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DARWIN REVIEW

Tensional stress generation in gelatinous fibres: a review and possible mechanism based on cell-wall structure and composition

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Abstract

Gelatinous fibres are specialized fibres, distinguished by the presence of an inner, gelatinous cell-wall layer. In recent years, they have attracted increasing interest since their walls have a desirable chemical composition (low lignin, low pentosan, and high cellulose contents) for applications such as saccharification and biofuel production, and they have interesting mechanical properties, being capable of generating high tensional stress. However, the unique character of gelatinous layer has not yet been widely recognized. The first part of this review presents a model of gelatinous-fibre organization and stresses the unique character of the gelatinous layer as a separate type of cell-wall layer, different from either primary or secondary wall layers. The second part discusses major current models of tensional stress generation by these fibres and presents a novel unifying model based on recent advances in knowledge of gelatinous wall structure. Understanding this mechanism could potentially lead to novel biomimetic developments in material sciences.

Occurrence of gelatinous fibres

Gelatinous fibres are specialized sclerenchyma cells, characterized by their elongated shape and the presence of an inner cell-wall layer that exhibits gel-like shrinkage during drying (Clair *et al.*, 2008), looks more or less transparent in many types of histological preparations, and hence was called 'the gelatinous layer'. The weak interaction of this layer with most histological stains is explained by its high content of crystalline cellulose, which is not very reactive and thus remains unstained.

Gelatinous fibres are found in various plant organs, including thorns, tendrils, contractile roots, corms, peduncles, and stems. They occur in phloem and xylem of both primary and secondary origin, and sometimes in non-vascular tissues (Zimmermann *et al.*, 1968; Jourez, 1997; Tomlinson, 2003; Gorshkova and Morvan, 2006; Toghraie *et al.*, 2006; Fisher, 2008; Bowling and Vaughn, 2009). They may form either the bulk of a tissue, as in tension wood, be grouped in bundles, or sometimes even occur singly among other plant cells. They have important functions, since they can generate high tensional stress within mature organs, thus either enabling the movement of these organs or reinforcing their structure and stability (Yoshida *et al.*, 2002; Clair *et al.*, 2003; Fang *et al.*, 2008; Fisher, 2008; Abasolo *et al.*, 2009). The best known examples of extraxylary gelatinous fibres are those in major fibre crop plants, such as flax, hemp, and ramie. Although tension has never been directly measured in such fibres, the basic wall structure, composition, and function of these cells give reason to group them together with tension wood fibres (Gorshkova and Morvan, 2006; Gorshkova *et al.*, 2010).

The efficacy of gelatinous fibres can be quite remarkable; in some plant species they can pull entire shoots underground, where they can survive adverse conditions such as freezing temperatures or fires (Fisher, 2008; Schreiber *et al.*, 2010), and the gelatinous fibres in aerial roots of *Ficus*

Abbreviations: MFA, microfibril angle; G-layer, gelatinous layer; S-layer, secondary layer; P-layer, primary layer; AGP, arabinogalactan protein; XET, xyloglucan-endotransglycosylase; RG I, rhamnogalacturonan I; MXE, mixed-link XET.

benjamina generate tension capable of lifting pots filled with soil (Zimmermann *et al.*, 1968). Probably best known is the action of gelatinous fibres in tension wood of deciduous species, where, during the first few years of growth of woody stems, such fibres are frequently formed and can bring the leaning stems to the upright position (Badia *et al.*, 2006). In older trunks and branches they create tension that helps maintain proper stem orientation (Fisher and Stevenson, 1981). Gelatinous fibres may also develop in peduncles in response to increases in weights of attached fruits, and thus help to support them (Sivan *et al.*, 2010); for example the long peduncles of the sausage tree, *Kigeliapinnata* (Jacq.) DC., support fruits weighing up to 10 kg.

