension wood both in chestnut and poplar, which produce typical G-layers, and *Simarouba amara* having tension wood without a G-layer (Ruelle et al. 2007b). Whereas normal wood exhibits swelling linked to the difference of molecular size between water and ethanol, tension wood exhibits 0.05 % shrinkage. In the tangential direction no differences were found between wood types. When cycling between water and ethanol is performed, poplar tension wood samples always continue to shrink in the longitudinal direction both in sorption and desorption up to an asymptotic limit (Clair, unpublished data).

6.5 Drying Behaviour

Wood is subject to dimensional changes during drying. Normal wood shrinks about 4–6 % in the radial direction and around 8–10 % in the tangential direction, but much less in the longitudinal direction (around 0.1 %). Shrinkage along the radial and tangential directions is due to a combination of effects at the cell wall level and effect of structure linked to the organization and the shape of the cells. In the longitudinal direction, differences in shrinkage are explained mainly by differences in MFA in the S_2 layer (which is the thickest layer of the cell wall). In fact, the crystalline nature of cellulose make it quasi-non-deformable, thus, water movement only affects dimensions perpendicular to microfibrils (shrinkage is quasi-null along cellulose microfibrils and maximum transverse to them). The larger the MFA, the more the axial shrinkage and the less the transverse shrinkage of the wall. This has been largely verified experimentally and modelled (e.g. Barber and Meylan 1964; Barber 1968; Cave 1969, 1972a, b; Barrett et al. 1972; Boyd 1977; Gril et al. 1999; Yamamoto 1999)

6.5.1 Drying Shrinkage of Compression Wood

Because compression wood has a very high longitudinal shrinkage compared to normal wood, its presence in wood products decreases its value considerably. Heterogeneity in wood products produces warps and splits. Thus, numerous studies have been made on the drying shrinkage of compression wood. Timell (1986) reviewed the earlier ones and from the huge number of experiments presented the following conclusions can be drawn (shrinkage being calculated as the difference in length between green and oven-dry condition compared with green length): longitudinal shrinkage has been found generally to lie between 1 and 3 % with maximum values in some cases up to 6 %. Thus longitudinal shrinkage is generally 8–15 times higher than in normal wood and up to 40 times higher in some cases. In the radial direction, shrinkage is around 1.5–3 %, between a third and a half of what was found in associated normal wood. Tangential shrinkage is around 3–4.5 %, e.g. 40–60 % less than in normal wood. Although authors have considered the contribution of the high lignin content, the lower crystallinity of cellulose and some structural aspect such as the lack of S_3 and the circularity of the cells in compression wood, the key role of the larger MFA is regarded as the main factor influencing the high longitudinal shrinkage of compression wood.

6.5.2 Paradoxical Longitudinal Shrinkages of Tension Wood

6.5.2.1 Historic

In 1949, Onaka (1949) remarked on the lack of studies on the shrinkage of tension wood and reported an experiment comparing flexion during the drying of 30 cm long portions of branches of various hardwood and softwood species (Fig. 6.9). He noted that the bending is towards the lower side in conifers (compression wood side) and on the upper surface in deciduous trees (tension wood side). And he adds: "however, in such species as *Meliosma tenuis* Maxim. *Meliosma myriantha* Sieb. and Zucc. and *Tilia japonica* Smik. In which the gelatinous layer does not appear, no such bending is observed. Thus, such changes could be attributed to the presence of the gelatinous layer. It must shrink markedly in the longitudinal direction in drying".

From 1937 up to the 1950s, several studies of high longitudinal shrinkage in tension wood with a G-layer have been described (Clarke 1937; Chow 1946; Onaka 1949; Akins and Pillow 1950; Pillow 1956; Terrell 1953; Wardrop and Dadswell 1955). These studies highlight the relationship between this particular layer and the high shrinkage but there is no microscopic interpretation of the phenomenon. The link with density has been studied by several authors and confirms the absence of any clear relationship (Terrell 1953). The G-layer is widely believed to be the cause of the high shrinkage of tension wood, which is responsible for numerous technological problems such as splitting and distortion.

The G-layer was discovered by Hartig in the late nineteenth century and has been variously called by different authors "cellulosic layer", "mucilaginous layer", "cartilaginous layer", or "gelatinous layer" due to its high cellulose content, its detachment from other layers and its gelatinous appearance that gives it its irregular and swelled appearance (Sanio 1860a, b, 1863; Metzger 1908; Potter 1924). The name gelatinous layer (or G-layer) has now been generally adopted. Its structure has been described as highly cellulosic, highly crystalline and with a very low angle of microfibrils relative to the direction of the fibre. Despite extensive study, Wardrop and Dadswell (1955) were unable to explain the longitudinal shrinkage from the microstructure point of view since the classical conception of wood behaviour would predict a very low longitudinal shrinkage because of the low MFA.

In 1966, Norberg and Meier (1966) were the first to make shrinkage measurements at a microscopic level. They isolated portions of G-layers using an ultrasonic method and measured their length under the light microscope first in water and then after drying (Fig. 6.10). They concluded that the G-layer shows no longitudinal shrinkage (or too weak to be the cause of macroscopic longitudinal shrinkage) and they looked for an explanation for the shrinkage in other parts of the structure. As the G-layer often appears detached from the other wall layers they assumed it did not contribute to the shrinkage, but that it did not limit it either. They concluded that as the S_2 layer is generally thinner in the presence of a G-layer, the proportion of S_1 layer becomes significant in the wall and the MFA being greater than 40° in S_1,

Fig. 6.9 Bending of branches during drying for several species. *Full line*: green state, *dotted line*: dry state (adapted from Onaka 1949)
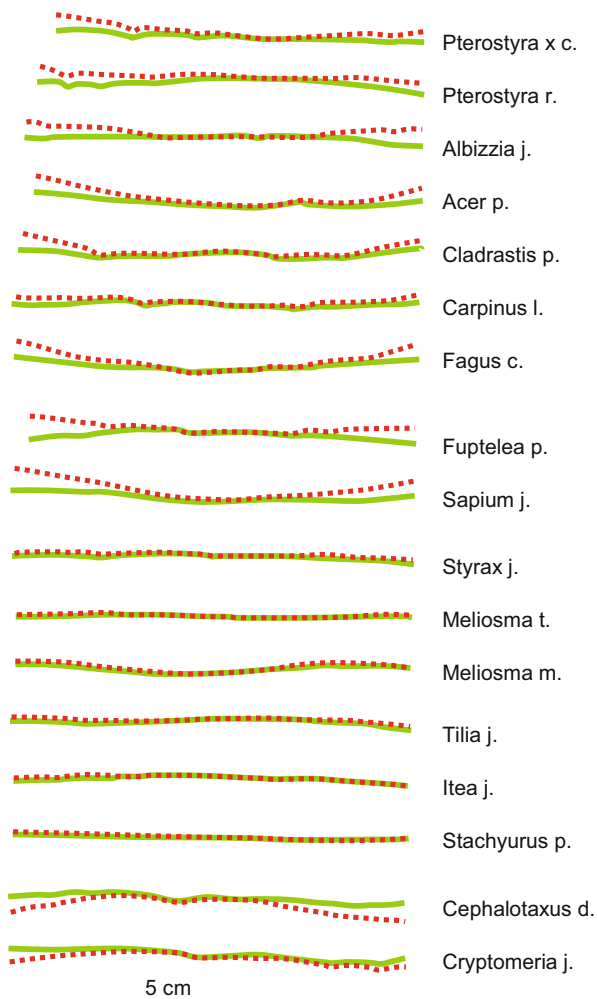
the authors attribute to the S_1 the high shrinkage observed in tension wood containing a G-layer. Later, Boyd (1977) also supported this hypothesis. These two articles are widely cited and the issue seemed settled.

6.5.2.2 The Key Role of G-Layer in Macro-Shrinkage

Amount of G-Layer Governs the Magnitude of Longitudinal Shrinkage

At the end of the 1990s, studies continued to report the higher longitudinal shrinkage of tension wood and its relationship with the amount of G-layer
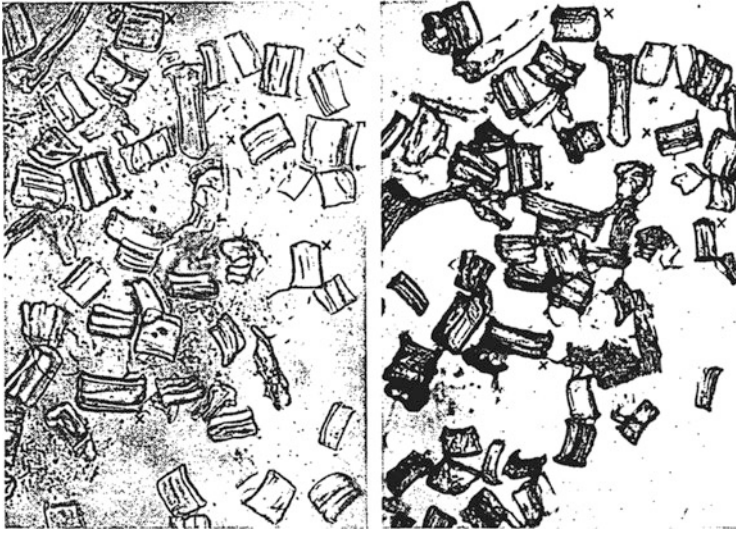
Fig. 6.10 Isolated portion of G-layer in wet (*left*) and dry (*right*) conditions (from Norberg and Meier 1966)

(Grzeskowiak et al. 1996; Jourez et al. 2001a; Washusen et al. 2001; Washusen and Evans 2001; Washusen and Ilic 2001). Clair et al. (2003c) investigated longitudinal shrinkage, the MOE and the proportion of fibres with a G-layer using the same chestnut samples as used to measure MOE (see Sect. 6.3.1) (Fig. 6.11). Using a simple mixture model it was possible to determine the longitudinal shrinkage of both fibres with a G-layer and fibres without a G-layer. They found 0.34 % longitudinal shrinkage for non-G-layer fibres and 0.71 % for G-layer fibres. Latter, Yamamoto et al. (2005) did similar work but with a more detailed description of the anatomy in which the G-layer itself was compared to the other layers. They found 1.30 % longitudinal shrinkage in the G-layer compared to 0.28 % in other layers.

Observation of Longitudinal Shrinkage at the Cell Wall Level

Following an original idea from Professors Yamamoto and Okuyama (Nagoya University), Clair and Thibaut (2001) measured the longitudinal shrinkage of the G-layer using stereo imaging with scanning electron microscopy and atomic force microscopy (Fig. 6.12). They showed that the G-layer shrinks more than other layer in passing from water saturated conditions to the air-dry condition. This shrinkage, partially reversible, was found to be about 4 % but a later study proved that this value was overestimated since part of the strain had already occurred before drying due to the release of maturation stress in the G-layer during sample sectioning (Clair et al. 2004, 2005b). The longitudinal shrinkage of the G-layer itself being proved, in order to contradict the Norberg and Meier hypothesis it was necessary to
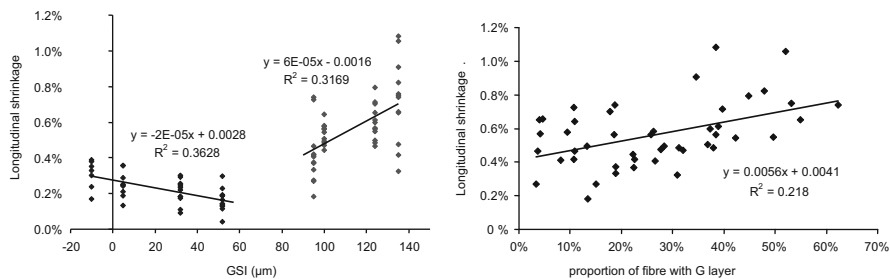
Fig. 6.11 Disjointed relationship between longitudinal shrinkage and growth stress indicator (GSI) and relationship with the amount of fibres with a G-layer (Clair et al. 2003c)
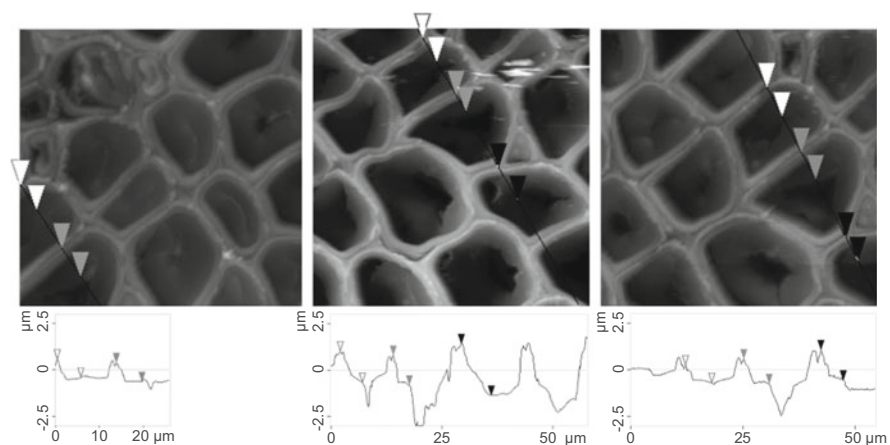
Fig. 6.12 Measurement on topographic profiles on AFM images of the same poplar tension wood cells in never-dried condition under water (*left*), air-dry (*centre*), and wet again conditions (*right*) (from Clair and Thibaut 2001)

verify the adherence of the G-layer to the S_2 layer. In a dedicated study it was proved that detachment of G-layer is an artefact arising during sample preparation and that it is not present in the core of wood sample, even after drying (Clair et al. 2005a). Therefore, the G-layer does appear to have a role in the high longitudinal shrinkage in tension wood.

6.5.2.3 Origin of G-Layer Shrinkage: Its Porous Structure

Earlier investigations seemed to prove that the G-layer is the driving force for longitudinal shrinkage, but the mechanism of the shrinkage remains enigmatic. In normal softwood, Abe and Yamamoto (2005) showed that cellulose microfibrils

shrink linearly with decreasing moisture content. In a later study concerning the contribution of cellulose microfibrils to growth stress generation, Clair et al. (2006a) investigated the strain of cellulose during drying of poplar tension wood. They observed that cellulose shrinks between 0.008 and 0.04 % when macroscopic shrinkage is about 0.8–1 %. Therefore, in tension wood, cellulose should buckle during the shrinkage process and thus cannot be the driving force behind shrinkage.

Thus, interest must be turned to the texture of the matrix. Thanks to the technique of nitrogen adsorption–desorption isotherms, supercritically dried chestnut tension wood has been characterized as having a gel-like mesoporous structure characterized by a pore surface more than 30 times higher than that in normal wood and attributed to the G-layer (Clair et al. 2008). When isotherms were done using oven-dry tension wood, all mesoporosity of the gel disappeared indicating that the gel collapsed during drying. This gel collapse is strong enough to be the driving force of cellulose microfibril buckling. Then the high longitudinal shrinkage of the G-layer and its transmission to the whole fibre, thanks to its adhesion to the other layers of the secondary wall, produces a macroscopic longitudinal shrinkage that sometimes exceeds 1 % in tension wood. In recent years several research teams (Lafarguette et al. 2004; Nishikubo et al. 2007; Bowling and Vaughn 2008; Ikushima et al. 2008; Mellerowicz et al. 2008; Baba et al. 2009; Kaku et al. 2009; Hayashi and Kaida 2010) have worked on the description of the nature of the non-cellulosic part of the G-layer among which pectins and xyloglucans appear to be good candidates for the origin of the gel structure of the G-layer. These ideas were previously hypothesized by Sachsse (1965) but latter forgotten after being rejected by Norberg and Meier (1966).

6.5.2.4 What About Longitudinal Shrinkage of Non-G-Layer Tension Wood?

Many species form tension wood without a G-layer (Onaka 1949; Fisher and Stevenson 1981). However, all of them have lower MFAs in tension wood than in normal wood (Clair et al. 2006b). In experiments on branches (see Fig. 6.9 at the beginning of this section), Onaka (1949) noted that some species do not bend during shrinkage when no tension wood was formed (i.e. no G-layer observed). Even so, Ruelle et al. (2007a) comparing properties of ten tropical species reported that "longitudinal shrinkage was often the most significantly different property between tension and opposite wood, four to seven times higher in tension wood for seven species, but less than two times higher for *Simarouba amara* Aubl., *Eschweilera decolorens* Sandw. *and Qualea rosea*". Two of these genera (*Simarouba* and *Eschweilera*) are known to produce tension wood without a G-layer (Clair et al. 2006b; Ruelle et al. 2007b). More recently, Ruelle et al. (2011) confirmed that *Simarouba amara* has a longitudinal shrinkage around two times higher than its normal wood. This longitudinal shrinkage is much less than in tension wood with a

G-layer but is still paradoxical considering the lower MFA than in normal wood. Recently, Chang et al. (2009a) tried to find some mesoporosity in tension wood without a G-layer. However, their results showed that tension wood without a G-layer does not have mesoporosity and that even some species with a thin, un-swollen G-layer do not show mesoporosity. Recently, a study produce evidence that the release of maturation stress during drying is an answer to this paradox.

Following ethanol exchange in chestnut and *Simarouba* never-dried tension wood, Chang et al. (2009b) shown that tension wood of both species (with and without a G-layer) shrinks about 0.05 % in the longitudinal direction. The observed phenomena are explained as a kind of hygrothermal recovery occurring during the departure of water molecules due to exchange with ethanol. A similar process could occur during drying. Previously, Abe and Yamamoto (2007) show on *Zelkova serrata* tension wood that longitudinal drying shrinkage is less after boiling compared to unboiled samples. Similarly, it has been recently showed that the longitudinal shrinkage of chestnut tension wood is lower when it has been previously heated. Measurement using replicates, dried with or without heat treatment (HT), gave the following results: HT strain $= -0.14$ % $\pm$ 0.05 %; drying strain after HT $= -0.64$ % $\pm$ 0.05 %; drying strain without HT $= -0.78$ % $\pm$ 0.08 %. This means that the strain released during heat treatment was part of the total strain generally measured in tension wood. Thus, longitudinal shrinkage could be a combination of three effects at the cell wall level: (1) the effect of the loss of spaces occupied by water (which depends highly on MFA and so does not affect tension wood very much), (2) the effect of stress recovery which is visible mainly in high tension stressed wood (tension wood), and (3) the effect of gel collapse, far higher than the previous effects, but occurring only in G-layer tension wood (Fig. 6.13).