Gelatinous fibres have been observed in diverse taxa, but primarily in angiosperms. They are formed in, and appear to be involved in the creation of tensional stress in, tension wood, in ≈50% of angiosperm woody species (representing diverse families) surveyed to date (Onaka, 1949; Höster and Liese, 1966; Fisher and Stevenson, 1981; Clair *et al.* 2006). However, the abundance of gelatinous fibres in tension wood can vary considerably even in closely related species. For example, some *Eucalyptus* species produce typical G-fibres in tension wood whereas others have little or no apparent ability to produce them. Examples of the former include *Eucalyptus regnans* F. Muell. and *Eucalyptus gigantea* Hook (Wardrop and Dadswell, 1948), *Eucalyptus gunii* Hook. f. (Toghraie *et al.*, 2006), *Eucalyptus grandis* Hill ex Maiden, and *Eucalyptus globules* Labill (Washusen *et al.*, 2005), while examples of the latter include *Eucalyptus nitens* H. Deane & Maiden (Qiu *et al.*, 2008) and the hybrid clones UAIC 1-45, 2-3, L2-131, and 18-14 (Baillère *et al.*, 1995).

G-fibres have also been observed in plants outside the angiosperms, including several coniferous genera (Jourez, 1997), as well as in *Ephedra* (Lev-Yadun, 1999) and *Gnetum* (Tomlinson, 2003), representing advanced gymnosperms, and more recently they have been discovered even in a horsetail, *Equisetum hyemale* L. (Gierlinger *et al.*, 2008). Although detailed information on the composition of the gelatinous layer in these plant taxa is not yet available, common characteristic features of this layer appear to be a high content of axially oriented cellulose and low contents of xylan and lignin. Thus, the G-fibres may have evolved in an early stage of land plant evolution and their occurrence seems to be more widespread than previously thought.

In recent years, gelatinous fibres have attracted considerable attention for several reasons. Firstly, their walls have very desirable chemical composition for use as feedstocks for saccharification. Their low lignin, low pentosan, and high cellulose contents are key characteristics that are thought to facilitate decomposition of cell walls by microbial enzymes (Engels and Jung, 1998). Secondly, understanding the molecular mechanism responsible for the generation of tensional stress in these fibres could potentially lead to the development of novel materials with similar (biomimetic) properties. The nature of this mechanism has been debated for many decades and several hypotheses have been proposed to explain it (Bamber, 2001; Yamamoto, 2004; Alméras *et al.*, 2006; Goswami *et al.*, 2008; Mellerowicz *et al.*, 2008; Bowling and Vaughn, 2009). Historically, researchers attempted to apply the same model to explain both tension generation in the tension wood of angiosperms and compression generation in the compression wood of conifers (Boyd, 1985; Okuyama *et al.*, 1994; Yamamoto, 1998; Bamber, 2001). However, although the architecture of cell walls in these two types of wood may superficially seem to represent two extremes of the same continuum – the former with low lignin contents and low cellulose microfibril angles (MFAs), and the latter with high lignin contents and high cellulose MFAs – there are major differences in the types of matrix polymer present and their organization. These differences lead to strong differences in cell-wall architecture, clearly indicating that these wood types represent very different materials and that a common model might not be applicable.

Basic cell-wall structure in gelatinous fibres

Tensional stress in gelatinous fibres arises from the cell-wall structure, therefore understanding the cell-wall architecture, including all the intricate interactions between the constituent polymers during their deposition and subsequent modification *in muro*, is essential for elucidating this physical phenomenon. The development of gelatinous fibres begins with the elongation of young fibres, which occurs either entirely (in secondary fibres) or partially (in primary fibres) by intrusive growth (between cells) (Gorshkova *et al.*, 2011). At this stage the fibres have primary (P) cell walls and are cemented together by acidic middle lamella (Snegireva *et al.*, 2010). During or following the final stages of elongation, successive secondary wall layers (S1, S2, to … S*n*) are deposited. After formation of one to three S wall layers of varying thickness (Onaka, 1949; Araki *et al.*, 1983), the tertiary, gelatinous layer (G) is laid down. Due to historical reasons, and the fact that S-layers are barely distinguishable in some fibres (notably flax and ramie), G-layers are sometimes referred to as parts of the secondary cell wall. However, careful observations indicate that deposition of S-layer(s) precedes that of G-layers, so an S-layer is never completely absent (Gorshkova *et al.*, 2010); hence G-layers of cell walls are tertiary. The proportions of S- and G-layers vary widely among phloem fibres of different species: ranging from only S-layers in jute and kenaf (McDougall, 1993; Lam *et al.*, 2003) to a high predominance of G-layers in ramie and flax (McDougall, 1993; Gorshkova *et al.*, 2010). Interestingly, G-layers seems to be specific to fibres and are not found in other cell types (Gorshkova *et al.*, 2010).

Thus, the cell wall of a mature gelatinous fibre has layers of three distinct types: the P-layer, including the pectin-rich outer region of the middle lamella; a variable number (usually on to three) of S-layers, and a tertiary G-layer (Wardrop and Dadswell, 1948; Onaka, 1949). In contrast, walls of normal wood fibres usually contain three S-layers deposited over the P-layer. The two types of cell-wall structure are diagrammatically presented in Fig. 1, and the two types of fibre with these cell-wall arrangements are

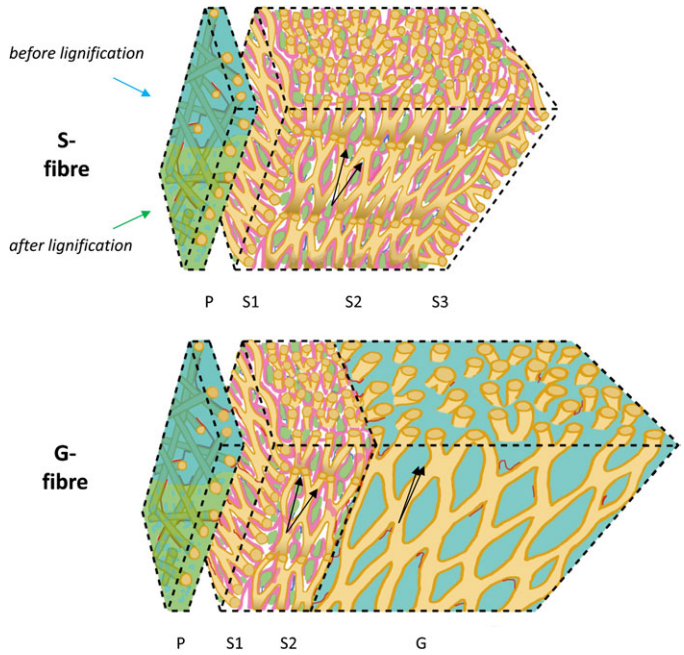

Fig. 1. Two types of cell-wall organization found in S- and G-fibres. The striking differences in composition and structure between P-, S-, and G-layers are depicted. The P-layer is initially an unlignified wall layer, and the only wall layer in expanding fibres. It is composed of highly hydrated pectins (blue), cellulose fibrils (beige), and xyloglucan chains (red), and it becomes filled with a cross-linked lignin network (green) after deposition of the S-layers. Cellulose fibrils run in a coordinated fashion within a single stratum of the P-layer, but change orientation between strata. In S- and G-layers the fibrils have the same orientation within a layer. Their angles relative to the cell axis (MFAs) in S2- and G-layers are indicated by long arrows. The fibrils consist of microfibrils and their aggregates (macrofibrils), which form a twisted honeycomb structure. S-layers are composed of cellulose, xylan (pink), and lignin, with a smaller proportion of mannan chains (dark blue). G-layers are mostly composed of large cellulose macrofibrils, with greater overall porosity. The pores are probably filled with hydrated pectins and arabinogalactan proteins. Xyloglucan chains (red) are present in the P- and G-layers and between different cell-wall layers.

hereafter called S- and G-fibres, respectively. In developing gelatinous fibres of some plant species, for example flax and hemp, the G-layer has two distinct sub-layers. The newly deposited sub-layer, closest to the plasma membrane, designated Gn, appears to have a loose structure in micrographs, while the outer part of the G-layer is more homogenous and 'solid'. The relative thicknesses of the sub-layers changes with time, and at fibre maturity the Gn sub-layer disappears, indicating that the deposited cell-wall material is extensively remodelled (Gorshkova *et al.*, 2004, 2010).