6.5.3 Transverse Shrinkage of Tension Wood

Fewer studies have been done on the transverse shrinkage of tension wood and the results are contradictory. Washusen et al. (2001, 2001) on eucalyptus and Clair et al. (2003c) on chestnut reported a higher tangential shrinkage in tension wood, while Arganbright et al. (1970) observed a lower shrinkage in silver maple (*Acer saccharinum*) tension wood both in tangential and radial direction. In beech tension wood, Clarke (1937) reported that shrinkage was higher than in normal wood in the tangential direction but not in the radial direction. Clair et al. (2003b) measured a higher shrinkage both in the radial and tangential directions in mani (*Symphonia globulifera*), but found that the difference was clearer in the tangential direction. In the case of the ten tropical species studied by Ruelle et al. (2007a), tangential shrinkage was always higher in tension wood, except for *Eperua falcata* in which tangential shrinkage was very low, and the difference was significant for only four trees. Along the radial direction, shrinkage was found to be sometimes higher, sometimes lower in tension wood, with only three significant differences (one lower
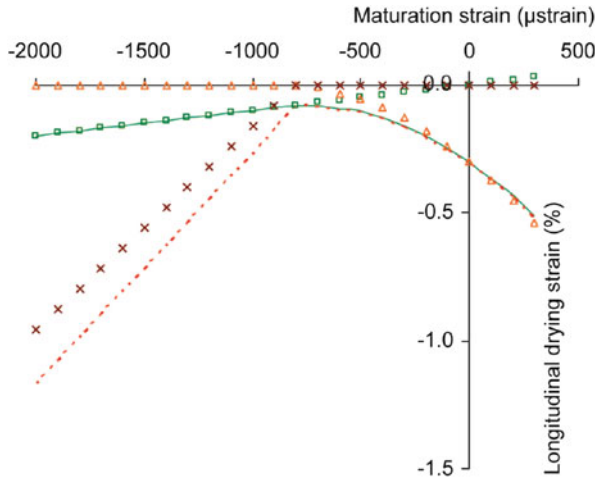
Fig. 6.13 Schematic model of the three cumulative contributions to longitudinal shrinkage as a function of the maturation strain. *Green squares*: strain caused by stress release; *orange triangles*: strain caused by water departure; *crosses*: strain caused by G-layer collapse. *Plain line* (*green*): resulting shrinkage on wood from species not producing a G-layer; *dotted line* (*red*): resulting shrinkage on wood from species producing a G-layer. Release strain is proportional to growth strain, water departure strain depends on MFA and gel collapse depends on the amount of gel in the wood sample (from Clair 2012)

and two higher for tension wood). Recently, Fang et al. (2007) carried out shrinkage measurement on 20 μm thick sections. They recorded a significant negative correlation with the measured growth stress (directly linked to the amount of G-layer (Fang et al. 2008b) in the tangential direction whereas no relationship was found in the radial direction).

In a recent study about the contribution of maturation stress on shrinkage, it was found that the release of transverse compressive stress during drying contribute to reduce the tangential stress in tension wood of chestnut (Clair 2012). At the cell wall level, several authors point to the very large transverse shrinkage of the G-layer. Norberg and Meier (1966) found it to be around 15–25 % on isolated G-layers. Recently, Fang et al. (2007) measured the shrinkage of the G-layer keeping it in its cellular context and avoiding preparation artefacts (Clair et al. 2005c). They found that G-layer shrinkage is around 12–27 % and that this shrinkage remains constant for several levels of growth stress (and therefore different G-layer thicknesses). Their observations showed that in G-fibres, lumen size increased during drying and this increase was positively related to G-layer thickness whereas in normal wood fibres, lumen size decreased during drying. These findings suggest that the G-layer shrank outwards (i.e. its internal perimeter increased) because of the absence of S_3 layer (Fig. 6.14), so that its shrinkage weakly affected the total cell shrinkage. Thus, in tension wood with a G-layer,
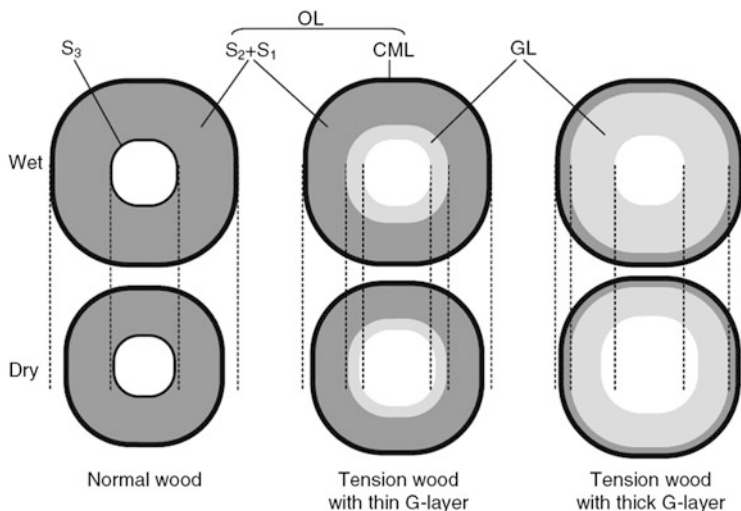
Fig. 6.14 Three shrinkage scenarios starting from the same cell and lumen size, depending on the proportion of the cell wall layers. *OL* other layers, *CML* compound middle lamella, *GL* gelatinous layer (Fang et al. 2007)

the macroscopic shrinkage would be mainly controlled by the shrinkage of other layers (ML, S_1, S_2: see Chaps. 2 and 3 for discussion of wood cell structure) which shrank inwards (i.e. its external perimeter decreases).

6.6 Conclusion

Both compression and tension wood are produced by the cambium at a given place within a woody axis in order to create a dissymmetry in forces on opposite sides of this axis. It is very likely that wood genesis by the cambium in these cases is strongly different from wood genesis for all "normal" woods, juvenile or mature, side or opposite, with specific genes involved and quite different chemical composition of basic cell wall constituents. Reaction wood does not seem to be an extreme case of normal wood.

The basis of different forces on opposite sides of the axis is a very different value of maturation strain in fibre cell wall during cell differentiation: opposite to normal for compression wood, three to six times higher for tension wood. This is achieved by both a radical change in chemistry and the peculiar orientation of MFA (always very high values for compression wood and always very low values for tension wood).

Forces result from the combination of maturation strain, density, and width of the newly formed ring. Thus reaction wood formation can be often associated with

changes in density and ring width, peculiarly in the case of compression wood, in order to compensate the huge reduction in specific MOE associated with high MFA values.

Thus physical and mechanical changes in properties from normal to reaction wood are more important for compression wood: higher density associated with higher rupture strength in compression and hardness, lower MOE, lower radial, tangential and volumetric shrinkage but much higher longitudinal shrinkage (ten times more at least).

For tension wood, the only common change is a higher longitudinal shrinkage (up to 1 % in many cases). Sometimes density and transverse shrinkage is also higher but may also be lower. This is the reason why tension wood is often not considered as a big problem, whereas the high level of residual stresses and stored elastic energy in the log are the biggest problem for hardwoods leading to, for example, log splitting and lumber twisting.

It should be noted that the very essence of the reaction wood mechanism, dissymmetry between two faces of a log, leads to strong heterogeneity within lumber pieces containing both normal and reaction wood. Associated with the differences in longitudinal shrinkage between the two types of wood this leads to the main problem with reaction wood in use. These issues will be dealt with in more detail in Chaps. 8 and 9.

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Chapter 7
Detection and Grading of Compression Wood

Philipp Duncker

Abstract The motivations to detect and identify compression wood are manifold and so are the demands on a proper solution of this problem. At present, biological detection and classification offer the most detailed grading of compression wood against normal wood, while visual inspection enables fast detection of compression wood in its spatial orientation on large sample sizes in most two degrees of severity. Chemical analysis complements these methods in providing quantitative measures of compression wood severity though losing its orientation in space. Various methodological approaches are discussed, intended to help in selecting an appropriate compression wood detection method according to the specific problem encountered.

7.1 Introduction

Humans have been able to identify compression wood since the first coniferous tree was harvested and worked. Tree-fellers, from Neolithic times to the present day have been familiar with the way their axes or saws jammed, while craftsmen would be aware that reddish regions of the wood twisted, warped and became brittle as the piece dried. The vernacular terminology which relates to this wood type reflects this experience and the ability which developed to identify and categorize it since ancient times. It were the properties of the wood which gave rise to the early names for compression wood: "red-wood", "glassy wood", or "hard streak". Further, its resistance to nails gave rise to another name, "Nagelhart", which means "hard *or* impossible to nail" (Timell 1986). Of these properties hardness and reddish colour are the most obvious and together with others provide a key to a methodological approach for its detection and identification.

P. Duncker (✉)
Institut für Waldwachstum - Institute for Forrest Growth, Albert-Ludwigs-Universität Freiburg, Tennenbacher Straße 4, 79106 Freiburg, Germany
e-mail: pduncker.faz@gmx.de

The methodological approach taken to detect and identify compression wood as well as the degree of precision of classification will vary according to the motive for the analysis. Although the reasons are manifold, two typical scenarios might be identified:

In industrial wood processing the rapid detection of compression wood on longitudinal or radial sections enables better sorting of lumber for utilization (Johansson 2002; Warensjö 2003; Müller 2003). The relative amount of compression wood within the board may provide sufficient information for its proper allocation to subsequent processes (Öhman 1999).

The level of identification required might be different in studies aimed at understanding the formation of compression wood in trees responding to environmental forces such as gravity and wind (Hartmann 1942; Sinnott 1951, 1952; Spurr and Hyvärinen 1954; Firn and Digby 1997; Duncker 2006). Here, precise classification of compression wood in cross sections is needed and requires a greater input of resources and time.

Whatever the reason for requiring accurate detection of compression wood, a thorough characterization (definition) of this tissue is required and is a pre-condition for any objective and reproducible compression wood detection method. The characterization requires a closer look at the reddish and hard crescent-shaped zones of growth rings. At one level, compression wood is best characterized by its anatomical structure. The most detailed grading scheme for the classification of compression wood into different degrees of severity along the transition from normal wood to severe compression wood was possibly presented by Yumoto et al. (1983). It is based on morphological characteristics determined by scanning electron microscopy (SEM). Accordingly, a thorough morphological characterization of compression wood by its cell structure as well as by its chemical and mechanical properties will provide a reference point for developing methodological approaches to detection methods.

7.1.1   Gross Anatomy of Compression Wood

The morphological structure of compression wood is radically different from that of normal wood. When compared to normal wood it is largely the tracheids that display a different anatomy, whereas other cell types are less changed (Timell 1986). The morphology of typical compression wood tracheids is distinctly different in that they are shorter than normal wood tracheids (Hartig 1896, 1901; Ollinmaa 1955, 1961) (Fig. 7.1) while their tips are often truncated and bent (Münch 1938, 1940) (Fig. 7.2). In transverse sections, compression wood tracheids have a round to oval outline, rather than being quadrangular or hexagonal (Cieslar 1896). The rounded outline is associated with the appearance of intercellular spaces (Sanio 1860), except in those cases where the rounding is limited to the boundary between the S_1 and S_2 layer within the secondary cell wall (Yumoto et al. 1983). While the tracheid diameter is slightly reduced compared to that in normal wood, cell wall thickness is considerably increased resulting in a correspondingly reduced
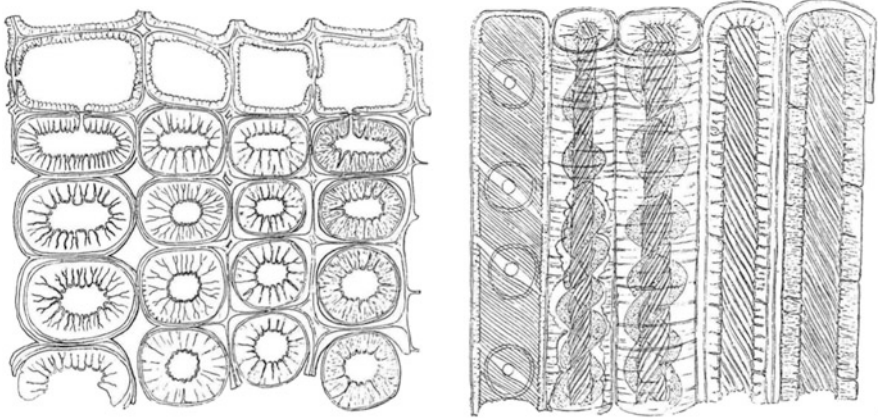
Fig. 7.1 Typical compression wood tracheids in radial and longitudinal section (Hartig 1901)

lumen diameter (Cieslar 1896; Ollinmaa 1961; Petric 1962). Tracheid diameter and the ratio of cell wall thickness to lumen diameter in compression wood show intra-annual variation analogous to the within-ring transition from normal earlywood to latewood although the structural changes are less distinct (Necesaný and Oberländerová 1967; Kibblewhite 1973; Yoshizawa et al. 1981; Donaldson et al. 2004).

The chemical composition and the anatomical structure of compression wood determine its physical and mechanical properties. Severe compression wood is characterized by a dark reddish colour, high wood density, high longitudinal shrinkage, hardness, high compressive strength, and elasticity, and its low tensile strength and rigidity (Timell 1986).

7.1.2 Ultrastructure of Compression Wood

The typical cell wall structure of tracheids consisting of primary (P) and secondary (S) cell wall, the latter being composed of outer (S_1), middle (S_2), and inner layer (S_3), is modified in compression wood. However, the most obvious difference between normal and compression wood lies in the existence of intercellular spaces which, together with the fragmentary lignified middle lamella (M) result in weak cohesion of compression wood tracheids (Hartig 1896; Münch 1938). While the primary cell wall is only subject to minor changes at most, the S_1 layer of the secondary cell wall is thicker in compression wood compared with normal wood (Côté et al. 1967; Timell 1986). The S_2 differs most from the one in normal wood and its structure is summarized below. The extensive literature on this subject up to 1986 was carefully compiled and analysed by Timell (1986), and the reader should
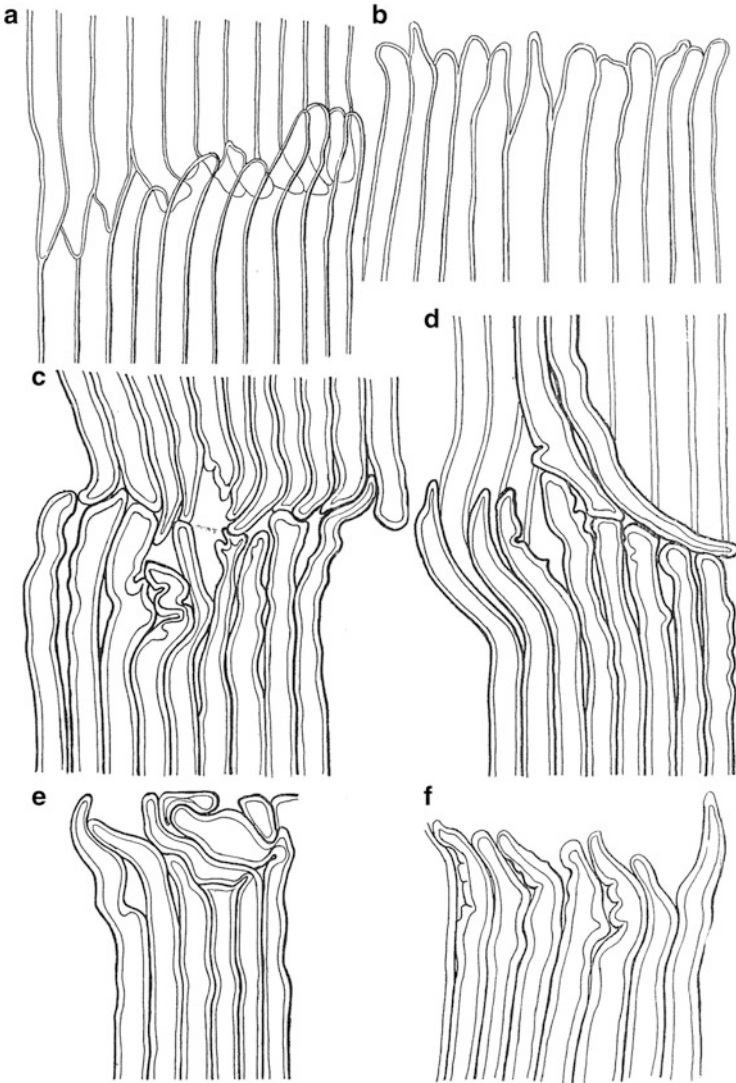
Fig. 7.2 Truncated and bent tips of compression wood tracheids (**c–f**) in contrast to normal wood (**a** and **b**) (Münch 1940)

consult this book for a more extensive account. The cellulose microfibril angle (MFA) in the S_2 is larger in compression wood tracheids (Ollinmaa 1961; Andersson et al. 2000; Gindl 2002; Donaldson et al. 2004) and the S_2 is deeply fissured with helical cavities between ribs (Hartig 1901; Casperson and Zinßer 1965) whose orientation reflects the MFA. While penetrating deeply into the S_2 the cavities never reach the S_1 layer. The outer portion of the thick S_2 layer (that adjacent the S_1 layer) has a high concentration of lignin. This outer portion of the S_2

layer is referred to as $S_2(L)$. The $S_2(L)$ is possibly the only feature unique to compression wood and is present in all degrees of compression wood severity (Yumoto et al. 1983). The inner portion of the S_2 layer forms the boundary against the lumen since all but very mild compression wood lacks an S_3 layer (Hartig 1901; Münch 1938; Ollinmaa 1955, 1961; Casperson 1959, 1962, 1963; Wergin 1965; Casperson and Zinßer 1965). If the cambium is stimulated to form compression wood instead of normal wood tracheids, the S_3 layer is the first feature to be eliminated (Yumoto et al. 1982). This is an important phenomenon in the formation of compression wood.

7.2 Classification of Compression Wood by Biological Features

The morphological characterization described above corresponds to the "typical" or severe form of compression wood tracheids and permits its identification by microscopy. However, not all compression wood tracheids show all these structural features. Instead, a gradual transition exists from normal wood via intermediates to severe compression wood tracheids. Further, close examination often reveals substantial differences among tracheids in the degree of development of the characteristic features even in a limited area of wood (Harris 1977). The intermediate degrees of compression wood tracheids are especially difficult to detect macroscopically and as part of an experiment involving experimentally induced compression wood formation Yumoto et al. (1983) devised a grading system for compression wood tracheids in the middle of a growth ring of *Picea glauca* based on anatomical features. This grades the wood into six severity classes by microscopic means. Ranked by importance the primary properties used are: helical cavities, ultraviolet absorption (lignin), cell wall thickness, and more variable secondary traits including outline of boundary between S_1 and $S_2(L)$, bordered pits, intercellular spaces, and the S_3 layer.

Markers chosen by Yumoto et al. included quantitative features: roundness in cross section, excessive lignification, and enhanced cell wall thickness. The level of development of these features is represented by the quantity of deviation from normal wood tracheids. Other features were qualitative, such as spiral grooves, the form of bordered pits and the distribution pattern of UV-absorption. It is difficult to describe their behaviour along a severity gradient and interdependences between some features were noted. However, the occurrence of spiral grooves, excessive lignification, and cell wall thickness, the last expressed as a ratio to cell diameter, are of primary importance and independent of any other feature. They chiefly determine other features, such as form of bordered pits, occurrence of intercellular spaces, and lignin distribution pattern and together with more variable features, i.e. outline of the boundary between the S_1 and $S_2(L)$, and S_3 layers are considered to be secondary traits.

The suggested classification by SEM is based firstly on the degree of development of spiral grooves, separating tracheids into three severity classes. These are further complemented and subdivided by the strength of UV-absorption (degree of excessive lignification), cell wall thickness, and by the degrees of development of other features into six grades (Table 7.1).

As a result of his work Yumoto et al. (1983) concluded that the grading scheme was too sophisticated for light microscopy. Using light microscopy, compression wood tracheids of Grades I and I′ were judged to be typical or severe, those of Grades II and III to be moderate, those of Grade III′ to be possibly slight or together with Grade IV to be normal. Thus, the SEM-gradation was reduced to fewer grades but more importantly it became evident that "normal wood" might actually include light microscopically indiscernible compression wood tracheids.

The scheme provides a reasonable basis for biologically grading the severity of compression wood tracheids and is often used as a reference for compression wood when detection methods are being developed. However, the specific properties of compression wood suggest the possibility of complementing this system using chemical or physical properties, possibly throwing new light on the biological classification while also reflecting its nature (Yumoto et al. 1983).