Chemical composition of gelatinous fibres

Gelatinous fibres are often analysed without separating P, S, and G cell-wall layers, or even separating fibres from surrounding tissues. When analysed in bulk, the chemical composition of normal wood and tension wood, which contain S- and G-fibres, respectively, does not notably differ, as exemplified by the composition of these composite tissues in poplar (Table 1). However, layer-by-layer analysis of S- and G-fibres, using techniques such as immunohistochemistry (Bowling and Vaughn, 2009) or microscopy-coupled spectrometry and spectroscopy (Gierlinger and Schwanninger, 2006; Gierlinger *et al.*, 2008; Gorzsás *et al.*, 2011), reveals remarkable differences among P-, S-, and G-layers. Especially important information has been obtained from isolated G-layers, collected after sonication of tension wood (Norberg and Meier 1966; Furaya *et al.*, 1970; Nishikubo *et al.*, 2007; Kaku *et al.*, 2009) (Table 1). Additional data have been gathered from numerous analyses of cell-wall polymers in isolated fibre bundles of fibre crops (Davis *et al.*, 1990; van Hazendonk *et al.*, 1996; Mooney *et al.* 2001; Cronier *et al.*, 2005), especially when combined with tracing polysaccharide deposition during the course of G-layer formation (Gorshkova *et al.*, 2010).

Sets of polymers in both P- and S-layers of S- and G-fibres are similar and correspond, respectively, to the general composition of primary and secondary walls in many plant tissues. In contrast, the G-layer is very distinct from other cell-wall layers in three major respects: the orientation, content, and structure of cellulose fibrils; the set of matrix polysaccharides; and lignin content.

G-layer polymers and their arrangement

Early studies of G-layers isolated by sonication from tension wood showed that they have very high cellulose contents (Norberg and Meier 1966). Recent analyses indicate that G-layers in *Populus* are largely composed of crystalline cellulose and matrix polysaccharides, accounting for ≈75 and 25%, respectively, of their total dry weight (Nishikubo *et al.*, 2007; Kaku *et al.*, 2009), with minor proportions of proteins (approx. 3%) and ash, but no detectable lignin (Kaku *et al.*, 2009). Similar proportions of major polymers have been found in isolated fibre bundles of flax (Mooney *et al.*, 2001) and ramie (McDougall, 1993), which are almost devoid of S-layers. Although lignin is present in P- and S-layers of gelatinous fibres, the close to zero lignin content of G-layers, in various species, has been confirmed by several approaches (Love *et al.*, 1994; Gorshkova *et al.*, 2000; Plomion *et al.*, 2001; Pilate *et al.*, 2004; Meloche *et al.*, 2007; Bond *et al.*, 2008; Kaku *et al.*, 2009; Schreiber *et al.*, 2010). However, there are several reports that some aromatic compounds may be present, either throughout G-layers (Joseleau *et al.*, 2004; Lehringer *et al.*, 2008) or in their inner parts (Gierlinger and Schwanninger, 2006). Overall, the composition of G-layers contrasts strongly with that of S-layers, which 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).

Table 1. Composition of normal wood, tension wood, isolated G-layers of poplar (*Populus alba*) tension wood fibres, and isolated primary phloem fibres of flax (*Linum usitatissimum*).

Sample	Constituent, % (w/w)				Reference
	Cellulose	Lignin	Matrix polysaccharides	Other	
Normal wood	42.5	19.4	36.4	1.7	Baba *et al.* (2009)
Tension wood	53.9	14.4	29.8	1.8	Baba *et al.* (2009)
G-layers	78	Not detected	19	2.6	Kaku *et al.* (2009)
Flax fibres	80	<1	15	4	Morvan *et al.* (2003)

Structure of cellulose

The cellulose network consists of fibrils that are vertically orientated with respect of fibre axis and are not completely straight, waving and merging with adjacent fibrils then splitting again (Fig. 1). Such a pattern was first observed in G-layers in the 1960s (Sachsse, 1964) and later detected in both S-layers (Côté *et al.*, 1969; Salmén and Burgert, 2009) and P-layers (Ding and Himmel, 2006). This implies that cellulose is present as both single microfibrils (fibrils secreted by individual rosette complexes) and their aggregates (macrofibrils); thus the diameter of fibrils varies. The diameter of a cellulose fibril is difficult to assess by microscopic techniques as measured sizes include unspecified amounts of fibril-coating matrix components. Therefore, reported sizes, ranging between 6 and 55 nm in various species, represent upper limits of cellulose macrofibril diameters (Daniel *et al.*, 2006; Donaldsson, 2007, Ruelle *et al.*, 2007*a*; Clair *et al.*, 2008; Lehringer *et al.*, 2009).