While microscopic grading of compression wood tracheids is the most reliable method, it has some associated disadvantages. First, being laborious, it requires high resource and time input in terms of specimen preparation. And although the grading is based on quantitative features, identification often depends on subjective analysis by the operator. Automatic detection of compression wood tracheids using microscopic image analysis has been attempted making use of the roundness of lumen and cell outline. Fast Fourier Transform methods have been applied to reduce image data in order to increase processing speed. Net map angular distribution functions indicate tracheid and lumen shape. When compared to a reference image, i.e. normal wood, severe forms of compression wood were successfully detected, while the result was not satisfactory for mild forms (Moëll and Fujita 2004). The reason might be that the grading scheme reveals that the rounded outline in moderate and mild compression wood is limited to the boundary between the S_1 and $S_2(L)$ layer within the secondary cell wall (Yumoto et al. 1983).

7.3   Detection of Compression Wood in Reflected Light

The most eye-catching characteristic of compression wood at the macroscopic level is its reddish-brown colour. Colour is the sensation resulting from a given spectral distribution of light, i.e. electromagnetic radiation in the wavelength range of 380–780 nm, stimulating the cone and rod cells in the retina and interpreted by the brain. Accordingly, colour is not an intrinsic property of the objects we see around us. The property is rather the interaction through selective absorption and scattering of these objects with electromagnetic radiation which causes the specific colour stimulus. Thus, the "colour" of wood is determined essentially by two factors, namely the light-scattering characteristics of the wood surface and the absorptive

Table 7.1 Criterion for the gradation of compression wood tracheids in the middle of growth rings (Yumoto et al. 1983)

Grade	Spiral grooves	UV-absorption	Cell wall thickness	Roundness of boundary line between S_1 and S_2 (L)	Form of bordered pits	Unstable features
I	Distinct	Totally strong; lower contrast between S_1 or inner region of S_2 and S_2 (L); distributed nearly evenly around entire circumference of tracheid	Very thick	Highly round nearly circular	No or slight rising of pits dome	Intercellular spaces generally present
I′	Distinct	Strong but less in S_1 and inner region of S_2 to stand out the presence of S_2 (L)	Thick	Round but not circular	Pit dome rising	Intercellular spaces generally present
II	Poorly developed	Considerably strong	Considerably thick	Round but variable depending on the presence or absence of intercellular spaces	Generally no grooves around pit openings	Intercellular spaces present or absence
III	Absent	Strong absorption not confined at cell corners	Thicker than normal	Fairly round	–	Intercellular spaces generally absent; S_3 layer generally absent
III′	Absent	Strong absorption confined at cell corners	Slightly thicker or similar, or even a little thinner than normal	Slightly round	–	Intercellular spaces generally absent; S_3 layer absent or present
IV	Absent	No strong absorption in S_2 (L) but slightly higher absorption evenly distributed in S_2	Similar to normal	Trace	–	S_3 layer generally present

Characteristic features are arranged in the order of importance from left. Spiral grooves, UV-absorption, and cell wall thickness are of primary importance, though the availability of the third is limited. Roundness of the boundary line between S_1 and S_2 (L), form of bordered pits are in the second class in importance. Unstable features are also incorporated at right. Cell wall thickness and form of bordered pits are only available in the middle of growth increments

properties of its chemical constituents (Hagman 1996). Scattering coefficients and specific absorption are calculated with the aid of the Kubelka–Munk equation (Kubelka and Munk 1931; Kubelka 1948, 1954). Thus, latewood is darker than earlywood because its thick-walled tracheids scatter light less than the thin early-wood cells (Wilcox 1975). The second factor, light absorption, is an intrinsic property of the wood, determined by its chemistry and independent of shape or structure. This absorption is positively correlated with the chromophoric groups present in wood. Lignin contains many chromophoric groups and absorption coefficients of wood have repeatedly been found to be directly related to its lignin content (Wilcox 1975). When normal softwood is ground to a powder, the difference in colour between earlywood and latewood disappears, presumably because any differences in light-scattering ability have now been eliminated, while any possible differences in lignin content are too small to affect the absorption. Powdered compression wood, in contrast, is always darker than powdered normal wood. Obviously, the much higher lignin content of compression wood (Sanio 1860) is the decisive factor here. The helical cavities and ribs in compression wood probably have no effect on its colour; in fact they should lighten it since they ought to enhance the scattering of light (Timell 1986). Consequently, the degree of compression wood is characterized by how both changes in structure and chemical composition influence its appearance. Mild compression wood is less deeply coloured than the moderate grade which in its turn is paler than the severe form (Yumoto et al. 1982; Timell 1986).

Accordingly, the visual appearance of compression wood serves as a characteristic property enabling its detection. The normal procedure for estimating areas of compression wood in a specimen is to outline them with a pencil and measure them with a planimeter. This is a common procedure not only in research but is also accepted in various European standards for softwood grading (DIN 4074, 2003[1]; EN 1310, 1997[2]; EN 1611-1, 2002[3]; EN 1927-1, 2008[4]). This methodological approach assumes reliable contrast in appearance between normal and compression wood which can be recognized by an operator on visual inspection. While an operator might per se increase measurement uncertainty attributable to repeatability, the detection of compression wood by this method is further influenced by tree species and specimen condition. High contrast between normal and compression wood is observed in species with a white sapwood while compression wood areas are usually obscured in species with a deeply coloured heartwood (Timell 1986). Although, pronounced compression wood is almost always distinctly different from

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[1] DIN Deutsches Institut für Normung e. V. DIN 4074-1::2003-06. Strength grading of wood. Part 1: Coniferous sawn timber.

[2] European Committee for Standardization. EN 1310:1997-08. Round and sawn timber. Method of measurement of features.

[3] European Committee for Standardization. EN 1611-1:2002. Sawn timber. Appearance grading of softwoods. Part 1: European spruces, firs, pines, Douglas fir and larches.

[4] European Committee for Standardization. EN 1927-1:2008. Qualitative classification of softwood round timber—Part 1: Spruces and firs.

normal wood when freshly cut (Timell 1986) the red colour fades with drying of the wood obscuring the differences between compression wood and normal latewood and even earlywood (Mer 1888; Cieslar 1896; Hartig 1901). The change in colour seems to be related to drying since wetting restores the original colour (Lämmermayr 1901; Timell 1986). The same effect can be obtained by coating with Vaseline (Cieslar 1896). However, polishing is preferable to wetting because structural details are not blurred as through wetting and the wood assumes the appearance it had in its green state (Timell 1986).

The above considerations already point to problems likely to be encountered when detecting compression wood using light. In order to increase contrast between normal and compression wood for visual detection, staining methods have been applied (Knigge 1958; Mergen 1958; Aufseß 1973; Timell 1986). Most of the methods stain lignin rather than cellulose using dyes such as safranine acid green, malachite green in conjunction with methylene blue in alcohol, and phloroglucinol-hydrochloric acid. Alternatively, the wood may be viewed using blue light illumination at a wavelength of 480 nm (Westing 1965), or black and white film slightly overexposed to increase contrast (Timell 1986).

Other attempts to find objective methods for the visual detection of compression wood while increasing repeatability, speed of detection, and precision have involved application of digital colour imaging. Colour imaging in this case means the use of a camera sensitive to the red, green, and blue (RGB) part of light (Nyström 1999; Nyström and Kline 2000). These three ranges correspond to the sensitivity peaks of the human eye photoreceptors, the cone cells, in long (L, 560–580 nm), middle (M, 530–540 nm), and short (S, 420–440 nm) wavelengths. The underlying principle is the fact that three parameters are sufficient to describe and stimulate a colour sensation, known as the tristimulus. The tristimulus value of a spectrum can be calculated and expressed as the amounts of three primary colours needed in a three-component additive colour model to stimulate the corresponding chromaticity (Eichler et al. 1993). Any specific method for associating tristimulus values with each colour is called a colour space. The particular RGB colour space is defined by the three chromaticities of the RGB additive primaries, and can produce any chromaticity coordinate that is the triangle defined by those primary colours.

In seeking methods for fast non-destructive sawn timber grading, Nyström (1999) tested, among other approaches, the potential of automatic colour scanning for compression wood detection in dried spruce wood. A test set of nine pieces of wood planed in a longitudinal direction was illuminated with linear fibre-optic light guides and scanned with a line camera. However, colour scanning with an RGB camera was less successful than had been hoped because detected colour differences were too small to distinguish compression wood from normal latewood on dry surfaces. In contrast, visual appraisal of compression wood in 16 lumber pieces of southern yellow pine[5] revealed distinct colour differences from normal

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[5] Southern Yellow Pine doesn't refer to any one species of tree, but rather a group of species (e.g. *Pinus taeda*, *P. palustris*, *P. echinata* and *P. eliottii*) which are classified as yellow pine, and are native to the south of the United States.

earlywood and latewood in the green condition. A multivariate nonlinear prediction model was produced using the original colour image data from scanning, and expanded variables derived from this data. The linear weight coefficients for each variable in the prediction model were estimated with the multivariate image projections to latent structures (MIPLS) algorithm (Hagman 1996). The prediction model gives a prediction image vector for each of the classes considered. In addition to the class for compression wood four non-compression wood classes were defined, i.e. earlywood, latewood, background and knots, to account for the colour variation outside the compression wood class. Colour information showed significant and consistent differences between compression wood and clear wood and resulted in an average correct compression wood classification of 89 %. The better classification accuracy in the green condition compared to dried surfaces might be explained by free water enabling the light to penetrate deeper into the wood surface and thus derive more influence from light absorbing characteristics of the wood (Nyström and Kline 2000).

A colorimetric compression wood detection method for stem cross sections was presented by Wernsdörfer et al. (2004). Compression wood areas were marked in RGB images of Norway spruce stem cross sections. Adjacent pixels with similar chromaticity to the marked areas were selected according to fixed tolerances. In a second step, position and extent were automatically measured for each compression wood area. While this method still requires manual selection of compression wood areas, presumably because a general chromaticity for compression wood could not be identified, it is faster and helps in describing each separate region in location and size compared to planimetry. Further, repeatability is possibly increased through standardized tolerance setting.

The weak performance of three band colour imaging in compression wood detection might be explained by the chromaticity coordinates. The chromaticity coordinates of different wood features of spruce can be illustrated in RGB colour space (Wendland 2000). This reveals both, clustering of the wood features within the space and assembly of clusters along a notional line. Accordingly, most wood features in spruce have the same hue with different saturation. The features following this notional line are composed to a high degree of cellulose and lignin, whose chromaticity coordinates are located at the endpoints of the line. The general chemical composition determining the absorptive properties explains cluster overlapping while structural differences between the features alter light scattering (Wendland 2000). This illustrates the difficulty of discriminating the compression wood cluster from intersecting clusters of other wood features relatively rich in lignin, e.g. blight affected wood with decomposed cellulose or dark branches (Wendland 2000). Although clustering is apparent, the fuzzy boundaries drawn in colour space for separating normal wood features reduce detection accuracy.

The strength of hyperspectral image analysis lies in enabling the detection of minor differences of light intensity in numerous wavelength bands and offering more sophisticated classification algorithms. Hagman (1996) discussed the suitability of an imaging spectrometer as a sensor to evaluate soft wood quality moulding

features on longitudinal and transverse faces of a piece of dried lumber in reflected light. Among the models developed for predicting various wood features, compression wood modelling was weakest. Nonetheless, in a combined spruce and pine analysis, regression coefficient estimation revealed that three to five variables (429, 487, 575, 659, and 679 nm) are sufficient for high precision solution of the problem with linear prediction models (Hagman 1996). The distinguishing information is within the blue spectrum, which is probably due to the high content of lignin in compression wood (Hagman 1997). Nyström and Hagman (1999) described a method which used hyperspectral image analysis in real-time compression wood detection on the longitudinal face of sawn timber. The sample pieces were oriented with both radial and tangential section towards the surface. The wood surface was scanned with an imaging spectrograph, and compression wood was detected by analysing the spectral composition of reflected light. Multivariate image analysis (MIA), being principal component analysis (PCA) applied to images, served to identify separable wood features and to extract areas with a similar spectral pattern. Good representatives for the class of interest, i.e. compression wood, latewood, earlywood, and dark defects were distinguished in score scatter plots. The representative patterns were input as a key with the original data and modelled by multivariate image projections to latent struktures (MIPLS) for estimating the prediction coefficients.

The best models were achieved with four principle components. Comparative analysis with SEM showed that 11 samples out of 14 could be considered as correctly classified with the linear prediction models fitted. The classification was correct on areas with pronounced compression wood whereas it was uncertain with scattered pixels of compression wood.

Another method has been developed to detect compression wood in dried and polished stem cross sections of Norway spruce by means of hyperspectral analysis (Duncker and Spiecker 2009). Reflected light from the stem cross-sectional surface is recorded with an imaging spectrometer to obtain the spectral characteristics in the visible light and near infrared (400–1,000 nm) for every pixel. The spectra are standardized with black and white references. The detection and classification of compression wood severity classes is performed by the Spectral Angle Mapper algorithm. It determines spectral similarity by calculating the angle between two spectra treated as vectors in space with dimensionality equal to their number of bands (Kruse et al. 1993). Here, the standardized spectrum of each pixel is compared to reference spectra stored in a spectral library. The reference spectra are obtained from selected training areas of the different compression wood severity classes identified by cell characteristics under a light microscope. In accordance with the grading scheme of Yumoto et al. (1983) a severe and a moderate compression wood class was distinguished from normal wood. The examples of standardized reference spectra in Fig. 7.3 confirm spectral separability in the blue part of visible light as stated by Hagman (1997). Spectral separability between the mean spectra of the different classes was confirmed by the Jeffries-Matusita and Transformed Divergence measures (Richards and Xiuping 1999). Cross-sectional areas are automatically classified into severe compression wood, moderate
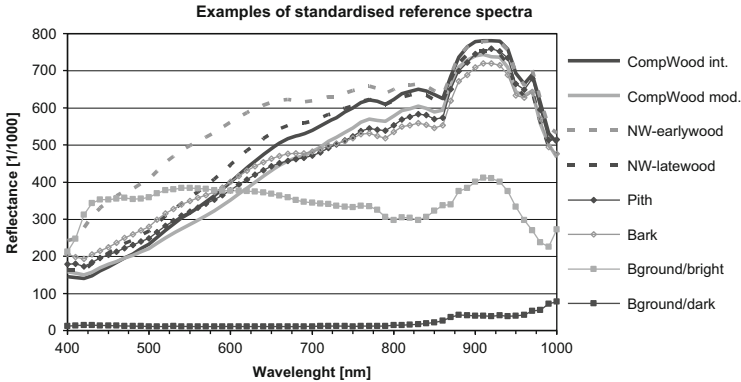
Fig. 7.3 Standardized reference spectra obtained from microscopically identified reference areas for different classes of interest. *CompWood int.* intensive compression wood, *CompWood mod.* moderate compression wood, *NW* normal wood, *Bground* background (Duncker and Spiecker 2009)

compression wood, normal wood, and background/cracks with an overall accuracy of 96 %. In addition, to quantify the spatial distribution of compression wood, the chronological pattern of its formation is recorded by cross linking the pixel classification to the tree ring sequence. The tree ring boundaries are located in a grey scale image which shows the spatial information at wavelength 435 nm and the annual radial increment is measured. The boundaries are detected by the sharp difference in brightness between dark latewood and the following year's bright earlywood. Cross-analysis of the classification result with High-Frequency Densitometry proved significant differences in the relative intra-annual micro-density patterns between the classes.

7.4 Detection of Compression Wood in Transmitted Light

The apparent contrast in reflected light between compression and normal wood is caused by the chemical composition and ultrastructure determining the adsorptive and light-scattering properties of the tissues. Transmitted light is influenced by the very same properties and thus compression wood can be detected by its relative opacity to transmitted light in comparison with the translucence of normal wood (Pillow 1941). When cross sections of wood about 1/8 to 3/16 of an inch thick (later only 2–3.5 mm) containing compression wood are held against a bright light, the summerwood (i.e. latewood) of the compression wood appears practically opaque, while other types of summerwood are translucent (Timell 1986; Andersson and Walter 1995). Springwood (i.e. earlywood) of both compression and normal wood is translucent in thin sections. Transmitted light is particularly helpful in identifying the less pronounced forms of compression wood which are sometimes difficult to

distinguish from normal wood by appearance on transverse sections (Pillow 1941). While extremely smooth surfaces on the cross sections are not necessary, sanded surfaces cannot be used because the cell cavities become filled with particles of debris that interfere with the inherent translucence destroying the contrast between compression and normal wood (Pillow 1941). According to Low (1964), a light green filter increases the contrast between normal and compression wood. The method is accurate and convenient (Timell 1986), and is accepted in TAPPI standard methods T20-m-55 and T267cm-05 (Technical Association of the Pulp and Pater Industry 1955, 1959,[6] 1972,[7] 2009[8]) to determine the relative or absolute amount of compression wood. The areas of compression wood on a specimen are outlined with a pencil and measured with a planimeter, which is time consuming and difficult to implement in cases when the compression wood is of various types (Andersson and Walter 1995). The use of image analysis increases repeatability. Discs are scanned with a digital RGB CCD-camera together with a black ruler for calibration. The blue band proves to contain very little information about variations of wood type. It is used to positively identify the wood region itself against the white background. Supervised maximum-likelihood classification is applied to discriminate between severe, moderate compression wood, and normal wood. This requires the operator to set 12 training pixels for reference. The amount of severe and mild compression wood, the angle from the pith to the centroids of the different wood types, and the average diameter of the wood disk are computed. A test against operator classification revealed poor estimation of severe compression wood, while repeatability in estimation of the milder form and total amount was good (Andersson and Walter 1995). A validation of the method in comparison with microdensitometry and cell structure analysis confirmed the classification results (Warensjö 2003).

The greater opacity of the summerwood of compression wood is evidently due to the discontinuous structure of the secondary cell wall of summerwood tracheids, which dissipates the light (Pillow 1941). In other words, the helical cavities in the inner portion of the secondary wall in compression wood tracheids scatter the transmitted light (Timell 1986; Andersson et al. 2000). The more pronounced the compression wood, that is, the more numerous the helical cavities, the greater is the opacity. However, it still remains to be explained why compression wood of species lacking helical cavities, such as *Ginkgo biloba* and *Taxus baccata*, are opaque in transmitted light (Timell 1986). Another explanation might be found in the shorter axial length of compression wood tracheids compared to normal wood tracheids (Hartig 1896, 1901; Ollinmaa 1955, 1961). The reduced length increases

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[6] Technical Association of the Pulp and Paper Industry, New York 1955, 1959 Compression wood in pulpwood. Standard method T 20-m-59. Tappi 38(1):174A–176A. 42(2):144A–145A.

[7] Technical Association of the Pulp and Paper Industry, Atlanta 1972 Compression wood identification in pulpwood. Proposed revision of T 20-m-59 as standard. Tappi 55:1119–1121.

[8] Technical Association of the Pulp and Paper Industry, 2009. Compression Wood Identification in Pulpwood, Test Method T 267 cm-05. Standards. Fibrous Materials and Pulp Testing. http://www.tappi.org/s_tappi/sec.asp?CID=7370&DID=547993.

the number of alternations between tracheids along the path of light when it is transmitted through the cross section. In addition, the axial end tips of tracheids are often truncated and bent (Münch 1938, 1940) which might further attenuate light transmission. This possible interpretation is in accordance with the tracheid-effect, whereby when light enters the longitudinal surface of timber it is scattered along the fibres and emitted with attenuated intensity at locations out of line with the region of entrance (Matthews and Beech 1976). Light radiated into timber transmitted along the grain is much less attenuated than across the grain. Thus, a small laser point on a clear wood surface will show an elliptic shape with the major axis in the grain direction (Nyström 1999). A knot or other areas with deviating grain will on the other hand have a much smaller ellipse or an ellipse of different orientation. This can be used to detect features on wood surfaces not readily distinguishable by appearance (Matthews and Beech 1976; Åstrand 1996; Nyström 1999; Fischer and Wendland 1999; Wendland 2000). While the physical phenomenon is not yet described in detail (Wendland 2000), fibre geometry and orientation have strong influence on the attenuation of transmitted light. Since the fibres of compression wood are shorter it has a significantly smaller longitudinal diffuse transmission compared to normal wood (Nyström 1999). This can be recorded by a scanning camera and compression wood differentiated against other wood features and might further explain its opacity in transmittance analysis.

7.5   Chemical Detection of Compression Wood and Severity Grading

Biologically grading the severity of compression wood tracheids revealed no distinct boundaries between normal and severe compression wood tracheids but a gradual transition in tracheid features (Yumoto et al. 1983). Some of the analytical detection methods presented above differentiate between mild and severe grades (as summarized by Gardiner and Macdonald, 2005). The most sophisticated attempt to assess compression wood severity differentiated six severity classes by morphological features (Yumoto et al. 1983). However, although no reliable quantitative measure of compression wood severity has yet been devised, grading of reference samples examined by fluorescence microscopy can provide a qualitative assessment of severity (Donaldson et al. 1999, 2004). Qualitative assessment means each classification system is subjective to the investigator and lacks an objective value that expresses the compression wood severity. Such an objective compression wood severity-value could improve correlations with other wood properties such as longitudinal shrinkage, stiffness, and chemical composition (Altaner et al. 2009). Chemical composition is an ideal characteristic to be correlated with compression wood severity due to its relative ease of measurement.

Chemical composition differs in quantitative terms between compression and normal wood and only to a minor degree in molecular structure (Timell 1986)

Table 7.2 Lignin and polysaccharide composition of normal and compression wood tracheids of an average conifer (Timell 1986, p. 393)

Constituent	Normal wood	Compression wood
Lignin	30	40
Cellulose	42	30
(1→3)-β-D-Glucan	Trace	2
Galactoglucomannans	18	9
Galactan	Trace	10
Xylan	8	7
Pectin	1	1
Other polysaccharides	1	1

All values as a percentage of extractive-free wood

(Table 7.2). As well as altered yields of monomeric sugars (Yeh et al. 2006; Nanayakkara et al. 2009) compression wood contains about 20–25 % less cellulose and 30–40 % more lignin (Cieslar 1896; Leary et al. 1986; Timell 1986). The quantity of acetyl groups is halved and compression wood contains up to 10 % of β-1-4-galactans not found in normal wood (Bouveng and Meier 1959; Yeh et al. 2006; Altaner et al. 2007; Nanayakkara 2007; Mast et al. 2009). In addition, not only is the relative share of lignin increased, but its chemical structure is altered (Önnerud 2003; Fengel and Wegener 2003; Nanayakkara 2007). Lignin concentration is especially high in the outer portion of the $S_2(L)$ layer against which the concentration in the primary wall (P) and the S_1 layer is considerably lower (Wergin 1965; Maurer and Fengel 1991). This modified lignin distribution within the tracheid cell wall is found in moderate compression wood (Donaldson et al. 1999, 2004).

It can be assumed that the changes from normal wood to severe compression wood in terms of chemical composition are as gradual as the changes in anatomical features (Altaner et al. 2009). If a chemistry-based parameter is being sought for the positive identification of the gradation of compression wood, i.e. the severity, a prerequisite is information about any quantitative relationship between chemical composition and compression wood severity. Moreover it is necessary to know whether this relationship is the same in all parts of the tree, i.e. whether they are independent of the morphological origin of the sample (Nanayakkara et al. 2009). In the following, possible chemical parameters are considered for compression wood detection and severity classification.

7.5.1 Lignin Content and Levels of Monomeric Sugars

Compression wood has a higher lignin content than normal wood. Moreover, the relationship between lignin content of *Pinus radiata* wood samples and microscopical compression wood classification shows good agreement between compression wood severity, as assessed by fluorescence microscopy, and the measured lignin content (Nanayakkara 2007). In samples of normal and opposite wood, lignin

content was below 31 % and none of the anatomical features associated with compression wood were present. Above a lignin content of approximately 31 % pronounced lignification began in the $S_2(L)$ layer at cell corners. At around 35 % the lignification in the $S_2(L)$ stretched all around the cell wall but was not detectable in cell corners and middle lamella and intercellular spaces became apparent (Nanayakkara 2007). This suggests that compression wood severity can be quantitatively predicted from the lignin content of the sample. Since lignin content changes consistently with increasing compression wood severity, it is useful to correlate other chemical parameters with the lignin content as an indication of how these other parameters change with compression wood severity (Nanayakkara et al. 2009).

Yields of neutral glucose, xylose, and mannose change approximately linearly with lignin content in opposite and compression wood (Nanayakkara 2007; Nanayakkara et al. 2009). While levels of glucose and mannose decrease with increasing lignin content, those of galactose remain comparatively constant for opposite wood (lignin content of 26–31 %) and only increase linearly ($R^2 = 0.79$) when lignin levels exceed ~31 %, i.e. as the compression wood severity increases. Accordingly, it is to be concluded that, even though compression wood formation was associated with changes in yields of monomeric sugars, only the galactose content is a useful indicator of compression wood severity (Nanayakkara 2007; Nanayakkara et al. 2009).

7.5.2   Levels of β-1-4-Galactan

The abundance of galactose in compression wood is representative of galactan (Bouveng and Meier 1959; Yeh et al. 2006). Compression wood contains up to 10 % of β-1-4-galactans not found in normal wood (Bouveng and Meier 1959). The anti-β-1-4-galactan monoclonal antibody LM5 was found to bind to certain wood tissues in Sitka spruce leading to a clear difference in the immunofluorescence level depending on its presence or absence (Altaner et al. 2007). Samples identified as distinct normal wood and compression wood differed clearly in the amount of LM5 binding. Strongest binding of LM5 was found in tracheids of compression wood tissue, indicating the presence of large amounts of β-1-4-galactan structures in these wood cell walls. In normal wood tissue, no binding of LM5 to the tracheids was observed (Altaner et al. 2007). Incorporating β-1-4-galactan into the cell wall is suggested as being the first physiological reaction of a tree to mechanical stress.

Correspondingly, LM5 labelling is able to detect very mild degrees of compression wood formation and is therefore a suitable biochemical marker for compression wood (Altaner et al. 2007). However, methods to routinely quantify β-1-4-galactan in wood are currently unavailable (Nanayakkara et al. 2009) and the physiological role of β-1-4-galactan is not yet well understood. In developing compression wood tracheids LM5 strongly and uniformly labels the S_2 region (Mast et al. 2009). In contrast, the signal for β-1-4-galactan is weak and patchy in fully developed compression wood tracheids. The signal might be masked by lignin

deposition in later tracheid development. β-1-4-Galactan is an abundant, integral component of compression wood tracheids, evenly distributed across the whole S_2 layer, an ultrastructural distribution different from that of lignin. Thus it is suggested that β-1-4-galactan plays no direct role in lignin distribution in compression wood tracheids (Mast et al. 2009). While galactose yields appear to be of limited value for assessing compression wood severity, the levels of β-1-4-galactan may still be a good predictor of severity as suggested by Altaner et al. (2007).

7.5.3 Ratio of Monomeric Sugars (Galactose/Glucose and Galactose/Mannose Ratio)

We have seen that the yield of different monomeric sugars changes with lignin content and thus with compression wood severity. The need to detect absolute amounts of lignin and sugar is avoided when the ratio of different sugars is used rather than a single sugar (Nanayakkara 2007). Among possible relationships the galactose/glucose ratio shows strong correlation ($R^2 = 0.86$) with lignin content in *P. radiata*. The ratio remains rather constant up to a lignin content of 31 % previously related to normal and opposite wood. Above this value the galactose/ glucose ratio is linearly related to increasing lignin content and compression wood severity (Nanayakkara 2007). This increased galactose/glucose ratio in juvenile and mature compression wood, being in the range of 0.21–0.26 against 0.03–0.04 in normal or opposite wood, has been confirmed for *Pinus taeda* L. (Yeh et al. 2006). However, the galactose/mannose ratio in normal and opposite wood is in the range 0.10–0.17, whereas the ratio in compression wood is 1.11–1.14. Although both galactose/glucose and galactose/mannose ratios are of similar magnitude in normal and compression wood, the galactose/mannose values are higher and more accurate, thus representing a better indicator for detecting the existence of compression wood (Yeh et al. 2006). Possibly, this observation is species dependent since in *P. radiata* the galactose/mannose ratio was less strongly correlated to lignin content than galactose/glucose (Nanayakkara 2007). Further, the latter appears somewhat less sensitive to the morphological origin of the sample (Nanayakkara et al. 2009).

7.5.4 Level of Lignin Structural Units

Lignin consists of three monolignol monomers, *p*-coumaryl alcohol, coniferyl alcohol, and sinapyl alcohol, which are methoxylated to various degrees. These lignols are incorporated into lignin in the form of the phenylpropanoids *p*-hydroxyphenyl (H), guaiacyl (G), and syringal (S), respectively. Compression wood has a higher content of releasable H-units than normal wood (Fukushima and

Terashima 1991; Önnerud 2003; Yeh et al. 2006) associated with a decreasing level of releasable G-units (Nanayakkara et al. 2009). Interestingly, the level of releasable H β-ether units again remained constant at a lignin content lower than 31 %, i.e. in normal and opposite wood. Thereafter, the levels increased linearly with higher lignin content and thus with compression wood severity (Nanayakkara 2007). The H/G ratio as determined by thioacidolysis also shows a linear correlation ($R^2 = 0.76$) with lignin content, but correlation was weaker than for releasable H β-O-4 units alone. However, the ratio spanned a larger range relative to levels in normal wood and is less sensitive to the morphological origin of the sample. The H/G β-ether ratio, as measured by thioacidolysis, may be the preferred parameter since it does not require the determination of absolute values (Nanayakkara 2007).

If chemical composition is to be quantified for measuring compression wood severity the spatial resolution should be preferably of sub-millimetre order and spatial orientation must not be lost. An analytical technique capable of meeting this demand is scanning FTIR micro-spectroscopy which has been employed to obtain compression wood severity measurements on Norway spruce (*Picea abies* (L.) Karst.) and Sitka spruce (*Picea sitchensis* (Bong.) Carrière) (Altaner et al. 2009). Samples were scanned with an FTIR-microscope in reflective mode and spectra gathered between 4,000 and 800 cm^{-1} at a resolution of 4 cm^{-1}. Spectra obtained from normal and compression wood differed in the intensity of the band centred at 1,730 cm^{-1} originating from carboxylic stretching vibrations and the band centred at 1,600 cm^{-1} related to aromatic skeletal vibrations. Further, in line with the higher lignin content the band centred at 1,510 cm^{-1} originating from aromatic skeletal vibrations was more intense in compression wood (Altaner et al. 2009). These bands are perfectly in agreement with those identified by Niemz et al. (1990) to reveal distinct differences between normal and compression wood. In order to describe the differences in severe compression wood, an indicator was calculated from the ratio between the linear baseline corrected spectral areas ranging from 1,635 to 1,556 cm^{-1} and from 1,778 to 1,705 cm^{-1}. Normal wood turned out to have a compression wood severity indicator of around 0.5, while for severe compression wood values of up to 1.7 were observed (Altaner et al. 2009). Cross-comparison with alternative compression wood detection methods revealed good agreement with absorption of transmitted light. Generally, absorption increased with the severity of the compression wood. However, all samples close to the bark were opaque. In this area the measurements in transmitted light were affected by dark bark extractives that had diffused into the outer growth rings. This was deduced from the fact that the alternative compression wood indicators, i.e. the microfibril angle and β-1-4 galactan content were not increased. While the β-1-4 galactan content agreed well with the transmitted light measurements, MFA measurements confirmed the presence of compression wood in mature wood. In normal juvenile wood MFAs were as high as in mature severe compression wood, making it difficult to use it as the sole indicator for compression wood (Altaner et al. 2009).

7.6   Conclusion

Irrespective of the reason for detecting compression wood, its detection necessitates a thorough positive identification of this tissue in contrast to other wood tissues. In principle, this identification could be based on its specific anatomical, physical, or chemical properties. Although the notion of compression wood is based on its optical and mechanical anomalies compared to "normal wood", its identification, according to anatomical properties is the best documented and understood. Further, anatomical identification almost exclusively serves as a reference when elaborating physical or chemical compression wood detection methods.

The most detailed grading scheme for classifying compression wood into different degrees of severity is based on morphological characteristics determined by microscopy after experimentally induced compression wood formation (Yumoto et al. 1983). However, the transition from normal wood to severe compression wood was revealed to be a gradual one and even adjacent tracheids may vary in their structural features. While anatomical analysis provides a reasonable basis for biologically grading compression wood and serves as reference when developing alternative detection methods it remains disadvantageous in being laborious, limited to relatively small sample sizes and often remaining subject to operator bias. Nevertheless, the specific properties of compression wood reveal the potential to complement this system by chemical or physical gradations, possibly throwing new light on the biological gradation while also reflecting its nature (Yumoto et al. 1983).

Alternatively, visual inspection at the macroscopic level might overcome limitations related to sample size and time required for biological compression wood detection. The visual appearance of compression wood in reflected light results from its specific interaction with electromagnetic radiation. This interaction is affected by its chemical constituents and anatomical structure which determine its absorption and light-scattering characteristics (Hagman 1996). Thus, visual analysis offers a promising approach for detection of compression wood. Moreover, it enables us to classify severity grades since the degree of compression wood is characterized by both changes in structure as well as in chemical composition.

Although outlining compression wood areas is accepted for softwood grading, diverse recommendations on how to improve contrast reflect the problems encountered when detecting compression wood in visual light. Digital colour imaging applications attempt to objectify the visual detection of compression wood while increasing repeatability, speed of detection, and precision. Distinct colour differences allow appraisal of compression wood in the green condition although they are too small to enable automatic separation on dry surfaces (Nyström 1999). Nonetheless, when compression wood areas are manually selected, regions with similar chromaticity are identified in RGB images and their position and extent can be automatically measured (Wernsdörfer et al. 2004).

The strength of hyperspectral image analysis is that it can overcome the difficulties associated with three band colour imaging. It enables the detection of minor

differences in light intensity in numerous wavelength bands. The spectral characteristics of reflected light are input to classification algorithms which permit the classification of longitudinal and transverse faces of dried lumber with high level of precision (Hagman 1996) and enable automatic classification of cross-sectional areas into severe, moderate compression wood, and normal wood with an overall accuracy of 96 % (Duncker and Spiecker 2009).

Compression wood can also be detected in transmitted light through its relative opacity (Pillow 1941). Microdensitometry and cell structure analysis have validated the decisive effect of compression wood on transmitted light (Warensjö 2003). Again, the application of digital image analysis improved the method and increased repeatability compared to operator decision-making (Andersson and Walter 1995). In addition, the fibres of compression wood are shorter, which results in a significantly smaller longitudinal diffuse transmission of light in transmittance analysis (Matthews and Beech 1976) compared to normal wood (Nyström 1999).

Often, visual detection methods differentiate between mild and severe grades of compression wood (as summarized by Gardiner and Macdonald, 2005). However, since biological grading does not revealed distinct borders (Yumoto et al. 1983) reference samples remain a qualitative measure of severity (Donaldson et al. 1999, 2004). In consequence, each classification system is subjective to the investigator and lacks a quantitative measure that expresses the compression wood severity objectively. Due to its relative ease of measurement, chemical composition is an ideal candidate to be correlated with the compression wood severity. Such an objective compression wood severity-value could improve correlations to other wood properties such as longitudinal shrinkage, stiffness, and chemical composition (Altaner et al. 2009).

The chemical analysis of compression and normal wood demonstrates that several parameters have the potential to identify compression wood and to complement other methods in quantifying compression wood severity. There is a good agreement between compression wood severity as characterized by fluorescence microscopy and lignin content: the higher the lignin content, the more severe the development of compression wood (Nanayakkara 2007; Nanayakkara et al. 2009). Although, lignin content was found to be linearly related to compression wood severity, the increase in lignin with compression wood severity was proportionately much smaller than that of galactose or the galactose/glucose ratio. This suggests that lignin content is less suitable as an indicator for compression wood severity than the monomeric sugar levels (Yeh et al. 2006; Nanayakkara 2007). Among the chemical characteristics found to be linearly related to compression wood severity *p*-hydroxyphenyl-related criteria appear the most suitable chemical indicators of CW severity, as they are least sensitive to the sample's morphological origin and their response to compression wood severity is high (Nanayakkara et al. 2009).

At present, biological detection and classification offers the most detailed grading of compression wood and discrimination against normal wood. Visual inspection methods enable fast detection of compression wood in its spatial orientation on large sample sizes in most two degrees of severity. Chemical analysis complements these methods in providing quantitative measures of compression

wood severity though losing its orientation in space. According to the specific problem encountered an appropriate compression wood detection method can be selected until a new and better method is developed which is likely to combine the strengths of these three principle approaches.

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Chapter 8
Effects of Reaction Wood on the Performance of Wood and Wood-Based Products

Rupert Wimmer and Marie Johansson

Abstract Compression wood in softwoods and tension wood in hardwoods have properties, which adversely affect its usefulness for wood products. This chapter shows that reaction wood can be associated with many unsuitable wood properties. The results vary due to the fact that definitions about occurrence and severity of reaction wood are scarcely documented. A few properties seem to be even benefitting from the presence of reaction wood: the higher smoothness of compression wood surfaces, better shear strength of compression wood, higher toughness and impact resistance when tension wood is present, lower water uptake and swelling in fibreboards containing compression wood, and higher durability against fungi of compression wood. However, these are outweighed by disadvantages, which is the reason why reaction wood has a bad reputation in industry. The problem with reaction wood is that it is in most cases mixed with normal wood, which leads to non-uniform and more variable properties. This may lead to non-uniform swelling and shrinking, causing distortions, with additional problems of reduced strength and unfavourable surface properties. Wood-based materials such as particle boards or fibreboards are generally less prone to problems associated with reaction wood than solid wood products. With knowledge-based production methods the utilization of different wood types, including reaction wood, might be feasible.

R. Wimmer (✉)
Universität für Bodenkultur Wien (BOKU), IFA Tulln, Institute for Natural Materials Technology, Sustainable Biomaterials Group, Konrad Lorenz Strasse 20, 3430 Tulln an der Donau, Austria
e-mail: rupert.wimmer@boku.ac.at

M. Johansson
Department of Building and Energy Technology, Linnaeus University, 35195 Växjö, Sweden
e-mail: Marie.Johansson@lnu.se

8.1 Introduction

Reaction wood, whether compression wood in softwoods or tension wood in hardwoods, has properties, which adversely affect its usefulness for many wood products. This includes solid wood products (Kretschmann and Bendtsen 1992), fibreboards (Roffael et al. 2005; Akbulut et al. 2004; Akbulut and Ayrilmis 2006; Ayrilmis 2008), oriented strand boards (OSBs, Stürzenbecher et al. 2010a, b), particle boards (Gunther et al. 1972; Lehmann and Geimer 1974), veneer-based materials (Maeglin 1987; Zhang et al. 1994), and pulp and paper (Timell 1982; Ban et al. 2004). It also has adverse effects on wood processing (Clair et al. 2005), in that it affects gluing (Frihart 2005), permeability (Spicer and Gartner 2002), or the drying process (Timell 1986; Walker 2006). The details of the mechanical properties and behaviour of reaction wood at a cellular level are discussed in Chap. 6 and the commercial and forest management implications of reaction wood are explored in Chap. 9 and provide complementary information to this chapter.