X-ray diffraction analyses provide more specific measurements of cellulose fibril diameters and other variables of cellulose crystallites in the G-layer. G-layer fibrils form monoclinic I_β cellulose, as in normal wood (Wada *et al.*, 1995), with lattice parameters *a*, *b*, and *c* defining the double inter-sheet distance, between-chain distance, and the repeat value along the chain corresponding to the cellobiose length, respectively. Analyses of samples from several species indicate that the cellulose forms crystallites of higher diameter in G-layers than in S-layers (Washusen and Evans, 2001; Hillis *et al.*, 2004; Müller *et al.*, 2006; Ruelle *et al.*, 2007*b*; Yamamoto *et al.*, 2009, 2011), consistent with a higher degree of microfibril aggregation. Based on crystal diameters obtained from synchrotron generated X-ray diffraction patterns of single G-fibres in poplar, it has been proposed that the cellulose fibrils of the G-layer contain four times more glucan chains than those of the S-layers (Müller *et al.*, 2006). The inter-sheet distance has been found to be smaller in tension wood or isolated G-layers (0.391 nm) than in normal wood (0.397 nm), as expected in fibrils with larger diameters (Yamamoto *et al.*, 2009). Thus, it is conceivable that the relative number of glucan chains is generally more than four times larger in G-layer than in S-layer fibrils.

Interestingly, the repeat value (c) in G-fibres does not vary with temperature, nor is it changed by boiling or drying, but co-varies with the tensional stress in the tissue (Clair *et al.*, 2006; Abe and Yamamoto 2007). Clair *et al.* (2006) found that the repeat value in large wood segments changed from 1.0035 nm to 1.0033 nm when tension stress was released by transversal cutting. In normal wood, the repeat value is usually 1.0033 nm, but values are higher in phloem fibres of ramie, cotton 'fibres', and algal cellulose, which has larger cellulose aggregates than wood (Davidson et al, 2004). Developmental modifications of cellulose structure in tension wood have been followed during fibre differentiation by focusing a narrow X-ray beam on G-fibres at successive stages of cell-wall development (Clair *et al.*, 2011). The results show that the appearance of the G-layer coincides with an increase in the glucan chain repeat value, suggesting that the extension of glucan chains occurs concomitantly or very soon after deposition of the G-layers. The implications of these findings are discussed below. The degree of polymerization (or DP) of cellulose in G-layers is unknown, but it is probably slightly higher than in S-layers, based on comparisons of normal and tension wood in *Populus "robusta"* (Grigoras *et al.*, 1971). Thus, G-layer fibrils contain at least four times more glucan chains, and the chains are slightly longer and have more widely spaced glucose units than S-layer fibrils, but the glucose spacing returns to that of typical normal wood after the release of longitudinal tension in the tissue.

Another distinct characteristic of G-layer cellulose fibrils is their almost axial orientation in fibre cells. Their MFA has been determined by various techniques, including microscopy (iodide precipitate observation by light microscopy, field emission scanning electron microscopy, and other techniques) and X-ray techniques including, among others, diffraction analysis and wide-angle X-ray scattering (WAXS), as reviewed by Donaldsson (2008). Although the uncertainty of X-ray measurements is high when MFAs are low, these methods have yielded similar results, with reported MFAs in the G-layers of various species ranging between 0 and 10 degrees (Yoshida *et al.*, 2000; Hillis *et al.*, 2004; Washusen *et al.*, 2005; Clair *et al.*, 2006; Müller *et al.*, 2006; Yang *et al.*, 2006; Ruelle *et al.*, 2007a; Goswami *et al.*, 2008; Lehringer *et al.*, 2009).

Non-cellulosic polysaccharides of the G-layer

The types of non-cellulosic polysaccharides present in G-layers differ strongly from those in S-layers (Box 1). Two matrix polysaccharides, which are often not present in

Box 1. Polymers of P, S, and G wall layers of fibres in dicotyledons.