8.1.1 Perception of Wood Quality

The task of understanding the selection of materials is complex, as it may be influenced by criteria coming from different end-users such as architects, engineers, contractors, or regular consumers. In many instances poor communication and misunderstandings exist on the part of manufacturers in recognizing end-user specifications and requirements (Emmitt and Yeomans 2008). According to ISO 8402, quality can be defined as "all the properties and characteristics of a product which enable it to fulfil explicit or implicit requirements". Wood quality is mostly formulated in negative terms by listing properties and characteristics that should not be present. These "defects" are, for example, defined by visual grading rules that are based on common strength reducing characteristics defined in Europe in Annex A of EN 14081-1. These characteristics include knots, slope of grain, wide rings, fissures, wane, warp, and other characteristics such as reaction wood, mechanical damage, bark inclusions, stem damage, or tree top rupture. Therefore, wood should be without knots, rot, reaction wood, warp and other features, even though they are naturally occurring features. It is less common to list the properties that are required, which could be a reason why some wood features are per se seen as unwanted. The building industry often uses the word "quality" with reference to "high quality" rather than the absence of defects.

8.1.2   Fitness for Use

Since wood quality can be defined as a measure of "fitness for use", individual wood properties are critical to given applications. These properties might also include optical appearance, haptics (feel, touch), natural durability, gluing performance, permeability for liquids, or the many mechanical performance properties in constructions or buildings (Johansson et al. 1994). Workability is a term that summarizes a number of properties important for transforming wood material to finished end-products. It includes cutting, sawing, drying, planning, sanding, and wood finishing using, e.g. a varnish. For these and other quality parameters straightness, shape and dimensional stability, surface roughness, tool wear, and dulling are all relevant factors. Today's consumers expect high quality standards from a natural product such as wood, combined with natural optical appearance, high biological and mechanical durability, and low maintenance.

8.2   Solid Wood Products

8.2.1   Wood Density

Density denotes the mass per unit volume of a substance and is the most commonly used quality indicator for wood raw materials. It is the most extensively researched wood property (Cown and van Wyk 2004). The density of the oven-dry cell tissue of all woody plants is reported as being roughly 1.5 g/cm^3 (Walker 2006). The density of 100 % crystalline natural cellulose lies between 1.582 and 1.599 g/cm^3, and amorphous cellulose ranges between 1.482 and 1.489 g/cm^3 (Sun 2005). For the G-layer isolated from tension wood of an oak a density of 1.52 g/cm^3 was found (Terashima et al. 2009). The density of lignin is reported as being lower than that of cellulose: the density of dioxane extracted lignin is 1.278 g/cm^3, and that of periodate dissolved lignin 1.350 g/cm^3, the latter retaining much of the original macromolecular structure (Ramiah and Goring 1965). For the hemicelluloses, the density is around 1.5 g/cm^3 (Mark 1967). Mark (1967) calculated a weighted density of 1.48 g/cm^3 for the proportions of cellulose, hemicellulose, and lignin found in the compound middle lamella region and the secondary wall. If the inorganic content is added to this value, a final value of 1.5004 g/cm^3 results.

Logically, wood having higher proportions of lignin should have also lower density. However, the density of compression wood is reported as higher than normal wood (Harris 1977; Nicholls 1982; Timell 1986), despite the higher lignin content. Reasons for the observation of increased density in compression wood include increased tracheid wall thickness, which results in lower porosity, and also shorter tracheids (Donaldson et al. 2004). Observations of lower latewood density such as, for example, by Shelbourne and Ritchie (1968) are explained by the increased radial diameter of tracheid lumens in the latewood of compression

wood compared to normal wood. As for tension wood, density is reported to be higher compared to opposite or normal wood. In many hardwood species investigated, wood density of tension wood was always higher than in opposite or normal wood (Arganbright et al. 1970). The increase of wood density in tension wood tissue is attributed to thickening of fibre walls with gelatinous layers. When wood density increased in tension wood in young poplar stems, a reduction of longitudinal shrinkage occurred in the opposite wood, while shrinkage increased on the tension wood side (Jourez et al. 2001a).

8.2.2   Mechanical Properties

Wood density is correlated to many wood parameters, including mechanical properties such as strength and stiffness (Timell 1986). Young's modulus, tensile and bending strength are distinctively reduced in compression wood, whereas compression strength remains the same or is increased (Timell 1986). Longitudinal shear modulus as well as shear strength had higher values for compression wood of *Larix decidua* compared with normal wood (Müller et al. 2004). After correction for the higher compression wood density, however, the differences disappeared. This indicated that the high microfibril angle (MFA) in compression wood is less important for shear properties than it is for the other mechanical properties (Müller et al. 2004). Knowledge of wood density is also essential for the estimation of embedding strength, which is the load carrying ability of a mechanical joint. This property is crucial for the design of wood connections (Anon 2004). Standards for testing embedding strength prescribe that the wood should be without visible defects such as reaction wood, with the actual knowledge about the influence of reaction wood on embedding strength being very limited. There are indications that reaction wood in connection/joints results in brittle failures at relatively low stress levels (Wolfe et al. 2000).

Gindl (2002) has shown that compression wood has reduced stiffness (Young's modulus) due to much higher MFA, although compression strength was not negatively affected by the high MFA as might be expected. Evidence was also shown that higher proportions and an altered composition of lignin in compression wood increased the resistance of the cell walls to compression failure as lignin has a reinforcing function. Kolseth and Ehrnrooth (1986) indicated that the content of hemicelluloses and lignin in the fibres only marginally reduces stiffness, and the reduction in stiffness is more due to an increase in the moisture content. At high MFA, the loss of the reinforcing effect is also dependent on the cell wall matrix properties, which are strongly dependent on hydration (Salmén 2004). With increasing MFA, the shear stresses in the matrix reach a maximum at an angle of $45°$ and therefore lower the tensile stiffness disproportionately for wet compression wood fibres, compared to normal wood fibres having lower MFAs (Eder et al. 2012).

For tension wood, the mechanical stiffness of oak was found to be different from that of normal wood; the modulus of elasticity was lower in spite of a higher density (Passard and Perré 2005). In contrast to compression wood, tension wood is cited as having higher tensile strength and Young's modulus than normal wood, although the variability of these properties is greater than in compression wood (Ruelle et al. 2010, 2011). Pechmann (1972) found that compression strength was 25 % reduced in tension wood compared to normal wood. In addition, tension wood has been found to have higher fracture toughness and impact resistance (Barnett and Jeronimodis 2003; Saadat-Nia et al. 2011).

Wood density is seen as dependent on all other anatomical characteristics (Wimmer and Grabner 2000). As tension wood shows a greater anatomical variability than compression wood, this should also be the case for density and consequently mechanical properties. Tension wood contains fewer and smaller vessels than normal wood, and in many species the fibres are modified by the deposition of the G-layer inside replacing the normal S_3 layer. The G-layer is cellulose-rich with the microfibrils deposited almost parallel to the longitudinal axis of the cell. See Chap. 6 for more details on the mechanical properties of reaction wood.

8.2.3   Dimensional Stability

The reaction wood property causing the greatest problem for wood utilization is the large shrinkage that occurs in the longitudinal direction (Skaar 1972). Juvenile wood, compression wood, and fibre deviations around knots are wood types that often display more longitudinal shrinkage than normal wood (Zobel and Sprauge 1998). For spruce studs showing compression wood the longitudinal differential shrinkage coefficient (the percentage shrinkage per percentage change in moisture) was studied by Johansson (2003). Differential shrinkage in the longitudinal direction varied greatly, with values between 0.0008 % longitudinal change per % moisture change, and 0.0903 % per % (a factor of 113 differences). The longitudinal shrinkage in tension wood is also reported to be higher but with a less pronounced difference to that of normal wood (Ruelle et al. 2010). Transverse shrinkage was significantly higher in tension wood than in normal wood of poplar, while tangential shrinkage was notably higher than that in the radial direction in both normal and tension wood (Fang et al. 2007). In the radial and tangential direction the shrinkage of compression wood is often smaller than the shrinkage of comparable normal wood, while for tension wood the transverse shrinkage is often larger than in comparable normal wood (Clair et al. 2003).

A hypothetical timber board consisting of pure reaction wood would not be a problem in use as it will shrink uniformly (Du Toit 1964). The problem occurs when the reaction wood is mixed with normal wood within a given board, as is usually the case, and this may lead to non-uniform shrinkage causing distortion in the form of bow, spring, or cup. The occurrence of compression wood in boards is often the explanation for severe deformation occurring during changes in moisture content

Fig. 8.1 Growth stresses in
reaction wood causing
(**a**) log-splitting in
hardwoods with tension
wood and (**b**) pinching in
softwood with
compression wood
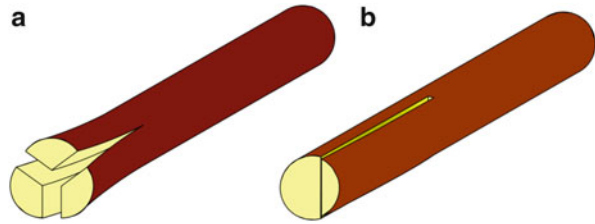
(Dogu and Grabner 2010). Warensjö and Rune (2004) concluded that the severity of bow and spring in boards sawn from 22-year-old Scots pine trees was significantly correlated to the amount of compression wood.

8.3 Effects on Wood Processing

8.3.1 Workability

Reaction wood has the potential to influence the workability of wood materials. Both tension wood and compression wood are associated with high growth stresses which are many times greater in tension wood than in compression wood (Dinwoodie 1966; Maeglin 1987; Bamber 2001; Fang et al. 2008). These large tension stresses give rise to end splitting of logs directly after cross-cutting (Castéra et al. 1994; Fig. 8.1).

Compression wood often gives rise to negative growth stresses (compression stresses). The release of these stresses during sawing may cause loosened boards that bend into the remaining log and pinch the saw blade (Timell 1986). The pinching effect during sawing can cause the saw blade to get locked and the sawing process needs to be halted in order to loosen the log. This pinching effect of compression wood has coined alternative terms for compression wood, including "timber bind" in English, or "tjurved" (stubborn wood) in Swedish. The release of growth stresses also causes warping problems directly after sawing.

8.3.2 Machining and Sawmilling

Machining problems (e.g. fuzzy surface or wooliness) are often related to the presence of tension wood (Balatinecz et al. 2010). Studies have reported higher energy consumption during cutting of tension wood (Coutand et al. 2004). The fuzzy grain surfaces make the wood difficult to sand, polish, or plane (Fig. 8.2; Lutz 1970). As compression wood is reported to be denser and harder it requires more energy for sawing (Timell 1986). Saw blades also have the tendency to deflect by following compression–normal wood borders. Pure compression wood cuts well

Fig. 8.2 Rejected wood products due to tension wood: wooden clog soles made from poplar and a clothes hanger made of beech, both showing expressed fuzzy grain surfaces

and forms a smooth surface due to its high lignin content (Lutz 1970). The smooth surface is probably one of the reasons why compression wood was used as skies and runners for sleighs (Timell 1986).

In the sawmilling industry compression wood is seen as a major defect, similar to knottiness (Wernsdörfer et al. 2004). Reasons include the overall lower strength of the boards, unfavourable drying properties, problematic gluing and impaired surface finishing properties. In addition, compression wood has higher hardness and tends to be more brittle.

Distortion in solid wood also takes place when high longitudinal growth stresses generated by tension wood are released (Boyd 1977, 1980; Wahyudi et al. 2000; Washusen et al. 2003). The high incidence of tension wood has a negative impact on all wood machining operations involved, including veneer peeling, flaking, stranding, or sanding (Balatinecz et al. 2001; Tarmian and Azadfallah 2009).

8.3.3 Wood Drying

Experiments in which tension wood from beech and compression wood from spruce were compared to normal wood (opposite wood) showed that drying, especially above fibre saturation, is significantly reduced (Tarmian et al. 2009a). Because the water content in green reaction wood is lower than in the adjacent normal wood, the dried timber may reach the final moisture content at the same time. However, the time period to reach uniform moisture was overall longer for the reaction wood samples (Davis et al. 2002).

8.3.3.1 Intervessel Pitting

The reasons for altered drying properties are found in wood anatomical differences (Tarmian and Perré 2009). The permeability of reaction wood was smaller

compared to normal wood in the longitudinal and radial direction of wood from the same species (Straze and Gorisek 2006; Tarmian et al. 2009a, b). Opposite wood dries significantly faster than compression wood during the free-water removal period owing to larger and more numerous bordered pits in axial tracheids. Similarly, the main reason for the lower drying rate of tension wood is that intervessel pits are smaller and less numerous in its vessel walls. In addition, an extended diffusion process in reaction wood is related to its higher wood density caused by less permeable, thick cell walls. In conclusion, reaction wood is found to be less permeable than normal wood, both in the longitudinal and radial directions. This difference seems to be more pronounced in compression wood, primarily in the longitudinal direction. The different drying behaviour of reaction wood, whether compression wood in *Picea abies* or tension wood in *Fagus sylvatica*, was more obvious during drying above fibre saturation when liquid free water is removed. In fact, the difference in the drying rate curves of reaction and opposite wood gradually decreases when drying progresses to the bound water domain (Williams 1971; Davis et al. 2002).

8.3.3.2   Drying Stresses

Drying studies with poplar that included tension wood revealed that boards suffered from higher drying stresses and a tendency to easily develop case-hardening (Tarmian et al. 2009b). Drying stresses can be especially severe in the longitudinal direction, which is associated with high longitudinal shrinkage. Tension wood is also responsible for buckling that occurs during veneer drying (Maeglin 1987). As shrinkage of compression wood is higher than in normal wood, compression wood should be trimmed off veneer sheets to reduce the risk of seasoning defects in machined joinery and cabinet pieces. The higher axial shrinkage found in reaction wood can be attributed to the existence of the G-layer in tension wood (Clair and Thibaut 2001; Washusen et al. 2001, 2002), or to the larger MFA in the S_2 layer of compression wood (Warensjö 2003; Timell 1986). These adverse effects of reaction wood on the quality of dried lumber can be addressed by increasing knowledge of reaction wood drying behaviour, in comparison to corresponding normal wood.

8.3.3.3   Reaction Wood Recognition

Mild reaction wood which macroscopically resembles normal wood is even more difficult to detect with the naked eye (Donaldson et al. 2004). Severe reaction wood is also visually difficult to recognize in the case of tension wood, or where compression wood is present in softwoods with coloured heartwood (detection methods for compression wood are discussed further in Chap. 7). Such wood pieces cannot be distinguished in the kiln stack by the kiln operator. In industry the presence of reaction wood is often ignored, or its proportion may be very low and

Fig. 8.3 Cross section of spruce with visible compression wood. The irregular occurrence of reaction wood creates additional variability, which causes most of the technological problems experienced in processing and use
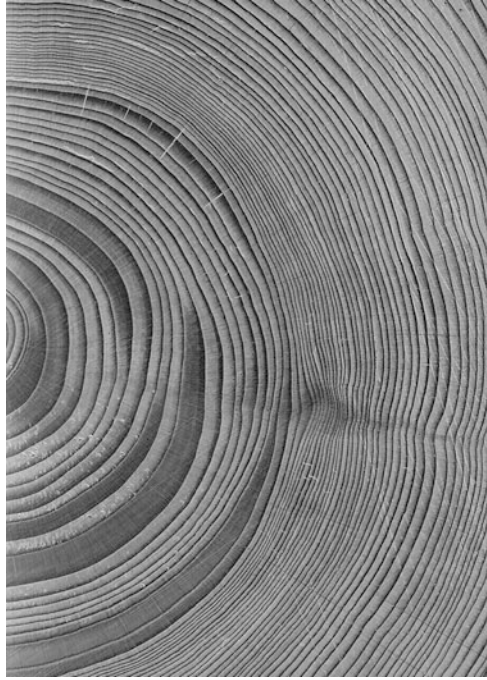
thus overlooked. It therefore seems reasonable to claim that reaction wood may be present in much greater quantities in dried lumber than expected, leading to unsatisfactory drying (Fig. 8.3 and Fig. 9.2).

8.3.3.4 Kiln Drying

While there is considerable work on the shrinkage behaviour of reaction wood during drying (Jourez et al. 2001b; Clair and Thibaut 2001; Perré 2007) and its influence on the occurrence of drying defects (Warensjö 2003), there is a lack of knowledge concerning the exact drying kinetics of reaction woods, particularly for tension wood. Most kiln drying studies have been focused on normal wood and have excluded reaction wood. Williams (1971) compared drying rates of compression wood and normal wood in *Pinus radiata* under two drying conditions. It was confirmed that compression wood had a slower drying rate than normal wood, and they simultaneously reached the final moisture content. Davis et al. (2002) showed that compression wood had also an adverse influence on the drying rate of *P. radiata*. These authors attributed the reduced drying rate of compression wood to an increased proportion of latewood, to narrower lumens, and to the reduced numbers of bordered pits.

8.3.4 Heat Treatment

Heat treatment is applied to solid wood to achieve better dimensional and biological stability (Hill 2006), with significant changes in colour seen as a side effect (e.g. Weigl et al. 2012). Heat treatment processes used in industry cause chemical alterations that depend on factors such as temperature, presence of water, duration of treatment, atmosphere, wood species, timber dimensions, or presence of catalysts (Hill 2006). During the treatment a number of chemical changes take place in the wood. The hemicelluloses degrade first at around 160° and above due to their low molecular weight and branching structure (Fengel and Wegener 1984). With heat treatment the equilibrium moisture content and the wettability of wood is lowered (Wang and Cooper 2005; Ates et al. 2009), the mechanical properties decline (Hanger et al. 2002; Shi et al. 2007), while dimensional stability often improve (Bekhta and Niemz 2003). For wood properties such as water uptake, volumetric swelling, or mechanical properties, all measured after heat treatment, no obvious differences are seen between compression wood and opposite wood (Dündar et al. 2012). However, heat treated compression wood of black pine showed a particularly high reduction in longitudinal swelling (up to 50 %). The results by Dündar et al. (2012) led to the conclusion that heat treatment of compression wood should ease the stability problem of compression wood, as it is caused by the excessive shrinkage or swelling in the longitudinal direction. Heat treatment has therefore a stabilizing effect on compression wood, which makes compression wood more suitable for end-products requiring dimensional stability.

8.3.5 Gluing and Bonding

Reaction wood is important for wood bonding (Frihart 2005). For any type of bond to form a molecular-level contact is required. Wood–adhesive interactions involve the entire complexity of wood anisotropy, with the added complication of differences between heartwood and sapwood, and earlywood and latewood. Because wood is a highly porous material, many factors control the wetting of the surface, including the relative surface energies of the adhesive and the substrate, viscosity of the adhesive, temperature of bonding, pressure on the bondline, and others. It is argued, that in juvenile, compression, and tension wood cell structures are "distorted", which should weaken the wood–adhesive interface region (Frihart 2005). However, in the study by Follrich et al. (2008) the adhesive bond strength of end-grain joints using compression wood was increased compared to normal wood. The morphology of these compression wood cells, with nearly circular or oval shapes and thick cell walls, was seen as a major factor. With increased density, the cross-sectional cell wall area is also increased, while the cell lumen size decreases. In the case of mechanical interlocking the higher density should reduce adhesive penetration into the pores resulting in lower penetration depth. However,

higher density also means an increase in the available area for adhesive bonding through the thicker cell walls (Reme and Helle 2002). It was concluded that the higher cell wall cross-sectional area is more important for a stronger bond than the mechanical interlocking due to adhesive penetration (Follrich et al. 2008).

Overall, hardly any studies have been found that refer to gluing questions with reaction wood. Most adhesive and gluing research has excluded reaction wood and works with "defect free" samples only. Since there is always reaction wood present, the wood industry would certainly benefit from more research in this area.

8.4 Wood-Based Composites

Wood-based composites fall into two categories from the standpoint of the end-application: panel applications, such as plywood, OSB, particleboard (PB), and fibreboard; and beam or header applications, including glulam, laminated veneer lumber (LVL), oriented strand lumber (OSL), parallel strand lumber (PSL), and scrimber based lumber (Ozarska 1999; Shi and Walker 2006). More recently wood has also been combined with synthetic polymers, mostly polyolefines, but also bio-polymers, to manufacture wood–polymer composites (Shi and Walker 2006).

Gindl (1997) investigated distortion problems of three-layered flooring panels made of poplar wood surface layers and a spruce core. He confirmed tension wood being the primary reason for severe out-of-plane warping (Fig. 8.4).

8.4.1 Fibreboards

Medium density fibreboard (MDF) is one of the most rapidly growing composite products on the market (Ayrilmis 2007). The physical and mechanical properties of MDF are strongly linked to the properties of the raw materials used, including wood species, adhesives, and additives (Akbulut and Koc 2004; Akbulut et al. 2004). For wood as a raw material, fibre structure and strength, anatomical and chemical fibre properties, and the composition of complete and broken fibres are considered as basic factors influencing the fibreboard properties (Suchsland and Woodson 1991; Maloney 1977; Groom et al. 1999).

Roffael et al. (2005) investigated MDF boards made with wood that included high proportions of compression wood. Fibres were separated in a thermo-mechanical pulping process using a lab refiner. Compression wood fibres were found to be 15 % shorter and with 15 % more lignin. The water retention value of the fibre furnish was reduced by 10 %. For the MDF boards containing compression wood, thickness swelling was lowered by 20 %, and water uptake was also significantly reduced. Internal bonding of the compression wood boards was 35 % higher than with normal wood boards. Bending strength of the compression

Fig. 8.4 Three-layer flooring panels with visible tension wood (iodine dye) in the upper surface layer made from poplar wood. Core layer is spruce (adopted from Gindl 1997)
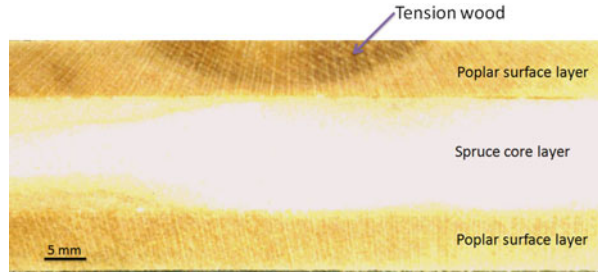
wood boards was lower, probably due to the reduced fibre length and other anatomical features of compression wood. Compared to normal wood boards the surface hardness of the compression wood-made boards was increased by 25 %. Overall, MDF boards containing a higher proportion of compression wood showed some degree of improvement in their properties. Results by Nicholls (1982) indicate that compression wood fibres dried under pressure (as in hardboards) do not have disadvantageous properties. However, insulation boards containing compression wood formed under low pressure had poorer bonding. Akbulut et al. (2004) reported that after 24 h water submersion the thickness swell coefficient of MDF panels made of black pine containing 10 % compression wood was 5.2 %, while for panels containing 75 % compression wood the coefficient was slightly higher (6.1 %). In a subsequent study by Ayrilmis (2008) MDF panels also made from black pine fibres containing 75 % compression wood had poorer dimensional stability than panels containing 10 % compression wood fibres. Surface roughness of MDF as studied by Akbulut and Ayrilmis (2006) increased as the compression wood proportion went up.

The refining process might result in a higher proportion of broken fibre fragments from compression wood, which are then less able to bond to each other compared to intact fibres from normal wood. Fibre composition is seen as a critical factor determining the properties of fibreboards (Suchsland and Woodson 1991). With the published results on the issue of compression wood in fibreboards, it can be concluded that careful refining is even more critical than with normal wood. With a gentle refining process fibres should stay intact and the higher lignin content may lead to boards with reduced thickness swell and water update. However, in the case of higher portions of fragmented and broken fibres adverse effects might be observed.

8.4.2 Particle Boards

Overall, fibre properties are less critical for particle board compared to fibreboards. Therefore, the presence of reaction wood in particle boards should be less important. Gunther et al. (1972) studied the utilization of branch wood from *Pinus sylvestris* with a high content of compression wood and found that particleboards

could be manufactured from this type of wood but that the physical properties, especially density, demonstrated high variability. It was recommended that in triple-layer particleboards, branch wood particles having higher proportions of compression wood should be used as the middle layer. Lehmann and Geimer (1974) also examined the properties of structural panel boards made from *Pseudotsuga menziesii* wood residues. Panels made from small branches with bark still attached were of lower quality. These panels had much higher water uptake rates than the control specimens and it is argued that the higher compression wood content could be the reason.

A few studies have looked at tension wood in panel boards. Particle mats formed with tension wood particles were seemingly more difficult to make, as the particle dispersion within the mat was less homogeneous (Bucholzer and Roffael 1987). The lower lignin content of tension wood resulted in more water sorption and thickness swelling of the boards. Likewise, bending strength and internal bond strength were lowered as a response to higher tension wood content (Buchholzer 1992). While tension wood is easy to detect in solid wood products, this seems to be difficult in wood-based composites where the wood is widely disintegrated (Husien et al. 1996). Even with the existing results it can be stated that no comprehensive understanding exists on the behaviour of tension wood in wood-based composites (Roffael and Dix 1988). Since higher amounts of short-rotation plantation hardwoods will be used in the near future, the issue of tension wood in wood-based composites will become of greater importance.

8.4.3 Oriented Strand Boards

OSB is an engineered wood product formed by layering strands or flakes of wood in specific orientations. A decline in plywood manufacturing in many countries, due to limited large log supplies and environmental concerns, has increased production of OSB, making it an effective substitute for plywood (Hiziroglu 2006). Starting in the mid-1980s, the demand for OSB is increasing at a fast rate.

There are hardly any studies that deal with OSB and reaction wood. Stürzenbecher et al. (2010a) have measured linear expansion of OSBs, which was found similar to that of plywood. At higher moisture exposures, juvenile wood had less impact on OSB than on plywood. Water absorption averaged slightly less in all exposure conditions for OSB made from juvenile wood compared to OSB made from mature wood (Geimer et al. 1997). Differences in mechanical properties resulted from varying mass densities, different contents of compression wood and also from different amounts of spiral grain of the four logs used in the study. This trend was most pronounced for the shear properties, leading to the assumption that the higher compression wood content of the strands, having also higher MFA, could have caused these results. Higher MFA in compression wood may have resulted in an increased shear stiffness of the strands, which ultimately affected the shear properties of the actual OSB boards (Stürzenbecher et al. 2010b).

8.4.4 Veneer-Based Products

The oldest known wood composite is plywood. Thousands of years ago Chinese
and Egyptians shaved wood and glued it together to achieve a stiffening effect with
veneered surfaces (Shi and Walker 2006). Commercial plywood started in the
seventieth and eighteenth centuries, as a result of work carried out in England
and France. Typically, early plywood was made from decorative hardwoods and
was most commonly used in furniture such as cabinets, chests, desktops, or doors.
Construction plywood made from softwood species did not appear on the market
until the twentieth century (Shi and Walker 2006). Specific features such as the
spatial distribution of tension wood and dimensional stability are the main quality
factors in veneers (De Boever et al. 2007). As far as reaction wood is concerned
veneer cutting of hardwoods containing tension wood leads to a blunting of the
knives (Noack 1979). Maeglin (1988) reports the combined effects of juvenile
wood and compression wood, which make the processing of lumber and veneer
difficult. The presence of both compression wood and normal wood in plywood has
caused warp. The longitudinal linear expansion of juvenile wood as well as
compression wood veneer was greater than that of normal mature veneer (Geimer
et al. 1997). Also, longitudinal shrinkage was found to be significantly greater in
veneers with cross-grain, in reaction wood, and in juvenile wood, compared to
normal straight-grain wood.

8.4.5 Wood–Polymer Composites

Wood fibres attracted attention as fillers in plastic composites because of their
abundance, renewability, non-abrasiveness, and low density. The use of wood fibres
lowers production costs and has the potential to positively modify mechanical
properties. Instead of widely used oil-based polymers, e.g. polypropylene,
bio-based and biodegradable plastics are increasingly utilized with the intention
of reducing environmental pollution and replacing petroleum-based plastics.
Polylactic acid (PLA) is a biodegradable, aliphatic, and semi-crystalline polyester
produced through direct condensation of its monomer, lactic acid, followed by a
ring opening polymerization of the cyclic lactide dimer (Sedlarik et al. 2007). PLA
shows stiffness and strength properties comparable to oil-based plastics and can be
processed by standard methods such as extrusion, injection moulding,
thermoforming, or compression moulding (Garlotta 2001). Despite these promising
properties, its applicability is restricted by higher production costs, brittleness, and
a low softening temperature. Brittleness can be lowered through incorporation of
plasticizers, while production costs can be reduced and the mechanical performance
improved by the addition of wood fibres. In a study by Gregorova et al. (2009) wood
fibres were prepared with high temperature thermo-mechanical processing (TMP)
from juvenile, mature, and compression wood tissues of Sitka spruce (*Picea*

sitchensis). Wood–plastic composite test samples were prepared through solution casting with subsequent hot-pressing. It was shown that mature fibres improved the Young's modulus of the tested PLA composites by 30 %, while juvenile fibres did so by only 12 %. The most effective reinforcing fibres originated from mature wood and not from compression wood fibres. Mature wood exhibited the highest content of glucose and lowest concentration of lignin. The study shows that variability of the fibre properties needs to be considered when wood fibres are to be used as the reinforcing phase in bio-composite materials. High proportions of compression wood seem to be a disadvantage; however, higher lignin content should improve fibre–matrix bonding (Thielemansa and Wool 2004; Graupner 2008).

8.5 Pulp and Paper

Tension wood from short-rotation *Populus* was found to produce paper of consistently inferior strength properties compared to normal wood of the same tree. It has been suggested that the presence in paper of gelatinous fibres of tension wood of any hardwood species will have this same general effect (Parham et al. 1977). The same is true for compression wood because mechanical pulping of compression wood produces only fibre fragments on grinding (Timell 1986). Likewise, compression wood is unsuitable for sulphite or alkaline pulping as more reagents and energy input are needed to obtain the required removal of lignin. The paper produced has lower tensile strength and higher stretch. In addition, compression wood fibres do not respond as well to beating as fibres coming from normal wood (Timell 1986). In general, for reaction wood pulps the consensus is that low-strength paper made from compression wood is a consequence of fibre morphology, while that from tension wood is more related to wood or pulp chemistry (Parham et al. 1977).

In juvenile parts of the tree it is known that there is a greater abundance of compression wood that is more difficult to pulp or bleach because it contains more lignin (Wadenbäck et al. 2004). Owing to the higher proportion of lignin in the fibre walls, the pulp yield of compression wood is lower compared to normal wood, with darker pulp and overall lower quality (Knigge and Schulz 1966). More obvious differences were observed in mature wood where the compression wood gave 20 % less screened pulp yield than normal wood. The pulp produced from juvenile compression wood had a higher kappa number than pulp produced from normal juvenile wood. Both the compression and normal mature wood had a significantly higher kappa number than juvenile wood.

The physical and chemical characteristics of juvenile and mature fibres obtained from the same black spruce tree (*Picea mariana*) have been shown to differ in their pulping and bleaching responses. Factors that may account for such differences include metals distribution, methoxyl content, lignin functional group characteristics, and wood density. The results demonstrate that juvenile compression wood has

pulping characteristics similar to those of juvenile normal wood, except that its delignification selectivity is slightly lower. On the other hand, mature compression wood exhibited poorer pulping properties such as a lower pulp yield, a higher kappa number, and lower delignification selectivity (Mancosky et al. 2005; Ban et al. 2004).

8.6 Other Wood Parameters

8.6.1 Visual Appearance

Colour and visual appearance are quality attributes that influence perceptions of wood products by customers and therefore affect strongly the commercial value and suitability of a given product (Sandoval-Torres et al. 2010). Severe compression wood appears as thicker darker late wood bands, at least in relative light coloured species; although in darker-coloured species it is not possible to detect compression wood visually. Compression wood is therefore normally not a problem for the visual appearance of wood.

In temperate hardwoods tension wood can have a silvery appearance; whereas in tropical woods dark streaks are sometimes visible. In green sawn timber fibres get pulled-out, resulting in a woolly surface (Lim 1998) after sawing, which in some cases is shinier than surrounding normal wood (Badia et al. 2005). Another aspect of visual appearance is the way in which the surface takes a coat of paint, but this has so far been studied only to a very limited extent. There are reports that compression wood may be difficult to paint with several types of paint, probably due to the higher lignin content.

8.6.2 Durability

A few studies have investigated the natural durability of reaction wood. Greater resistance of compression wood to decay fungi than normal has been reported (Coté et al. 1966; Timell 1986) while tension wood might be more susceptible to some wood destroying fungi than normal wood (Zabel and Morrell 1992). Blanchette et al. (1994) described an investigation of biodegradation of compression wood and tension wood by both white and brown rot fungi. In this study they tested softwood of the species *Abies balsamea*, *P. mariana*, and *Pinus strobus* as well as the hardwood species *Populus temuloides* and *Acer rubrum*. They found that while compression wood was more resistant than normal wood, hyphae were found to be colonizing both the cell lumen and the intercellular spaces whereas they could only be found in the cell lumen of normal wood. For the hardwood samples the amounts

Table 8.1 Property responses of wood and wood-based materials in the presence of reaction wood, compared to reaction-free materials (*MDF* medium density fibreboard, *PB* particle board, *WPC* wood–polymer composite)

Property	Responses compared to "normal wood"		References
	Compression wood	Tension wood	
Mechanical properties			
Cell wall density	Lower	Higher	Terashima et al. (2009)
Wood density	Higher	Higher	Jourez et al. (2001a), Timell (1986)
Young's modulus	Lower	Higher	Gindl (2002)
Bending/tensile strength	Lower	Higher	Saadat-Nia et al. (2011)
Compression strength	Higher, or no change	Lower	Timell (1986), Pechmann (1972)
Shear strength	Higher		Müller et al. (2004)
Embedding strength	Lower	Lower	Wolfe et al. (2000)
Impact resistance		Higher	Saadat-Nia et al. (2011)
Toughness		Higher	Saadat-Nia et al. (2011)
Hardness	Higher	Higher, variable	Timell (1986), Jayme and Harders-Steinhäuser (1953)
Dimensional stability			
Long. shrinkage	Higher	Higher	Johansson (2003), Jourez et al. (2001a, b)
Rad/tang shrinkage	Unchanged or lower	Higher	Clair et al. (2003), Washusen and Ilic (2001), Fang et al. (2007)
Warp, bow, spring	Higher	Higher	Warensjö and Rune (2004)
Workability			
Pinching on sawing	Higher		Timell (1986)
End splitting		Higher	Castéra et al. (1994)
Energy consumption		Higher	Coutand et al. (2004)
Surface smoothness	Higher	Lower (fuzzy)	Timell (1986)
Machining	More difficult	More difficult	Kretschmann and Bendtsen (1992)
Drying/gluing			
Drying stresses		Higher	Tarmian et al. (2009a, b)
Drying speed	Lower		Williams (1971)
End-grain bond strength	Higher	Weaker	Follrich et al. (2008)
Bondline interface	Weaker	Weaker	Frihart (2005)
Heat treatment			
Longitudinal shrinkage	Lower		Dündar et al. (2012)
Dimensional stability	Improved		Dündar et al. (2012)

Table 8.1 (continued)

| Property | Responses compared to "normal wood" | | References |
	Compression wood	Tension wood	
Wood-based composites			
Thickness swell	Lower to slightly higher (MDF)	Higher (PB)	Roffael et al. (2005), Akbulut et al. (2004), Buchholzer (1992)
Dimensional stability	Lower (MDF)		Ayrilmis (2008)
Internal bonding	Higher (MDF)	Lower (PB)	Roffael et al. (2005), Buchholzer (1992)
Water uptake	Lower (MDF) Higher (PB)	Higher (PB)	Roffael et al. (2005) Lehmann and Geimer (1974), Buchholzer (1992)
Shear stiffness	Higher (OSB)		Stürzenbecher et al. (2010b)
Bending strength	Lower (MDF)	Lower (PB)	Roffael et al. (2005), Buchholzer (1992)
Surface hardness	Higher (MDF)		Akbulut and Ayrilmis (2006)
Surface roughness	Higher (MDF)		Akbulut and Ayrilmis (2006)
Warping	Higher (OSB)	Lower	Geimer et al. (1997)
Fibre–matrix bonding	Higher (WPC)		Gregorova et al. (2009)
Pulp/paper			
Water retention	Lower		Roffael et al. (2005)
Fibre beatability	Lower		Timell (1986)
Paper strength	Lower	Lower	Timell (1986), Parham et al. (1977)
Pulp yield	Lower	Higher	Knigge and Schulz (1966)
Kappa number	Higher		Mancosky et al. (2005)
Others			
Visual appearance	Reddish	Silvery, fuzzy	Badia et al. (2005), Lim (1998)
Painting, sanding	Little change	Problematic	Lim (1998), Lutz (1970)
Durability	Higher	No change to lower	Timell (1986), Blanchette et al. (1994)

of degradation were similar in both tension wood and normal wood. In tension wood the degradation took place in the secondary wall layers and in the middle lamellae, while the G-layers were largely undisturbed.

8.7   Conclusions

This chapter shows that reaction wood, whether compression wood in softwoods or tension wood in hardwoods, can be associated with many unsuitable properties of wood and wood products. Table 8.1 summarizes the properties of compression and tension wood in comparison to normal wood. One reason for the varying results found in the different studies might be due to the fact that definitions about occurrence and severity of reaction wood are scarcely documented.

There are other properties of importance but many have never been examined in connection with reaction wood. A few seem to benefit from the presence of reaction wood: the higher smoothness of compression wood surfaces, better shear strength of compression wood, higher toughness and impact resistance when tension wood is present, lower water uptake and swelling in MDF panels containing compression wood, and higher durability against fungi of compression wood samples. However, these are outweighed by the disadvantages, which is the reason why reaction wood has a bad reputation in industry.

Solid wood board consisting of pure reaction wood would not be a problem per se, as it will shrink uniformly. The problem with reaction wood is that it is in most cases mixed with normal wood within a piece of wood, which leads to non-uniform and more variable wood properties. This leads to non-uniform swelling and shrinking, causing distortions, with additional problems of reduced strength and unfavourable surface properties as with tension wood. Wood-based materials such as particle boards or fibreboards are less prone to problems associated with reaction wood than solid wood products. There are opportunities to utilize reaction wood beneficially, but this requires investment in industrial reaction wood recognition techniques, material logistics, and quality control. With new sensor and detection devices such as near-infrared spectroscopic devices (Sykacek et al. 2006) or devices utilizing the "tracheid effect" (Nyström 2002), reaction wood could be rapidly recognized and separated. With knowledge-based production methods the utilization of different wood types, including reaction wood, should be possible.

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Chapter 9
Commercial Implications of Reaction Wood and the Influence of Forest Management

Barry Gardiner, Tom Flatman, and Bernard Thibaut

Abstract In general reaction wood creates problems in timber processing and service whether for solid timber, panel products or pulp and paper production. For example, timber containing reaction wood is more difficult to saw and takes a poorer surface. Compression wood also creates difficulties for papermaking because of its high lignin content although pulp is easier to produce from tension wood because of its lower lignin content. Part of the problems in performance of products incorporating reaction wood arises from the generally inferior mechanical properties of reaction wood compared to normal wood and part of the difficulty arises from the increased variability in wood properties introduced by the presence of reaction wood. Avoiding reaction wood formation in forest trees to reduce the problems in processing requires careful attention to site, species choice and management at all stages of the life of a tree. Although there can be sometimes conflicting evidence for the benefits or otherwise of a particular management option, in general any action that leads to unstable root systems, stem sweep or lean, unbalanced root to shoot biomass allocation, eccentric crowns or increased wind or snow loading will have a tendency to produce reaction wood. The key is for stand management to avoid, whenever possible, large scale changes that result in the requirement for major adaptation of the trees to new growing conditions.

B. Gardiner (✉)
Forest Research, Northern Research Station, EH25 9SY Roslin, Midlothian, UK

INRA, UR1263 EPHYSE, 33140 Villenave D'Ornon, France
e-mail: barry.gardiner@bordeaux.inra.fr

T. Flatman
Forest Research, Northern Research Station, EH25 9SY Roslin, Midlothian, UK

Biological & Environmental Sciences, University of Stirling, Stirling, Scotland, UK

B. Thibaut
Laboratoire de Mécanique et Génie Civil (LMGC), CNRS, Université Montpellier 2, Place
E. Bataillon, cc 048, 34095 Montpellier cedex 5, France

9.1 Introduction

Although a lot of work has been carried out on the detailed structure of reaction wood, understanding the influence of forest management operations on reaction wood formation and subsequent product performance is not straightforward. The reasons for this are the complex interactions between site, environment, genetics and tree history. Forest management can modify each of these factors through choice of planting stock and species, use of natural regeneration, cultivation methods, thinning or pruning. In this chapter we will attempt to unpick the consequences of different management actions on the location and level of reaction wood formation within trees.

The difficulty with forecasting the impacts of management on reaction wood formation is that anything that leads to stem lean, stem sinuosity, increased branch size, or increased wind and snow loading will tend to increase the level of reaction wood. Predicting the exact location of the reaction wood within a tree or a log is then very dependent on knowledge of the past history of the tree. Even then it is difficult because the exact mechanisms of reaction wood formation are not fully understood. Often there is little information on the past management, storm events or insect attacks associated with a stand, and predictions of levels of reaction wood can only be based on stand and tree conditions at harvest or the time of observation. Information such as tree lean, sinuosity, local slope and local competition can give some idea of levels of compression wood (Achim et al. 2006), although the relationships are usually weak, but tree morphology does provide an indication of the probable location of reaction wood, especially towards the base of trees. However, when dealing with logs there is usually little information on the growing conditions of the trees, and the orientation and shape of the tree is lost when the logs are cut, and it becomes much more difficult to predict the location of reaction wood from log external characteristics. Therefore, it is necessary to utilise systems such as X-ray scanners (Longuetaud et al. 2005), which can scan internal log characteristics including knot size and location, and pith eccentricity and sinuosity in order to predict probable reaction wood location.

Understanding the implications of forest management on the wood products themselves such as sawn timber, panel products, and pulp and paper has by necessity to be rather generic and "broad-brush". Partly this is because, as was discussed above, linking the history of a tree to the specific location and level of reaction wood within the stem or subsequent logs is difficult, and partly it is due to the impact that different levels and distributions of reaction wood have on product performance. For example, a uniform distribution of mild compression wood throughout a piece of sawn timber will have little impact on distortion, whereas compression wood on only one side of a batten could lead to serious spring or bow. In sawn timber the distribution of the reaction wood can be critical, whereas in panel products and pulp and paper the overall level within the mix is the most important factor.

9.2   Biological Reasons for Reaction Wood in Trees

Before beginning our discussion of the influence of forest management it is useful to remind ourselves why reaction wood forms in the first place. Walker (2006) states that the function of compression wood is to "act to correct the lean of the stem" and Low (1964) states that its purpose is to "oppose forces deforming the tree and to restore or maintain a specific pattern among tree parts". Trees make use of reaction wood to orientate their stems and branches in order to position their photosynthetically active components in the most advantageous manner. Wardrop (1964), as quoted in Barnett and Jeronimidis (2003), states that "woody stems have ceased elongation growth and must correct their orientation by bending the existing stem". In gymnosperms the primary agent is compression wood, which normally forms on the lower side of stems and branches, and in angiosperms the primary agent is tension wood, which in contrast normally forms on the upper side of branches and stems (Bowyer et al. 2007) (see Fig. 9.1). This arrangement may change if the tree is actively seeking light because it is being shaded, but normally compression wood is on the convex side and tension wood on the concave side of the bent section of stem or branch.

For each tree the level of reaction wood within the stem will be a function of its history and its location within the stand. The level of wind and snow loading, the steepness of slope and the influence of neighbouring competitors will all influence the response that individual trees make in order to maintain vertical alignment and to position their crowns in order to access light. Smaller sub-dominant trees may be forced to react to competition by aligning the stem to gaps in the canopy, which may result in poorer stem form and increased reaction wood. Larger dominant trees may be more exposed to wind loading and respond by producing reaction wood in order to ensure a vertical form. Dominant trees are also susceptible to loss of parts of the crown or leader and the subsequent requirement for branches to take over apical dominance and reorient themselves to the vertical by the production of large quantities of reaction wood (see Figure 8 in Longuetaud et al. 2005 for an excellent example). Therefore, the level and location of reaction wood within every log from a stand will be the result of complex interactions over the life of the tree and means it will only be possible to ever make generic comments about the influence of management.

A detailed explanation of the formation, structure and biological function of reaction wood is provided in Chaps. 3–5 of this book.

9.3   Implications for Commercial Exploitation

Even though reaction wood has important biological benefits for a tree the result is wood that is generally less desirable than normal wood for commercial exploitation. Some of the drawbacks of reaction wood are due to the inherent differences in its

Fig. 9.1 Tension wood on the upper side of a leaning chestnut (*Castanea sativa*) stem (photograph courtesy of Bruno Clair, Université de Montpellier 2, France)

properties and others are due to the variation introduced when reaction wood and normal wood occur in the same piece of timber.

Compression wood has higher specific gravity, a lower fibre saturation point, reduced permeability, lower radial and tangential shrinkage, but substantially larger longitudinal shrinkage than normal wood. It also has higher compressive strength but lower tensile strength, a lower modulus of elasticity and is more brittle in fracture than normal wood (Ni Dhubhain et al. 1988). Tension wood has less strength and has greater longitudinal shrinkage than normal wood, machines less well and has a greater tendency for its fibres to collapse (Bowyer et al. 2007). For a comprehensive description of the physical and mechanical properties of reaction wood please refer to Chap. 6.

The higher lignin content of compression wood causes problems in papermaking if the amount of compression wood is above approximately 15 %, producing darker pulp with short fibres that result in reduced yield and poorer strength properties (Ladell et al. 1968). Terziev et al. (2008) showed that paper made with fibres from compression wood had a reduced tear index because of the increased number of dislocations in the fibres. In addition, the shorter fibres of compression wood tend to lead to a reduced tensile and tear strength (Kellogg and Thykeson 1975; Fuglem et al. 2003).

In contrast, pulp made from tension wood is easier to produce from mechanical pulping as it has lower lignin content and it also produces a proportionally large quantity of chemical pulp. However, tension wood pulp contains many deformations in the fibres because of the lack of lignin (Ander and Nyholm 2000) and these produce weaknesses in the subsequent paper with reduced tear and tensile strength.

In addition the presence of tension wood can lead to a "woolly" appearance of the paper.

Compression wood can cause problems when sawing timber. In particular band saws can have severe problems with compression wood with the saw becoming pinched or the wood being thrown out with great force on the saw reaching the compression wood (Timell 1986). Tension wood is also difficult to cut because the fibres tend to be torn out rather than being cut leading to a "woolly" surface (BRE 1972). The presence of tension wood has also been found to lead to blunting of saws and to severe splitting in logs (Hughes 1965).

The major problem caused by compression and tension wood in sawn timber is distortion during drying (Bowyer et al. 2007; BRE 1972). In particular the presence of reaction wood can lead to high levels of bow and spring because of the higher longitudinal shrinkage of reaction wood compared to normal wood (typically 0.5–1 % compared to 0.1 % in normal wood). The reason is the high microfibril angles in compression wood but the reason for the increased shrinkage in tension wood is much less certain. One recent theory is that it is due to buckling of the microfibrils during drying (Clair et al. 2006). If the distribution of reaction wood across a piece of timber is not uniform one side of the timber can shrink much more than the other leading to bow or spring (Fig. 9.2). Twist on the other hand is not affected by the presence of compression wood and is much more a function of grain angle and ring curvature (Warensjö 2003). In addition to warping the increased shrinkage of reaction wood can lead to reduced accuracy in sawn timber dimensions (Hallock and Jaeger 1964) and it also leads to increased occurrence of cracks and checking in dried timber.

The presence of compression wood has serious consequences for the performance of sawn timber in service. Although compression wood reduces the stiffness and strength of wood the most important consequence is the reduced toughness and increased tendency for brittle failure (Mochan and Hubert 2005). This failure can be abrupt and potentially catastrophic (Fig. 9.3). The impact of tension wood on the strength properties of hardwood timber is less clear and the results are often more contradictory. In general tension wood seems to be tougher than normal wood but weaker in most measures of strength and is particularly true of compression strength parallel to the grain (Bowyer et al. 2007). However, as always appears to be the case with tension wood it is difficult to generalise because there are often exceptions.

For a comprehensive review of the impact of reaction wood performance of sawn wood and other wood-based products see Chap. 8 in this book.

Fig. 9.2 Severe bow due to compression wood (photograph courtesy of Mats Warensjö, SLU, Sweden)

Fig. 9.3 Brash failure in lodgepole pine (*Pinus contorta* Douglas) due to the presence of compression wood (reproduced with permission from Mochan and Hubert 2005 and covered by Crown copyright)

9.4   Review of Site and Management Influences on Reaction Wood Formation

9.4.1   Site Influence

The influence of site on the formation of reaction wood is a complex interaction of factors that leads to deviation of the stem from vertical and the subsequent production of reaction wood to return the stem to the vertical. Any factor that leads to stem lean will encourage the formation of reaction wood. In general the individual position of a tree relative to its neighbours is more important than the overall condition of the site (Low 1964; Hughes 1965) so that trees which are under severe competition will produce considerable amounts of reaction wood as they seek access to light.

9.4.1.1   Climate

The most important single climatic influence on reaction wood formation in trees is wind, although snow loading can also be important. In numerous references (summarised by Low 1964) the importance of wind direction in determining the location and extent of compression wood formation is emphasised (see also Larson 1965; Nicholls 1982; Watt et al. 2005). Because wind has an impact on trees throughout their lives, wind can have an influence on trees of all ages. For example, in young trees wind can induce toppling (Moore et al. 2008), which leads to reaction wood formation as the root system is rotated and the trees subsequently attempt to right themselves (Fig. 9.4). The tendency for young trees to topple can be due to poor rooting because of saturated soils, physical barriers (stones, iron pans, etc.), or an unbalanced crown to root growth particularly in fast-growing species [e.g. poplar (*Populus* sp.), maritime pine (*Pinus pinaster* Aiton) and Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco)]. In older trees wind can lead to compression wood formation in order to right trees that have been partially uprooted (Mochan 2002) or where a lateral shoot replaces a damaged or broken leader (see Fig. 9.13 and Sinnott 1952). The recent research of Dunker and Spieker (2008) arrives at the same conclusions and shows that except in the most sheltered locations the direction of the prevailing wind is much more important for determining the location of compression wood formation than slope direction (Fig. 9.5). Wind has also been found to influence tree lean in hardwoods and to lead to tension wood formation (Sorensen and Wilson 1964).

The impact of snow damage and snow loading on the production of compression wood in trees has been extensively reviewed by Timell (1986). The damage includes bending small trees past their elastic limit (Fig. 9.6), from which excessive sweep will occur (Del Río et al. 2004), displacement by avalanching (Fig. 9.7), branch breakage and leader damage.

Fig. 9.4 Toppling in fast-growing young Douglas fir (photograph courtesy of John Moore, SCION, New Zealand)
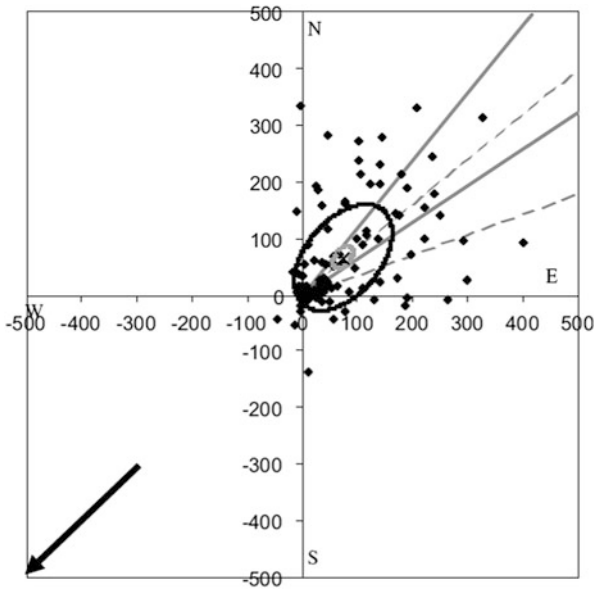

Fig. 9.5 Influence of wind direction on compression wood formation. Standard ellipse (*black*) and confidence ellipse (*grey*) for the compression wood distribution in the stem cross-sections at a steep south-western slope with prevailing south-westerly winds (upslope). The *arrow* indicates the downhill direction of the slope (reproduced with permission from Dunker and Spieker 2008)

9.4.1.2 Slope

The effect of slopes is mainly in the influence the slope has on the inclination of the trees (Plomion et al. 2001). This can be as a result of snow loading, or landslides (Timell 1986), or the response of trees seeking light. Snow loading and landslides

Fig. 9.6 Young radiata pine (*Pinus radiata*) in the Spanish Basque Country bent by wet snow (photograph B. Gardiner)

Fig. 9.7 Mountain pine (*Pinus mugo*) bent by snow loading and minor avalanches in the Pyrenees (photograph B. Gardiner)

can cause significant basal sweep, often with overcorrection higher up the tree producing a sinusoidal shape. For trees seeking the light there is a theoretical benefit of growing at an angle from the vertical away from the slope (Fig. 9.8 and Ishii and Higashi 1997). In a series of experiments in the west of Scotland the local slope at the tree location was found to be correlated (Fig. 9.9), albeit weakly, with the percentage of compression wood found in the tree (Achim et al. 2006). However, as found by Dunker and Spieker (2008) the effect of slope lean can be overcome if the prevailing wind is up the slope.

The role of tension wood in righting leaning trees on slopes is less clear and there is evidence that tension wood formation, eccentric growth and growth stresses can all contribute to stem righting (Wilson and Gartner 1996). However, there does seem to be a correlation between degree of lean and the steepness of the slope in hardwoods (Hibbs et al. 1994; Wiemann et al. 2004).

Fig. 9.8 Larch (*Larix decidua*) growing at an angle away from a steep slope in the Alps (photograph courtesy of Marco Fiorovanti, Università degli Studi di Firenze, Italy)
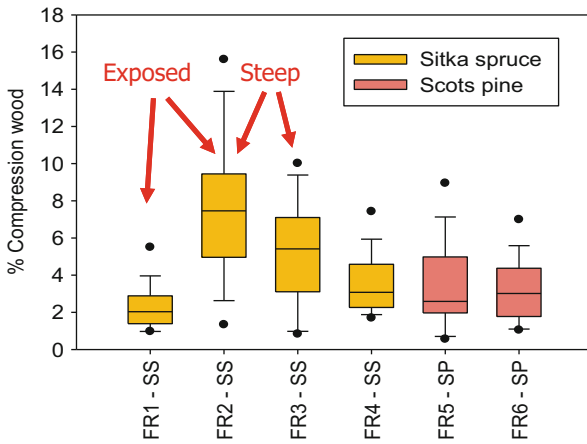

Fig. 9.9 Percentage of compression wood in Sitka spruce (*Picea sitchensis* (Bong.) Carr.) (FR1–FR4) and Scots pine (*Pinus sylvestris* L.) (FR5–FR6) on sites with different levels of wind exposure and slope steepness (from Achim et al. 2006). At site FR1 the prevailing wind was up the slope and counteracted the slope effect as found also by Dunker and Spieker (2008). Both the Scots pine sites were sheltered and relatively flat

Fig. 9.10 Scots pine
demonstrating sinusoidal
growth (photograph
courtesy of Shaun Mochan,
Forest Research, Roslin,
United Kingdom)

9.4.1.3 Fertility and Nutrient Status

Site fertility affects tree growth rate and the ratio of crown to shoot weights. On very nutritious sites it is possible to get a serious imbalance between crown and shoot that causes young trees to topple (Moore et al. 2008) in even moderate winds. Recovery from this toppling inevitably leads to sinuosity (Fig. 9.10) in the stem and production of reaction wood (Rune and Warensjö 2002). There is conflicting evidence as to whether trees tend to have better form on sites with good or poor nutrition. The evidence seems to be that on nutritious sites pines have poorer form, and tend to be forked and have heavy branches (Cown and McConkie 1981). However, other researchers have found that if there is damage to the leader then the replacement by a side branch (Archer 2000) on poor sites is slower than on nutritious sites leading to higher levels of compression wood formation (Westing 1965). Furthermore, on sites with higher growth rates branches are further apart giving lower levels of knottiness and associated reaction wood, although the branches themselves may be individually larger. However, on extremely nutritious sites trees may grow so fast that the stem growth becomes sinuous because the trees are unable to maintain good stem form (Downes et al. 1994; Spicer et al. 2000). Also on sites with high levels of nitrogen apical dominance can be lost with side branches competing with the leader and the formation of high levels of reaction wood on the side branches.

   On very nutritious sites with rapid growth there can be a large percentage (up to 80 %) of the stem cross-section occupied by juvenile wood (Bendtsen 1978; Bendtsen and Senft 1986) and juvenile wood is known to have higher levels of reaction wood than mature wood (Zobel 1981). It is also possible that the high levels of reaction wood found in rapidly growing trees even when the tree is

growing straight (Isebrands and Bensend 1972; Isebrands and Parham 1974; Crist et al. 1977) are caused by high auxin levels (Timell 1986; Bowyer et al. 2007).

The absence of certain key nutrients such as boron and copper can lead to extremely poor stem form, loss of apical dominance or even death of the leader (Harper 1989; Turvey and Grant 1990; Stone 1990). The inevitable result is increased reaction wood formation as stems straighten or side branches take over apical dominance.

9.4.2 Silvicultural Influence

9.4.2.1 Establishment

Probably the single most important silvicultural action in determining the levels of reaction wood in trees is establishment. Poor establishment, poor planting techniques or establishment on sites with poor rooting potential will almost inevitably lead to trees that lean when young, giving rise to basal sweep and subsequent reaction wood formation (Fig. 9.11). In some cases, such as when trees are planted using planting pots, rooting can be severely restricted leading to extreme deformation of the base of the tree and high levels of reaction wood in the trees as they grow (Rune and Warensjö 2002). On wet sites where rooting may be restricted toppling of young trees because of insufficient anchorage may occur with the same problems as identified in Sect. 9.4.1.1 (Low 1964; Mochan and Hubert 2005). The result is trees with large basal sweep and large volumes of reaction wood, particularly in the lower stem (Fig. 9.12).

The type of cultivation is also important and those that produce uneven root development and lead to instability (Nicoll et al. 2006) are also likely to cause problems with partial uprooting or toppling and subsequent compression wood formation (Mochan 2002). Stem lean has been found to be weakly correlated to amount of compression wood (Achim et al. 2006) but is a poor predictor of levels of compression wood because trees that have straightened following early instability may have substantial levels of reaction wood (Warensjö and Rune 2004).

9.4.2.2 Fertilisation

The impact of fertilisation on reaction wood formation is unclear based on the published studies. Although rapid growth rates are associated with increased levels of compression wood (Timell 1986) and much of the impacts of fertilisation are likely to be similar to that of growing trees on nutrient rich sites, there is conflicting evidence concerning the impact of fertilisation on the formation of compression wood. Barclay et al. (1982), Cown (1978, personal communication) as reported by Timell (1986), and Taira and Yasuda (1986) found no evidence that fertilisation led to increased compression wood formation whereas Livingston et al. (2004) and
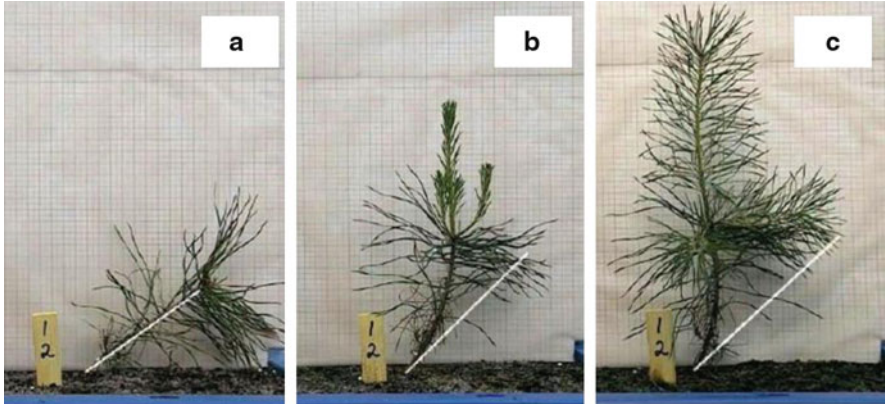
Fig. 9.11 Development of basal sweep in Scots pine. The seedling was planted in a box at an angle of 45° from the vertical and was grown for 30 days in a greenhouse. Image a=0 days, b=15 days and c=30 days after planting (reproduced with permission from Rune 2003)

Fig. 9.12 Compression wood formed in a Scots pine with large basal sweep (photograph courtesy of Mats Warensjö, SLU, Sweden)

Cameron and Watson (1999) found evidence that increased growth rates and fertiliser application may lead to higher levels (Table 9.1). Walker (2006) found that vigorous growth could lead to compression wood forming a ring around the stem. However, Jaakkola et al. (2007) found only slightly increased lignin levels

Table 9.1 Influence of nutrition in compression wood formation in Sitka spruce (reproduced with permission from Table 7, Cameron and Watson 1999). Standard errors are in parenthesis

Treatment	Whole disc density (g/cm^3)	Whole-disc ring width (mm)	Whole-tree compression wood content (%)
Fertilised regularly	0.406 (0.0094)	3.32 (0.227)	17.83 (1.314)
Fertilised periodically	0.416 (0.0129)	2.78 (0.191)	19.14 (1.706)
In nursing mixture with lodgepole pine	0.395 (0.0092)	2.81 (0.191)	16.66 (1.513)
In nursing mixture with hybrid larch	0.411 (0.0122)	3.19 (0.185)	14.41 (1.513)

(often associated with compression wood) in fertilised Norway spruce [*Picea abies* (L.) H. Karst] compared to unfertilised trees, but they did not specifically identify the existence of compression wood.

The likelihood is that application of fertiliser will have different impacts under different circumstances. On nutrient poor sites where trees are struggling there may be no impact from fertilisation application because the trees will then be growing at a sustainable rate, whereas on richer sites application of fertiliser may increase the growth rate so much as to cause leader loss with its associated problems (Baldwin 1993). The use of fertiliser may be a particular problem where trees are susceptible to leader loss because of windy conditions or where they are susceptible to toppling because soils are shallow or the water table restricts root development (Valinger 1990). On very dry sites fertiliser application may also cause leader damage (Fielding 1967), again producing an increased likelihood of compression wood formation.

There appears to have been no work on the impact of fertiliser application on tension wood formation in hardwoods.

9.4.2.3 Spacing

Initial spacing and planting pattern can have important consequences for tree growth, tree form and crown development, which are all important in reaction wood formation. In general at wider spacings trees grow faster, have larger crowns, self-prune more slowly, have larger branches and knots and are more likely to be swept and crooked (Low 1964; Timell 1986). The rougher form of trees grown at wider spacings is well known for conifers, in particular the increased size of branches (e.g. Moore et al. 2009) which leads to increased compression wood formation (Fig. 9.13) because knots are always surrounded by compression wood (Timell 1986).

Uneven spacing (e.g. rectangular spacing) or growing a species in mixture with a nurse species (Watson and Cameron 1995; Berry 1965) can lead to unbalanced crowns due to the asymmetry of light levels and also to stem lean as the trees attempt to maximise their individual light availability (Büsgen et al. 1929). This mechanical imbalance results in reaction wood formation in order to maintain the position of the crown and stem (Fourcaud et al. 2003).

Fig. 9.13 Compression wood formed around knots and a previously damaged leader in Sitka spruce (photograph B. Gardiner)

9.4.2.4 Thinning

Thinning potentially provides an opportunity to remove trees with high levels of compression wood and to improve the quality of the remaining crop. However, it also opens up the canopy which may lead to the formation of unbalanced crowns and to stem lean. As Jacobs (1937) noted many years ago the most important factor for determining the level of reaction wood within a tree is the individual location of the tree in relation to its neighbours. Unbalancing the competition surrounding a tree can lead to reaction wood formation as the trees change their orientation in order to obtain access to the increased light. A very detailed analysis of the impact of thinning on compression wood formation and other wood characteristics is provided in Timell (1986).

However, the results obtained from thinning trials are sometimes contradictory. Early work suggested that thinning, because it inevitably led to increased growth, crown and branch size and wind loading, encouraged the formation of reaction wood. This is believed to be the case for both compression wood (Larson 1972; Cown 1973, 1974) and tension wood (Hughes 1965). However, more recent work by Cameron and Thomas (2008) found compression wood to be lower in selectively thinned Corsican pine (*Pinus nigra* L.) than in unthinned pine on the same site. The differences may be due to differences in the wind exposure of the stand or the timing and intensity of thinning. On sheltered sites such as those studied by Cameron and Thomas (2008) careful thinning may be beneficial because the increased wind loading is unimportant and it permits crowns to become better balanced. On windier sites the increased wind loading after thinning may lead to high levels of stem bending and on highly nutritious sites the stem may be bent by vigorous crown growth. However, it is not possible to be absolutely certain about the impact of thinning, because the strong interaction between tree growth, light availability, wind loading and the individual growing conditions of each tree means that site and silvicultural factors can sometimes interact in unexpected ways in the formation of reaction wood.

9.4.2.5 Pruning

Pruning is used to remove branches from the stem and to produce higher quality clear-wood. Because of the association of knots and branches with compression wood pruning inevitably leads to a reduction in reaction wood levels. Pruning also reduces the juvenile core area, which is again associated with compression wood formation (Timell 1986) and stem taper. The only major restriction to the widespread use of pruning is the high associated costs (e.g. Curry and Endersby 1965) and, therefore, pruning is only an option in trees with a high final value. In contrast to conifers little has been written on the impacts of pruning on reaction wood formation in hardwoods.

9.4.3 Genetic Influence

9.4.3.1 Choice of Provenance

Provenance differences can be found in tree susceptibility to toppling, leaning, sinuosity and response to reaction wood formation. Probably the most comprehensive recent study of provenance differences is by Sierra-de-Grado et al. (2008) who looked at stem straightness in maritime pine from different parts of Spain. One of the key observations was that different provenances appeared to have different levels of effectiveness of the compression wood that formed in response to leaning. Some provenances became much straighter with time compared to others but seemed to do so by laying down very similar levels of compression wood. Initial selection based on younger trees would not have favoured the straighter provenances because of their poor early form and would have missed their effectiveness in straightening.

Any provenance that tends to be more prone to the type of growth that leads to reaction wood formation such as leader loss, toppling, sinuous growth (Downes, et al. 1994), heavy branching or loss of apical dominance will also tend to produce larger quantities of reaction wood. For example, in lodgepole pine (*Pinus contorta* Douglas) some provenances are very prone to early partial toppling leading to large basal sweep (Fig. 9.14) and large volumes of compression wood (Mochan and Hubert 2005).

Work in Australia has shown that there can be significant differences in the impact of tension wood in terms of transverse shrinkage between different provenances of Eucalyptus, even though there were no significant differences in the amount of tension wood (Washusen and Ilic 2001).

Fig. 9.14 Basal sweep in South Coastal (Longbeach) provenance of lodgepole pine (reproduced with permission from Mochan and Hubert 2005 and covered by Crown copyright)

9.4.3.2   Choice of Progeny

Many of the characteristics that can lead to increased levels of reaction wood formation are relatively heritable. Timell (1986) discussed these for compression wood in great detail. Other factors that could mitigate the impact of reaction wood may also be heritable, such as longitudinal shrinkage (e.g. Koshy and Lester 1994 found moderate heritability of the longitudinal shrinkage in the first few rings of Douglas fir) and fibre length (e.g. Gonzalez and Fisher 1998), so that it may be possible to breed for fibre characteristics that reduce the impact of reaction wood in service.

Separating the importance of direct genetic control of reaction wood formation and genetic control of the factors that predicate the formation of reaction wood such as tree lean or sinuosity is difficult. For example, trees with rapid growth also tend to have more reaction wood (see Sects. 9.4.1.3 and 9.4.2.2 above). Therefore, working out whether increased reaction wood is due to the improved growth of a fast-growing progeny or to its genetic susceptibility to reaction wood formation is difficult. Livingston et al. (2004) found that in faster growing progeny of Sitka spruce [*Picea sitchensis* (Bong.) Carr.] there were larger branches, less latewood and more compression wood in comparison with the control and the slow-growing progenies. On the other hand, trees from the fast-growing progenies had a smaller grain angle, which reduces the tendency of timber to twist. Timell (1986) quotes Shelbourne (1966) and Shelbourne et al. (1969) as suggesting there is a strong

genetic control for compression wood. There is also more recent clear evidence for the genetic control of key wood properties in angiosperms (e.g. Yu et al. 2001) and gymnosperms (e.g. Zubizarreta Gerendiain et al. 2009). However, the focus has been almost exclusively on characteristics such as fibre properties (length, cell wall thickness, etc.) and wood density and there is little field experimental measurement of the genetic control of reaction wood formation.

9.4.3.3   Clonal Material

The evidence for a genetic component to reaction wood formation is strong and, therefore, there is potential for using clonal material to minimise the production of reaction wood while retaining other features of benefit for timber or pulp production. It might also be possible to minimise other factors that can lead to reaction wood formation such as basal sweep, lean, sinuosity and heavy branching. However, it needs to be remembered that wood properties are a combination of genetic factors and the local environment, and although wood properties (e.g. wood density, fibre length) seem to be under stronger genetic control than growth properties (e.g. tree height, diameter, taper) there is still a strong influence of the local competition from surrounding trees (Zubizarreta Gerendiain et al. 2009). Therefore, selecting clonal material with superior performance regarding reaction wood formation will not totally compensate for the influence of the site and silviculture. All the benefits of clonal material can be lost if such material is used in an environmentally challenging location or is poorly managed.

9.4.3.4   Transgenic Material

Recent research has begun to unravel the mechanism of hormonal control of reaction wood formation and the genes involved in controlling its formation (see Chaps. 3 and 4). Since plant growth hormones such as auxins, gibberellins, cytokinins or abscisic acid (Herschbach and Kopriva 2002) also influence wood formation (Little and Savidge 1987) there has been considerable interest in the genes controlling the operation of these hormones. Particular attention has been paid to genetic control in aspen and poplars (e.g. Tuominen et al. 2000; Hu et al. 1999; Sterky et al. 1998) because of the potential to influence the fibre properties of importance for the paper making industries. Less work has been carried out on the genetic control of compression wood formation in conifers but this is beginning to change with increasing interest in controlling its formation (e.g. Chen et al. 2007).

9.5   Management to Reduce Reaction Wood in Trees

The management of trees and forests to produce timber or pulp with low levels of reaction wood follows the same general rules as producing good quality timber. These can be broken down as follows:

1. Site selection: Understanding the nature of the site and its probable impact on tree growth and form is critical. Trying to grow species on sites for which they are unsuitable is likely to lead to problems with tree growth and wood quality. The underlying characteristics of the site such as lithology, soil type and climate (e.g. accumulated temperature, moisture deficit, exposure, etc.) cannot be modified. However, soil nutrient status and moisture level can be changed through application of fertiliser and by cultivation, respectively. There are a number of systems available for identifying the existing nutrient and moisture status of a site using indicator plant species (e.g. Pyatt et al. 2001).

2. Species choice: Choosing the appropriate species for a particular site is based on a thorough knowledge of the site (see above) and the objectives for the site. Species which are best suited for a site may not be the most appropriate to choose because of generally poorer growth rates or timber quality than another species that is not ideally suited to the site. In addition the provenance and progeny of the selected material needs to be appropriate to the site (see Sects. 9.4.3.1 and 9.4.3.2) and tree breeders place a lot of emphasis on selecting the most suitable material for particular areas of a country. See, for example, Samuel (2007) and Fletcher and Samuel (2010) for the choice of planting material for Sitka spruce and Douglas fir, respectively, in the UK.

3. Establishment: Poor establishment is one of the key reasons for trees developing reaction wood (see Sect. 9.4.2.1 above). Trees that are carefully handled, well planted in a suitably prepared site (see, e.g., Paterson and Mason 1999), with adequate nutrition and freed of weed competition are more likely to establish quickly, have rapid early growth, develop stable root architecture and have a balanced biomass allocation between roots and shoots. The quality of the planted material is also crucial in order to avoid seedlings with swept stems or poorly developed root systems.

4. Management: As discussed above anything that leads to stem lean, stem sinuosity, increased knottiness, increased wind/snow loading or increased tree sway will tend to increase the level of reaction wood. The use of shelterwood systems for protecting young trees from the wind may be appropriate for certain species and in addition planting systems that produce large crown asymmetry (Watson and Cameron 1995) should be avoided. Careful and well-timed thinning is also required with a focus on selecting trees with the best form but at the same time avoiding the creation of large crown asymmetry or a large increase in wind exposure (Macdonald et al. 2010). Even so, it is impossible to totally avoid reaction wood formation and good management can only reduce the chance and severity of its formation.

A large number of textbooks are available on tree response to the environment and silvicultural practice for trees in both temperate and tropical forest (e.g. Büsgen et al. 1929; Dengler et al. 1990; Savill and Evans 1986; Smith and Read 1997; Evans and Turnbull 2004). These books all discuss the implications of different management treatments on wood quality. There are also numerous papers that discuss the implications of different forest management practices on the wood quality of specific commercial species (e.g. Macdonald and Hubert 2002; Macdonald et al. 2010; Seeling 2001; Guilley et al. 2004; Väisänen et al. 1989; Gonçalves et al. 2004; Houllier et al. 1995; Maguire et al. 1991; Bhat 2000; Zobel 1992).

A number of suggestions have been made in the past for modifying the way timber is cut to reduce the impact of reaction wood or to be treated chemically to reduce warping (e.g. Schwegmann 1964; King 1954; Konïg 1957; Hallock 1969). These systems have not been adopted, probably due to the increased costs and the problems of implementing such systems into the rapid throughput of modern sawmills. In contrast there are cases where industrial processors have stopped buying logs with too high a value of pith eccentricity because this was found to be correlated with increased reaction wood formation and plywood instability. Easily identifying logs containing reaction wood using readily accessible methods such as log geometry have proved difficult (Warensjö et al. 2002), but modern techniques such as X-ray scanning and colour scanning may offer improved possibilities in the future (Bowyer et al. 2007; Nyström and Kline 2000). A more detailed discussion of the detection of compression wood is presented in Chap. 7.

9.6   Commercial Benefits

Compression wood has been occasionally seen as beneficial in some small-scale commercial applications. Examples in the past have been the use of compression wood in sledge runners and skis (Mork 1928), in bows and crossbows (Wegelius 1939), the underside of boats and ships (Knuchel 1940) and in roof shingles (Timell 1986). Tension wood has benefits in the production of certain pulps (mechanical and dissolving pulps) because of the low lignin and high cellulose levels and this is discussed in more detail in Chap. 8. However, all types of reaction wood are generally seen as only creating problems in solid and laminated timber products.

9.7   Summary

Understanding the impact of forest management on reaction wood formation in trees is a complex and difficult process. This difficulty is common to understanding any aspect of growth and wood formation in trees because of the interactions between climate, site and genetics and the interactions between competing individuals within the forest or stand. These interactions make it difficult to give anything

other than generic observations regarding how forest management affects reaction wood formation in trees.

In general any action that leads to unstable root systems, stem sweep or lean, unbalanced root to shoot biomass allocation, eccentric crowns or increased wind or snow loading is liable to produce increased reaction wood. This includes the selection of genotypes with a propensity to poor form, poor rooting, heavy crowns and so on. The reason is that if there is any loss of stem straightness or any factor causing a tree stem to lean the tree can only correct this by forming reaction wood to straighten the stem and reassert apical dominance.

The difficulty for forest managers, timber buyers and timber processors is that it is extremely difficult to be sure which trees will have the greatest levels of reaction wood except in very extreme cases of lean, basal sweep or sinuosity. Even extremely straight trees can contain substantial reaction wood and their straightness may even be a testament to the effectiveness of the reaction wood. The same has been found for growth stresses (which can lead to log splitting after felling) where leaning trees have often been found to have lower levels than straight trees. The key for managers is to manage their stands "subtly", to not enforce large scale changes (e.g. heavy nutrition, deep cultivation, heavy or uneven thinning), and to remove during thinning those trees with the highest probability of containing reaction wood whenever possible. Once the trees are cut into logs it is very difficult to determine which are the most likely to contain reaction wood because much of the information regarding tree shape and lean is lost. The main hope for detecting reaction wood prior to processing is through new technologies such as X-ray scanning.

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