Layer	Polymer (% d.w. AIR)*	Structure
P-layer	Cellulose (30–40% BL)	[→4)-β-D-Glc*p*-(1→]$_n$; *n*=≈4000; glucan chains assembled into crystalline fibrils with ⩾2.6 nm diameters, the smallest fibrils corresponding to aggregates of glucan chains secreted by single rosettes.
	Homogalacturonan (up to 40% BL)	[→4)-α-GalAp-(1→]$_n$; C-6 of galacturonic acid may be methylesterified. In S- and G-fibres the degree of methylesterification is lower than in other types. The most abundant polymer before lignification.
	RG I (lower than that of homogalacturonan)	A backbone consisting of [→4)-α-D-GalAp-(1→2)-α-L-Rha*p*(1→] dimers, and side chains of variable structure, composed of galactans [→4)-β-D-Gal*p*-(1→], arabinans [→5)-α-L-Ara*f*-(1→] or type I arabinogalactans, which usually have terminal arabinose and linear or branched chains of galactans, in which the galactose residues are linked at C-4.
	Type II arabinogalactan (?)	[→3)-β-D-Gal*p*-(1→]$_n$-[→6)-β-D-Gal*p*-(1→]$_n$. Constitutes part of the sugar antennae of arabinogalactan proteins and may be linked to RG I.
	Rhamnogalacturonan II (?)	This polymer has a homogalacturonan backbone and conserved, complex side chain structure. Not yet characterized in S- or G-fibres.
	Xyloglucan (≈20% BL)	Backbone as in cellulose and a repeating sequence of side chains, including: α-D-Xyl*p*-(1→6), β-D-Gal*p*-(1→2)-α-D-Xyl*p*-(1→6), and α-L-Fuc*f*-(1→2)-β-D-Gal*p*-(1→2)-α-D-Xyl*p*-(1→6). The main hemicellulose in P-layers.
	Xylan (≈5% BL)	Minor hemicellulose in some species, including sycamore (Darvill *et al.*, 1980). Differs structurally from S-layer xylan in the presence of short arabinosyl and xylosyl side chains.
	Mannan(≈3% BL)	Structure probably similar to that of S-layer mannan. Minor hemicellulose (Marcus *et al.*, 2010).
	Lignin (60–70% AL†)	Complex amorphous polymer composed of aromatic monomers. Polymerizes at a later stage of fibre development and becomes the major component of the P-layer (Donaldson *et al.*, 2001).
S-layers	Cellulose (50%)	[→4)-β-D-Glc*p*-(1→]$_n$; *n*=≈10 000. Cellulose microfibrils are aggregated into macrofibrils of ≈3 nm diameter, or more, forming a twisted honeycomb structure. Fibril orientation changes from flat S (looking from the lumen), 45 °, in S1 to steep Z, 10–25 °, in S2 layer, and then to flat S, 30–40 °, in S3 layer.
	Xylan (20–30%)	[→4)-β -D-Xylp-(1→]$_n$; *n*=50–250. Branching: [→2)-β-4-*O*-Me-α-D-GlcAp-(1→]; with Xyl:GlcA molar ratios ranging from 4:1 to 16:1. Acetylation of xylose residues at C-2 or -3, or both, giving a total degree of acetylation of 0.6. Major hemicellulose in S-layers.
	Glucomannan (≈5%)	[→4)-β -D-Man*p*-(1→] and [→4)-β-D-Glc*p*-(1→] in a molar ratio of ≈2:1. Randomly distributed acetyl residues at the C-2 or C-3 position of mannose residues with a total degree of polysaccharide acetylation around 0.3.
	Type II arabinogalactans (?)	Similar structure, probably, to that of P-layers.
	Lignin (10–20%)	Complex amorphous polymer composed of aromatic monomers. Lignin concentration is lower in S-layers than in P-layers (Donaldson, 2001).
G-layer	Cellulose (≈75%)	Present as larger crystallites, containing at least four times more glucan chains of a higher degree of polymerization, than in S-layers. MFA between 0 and 10 °.
	Xyloglucan (≈15%)	Structure similar to that in P-layers. Convincingly reported presence in tension wood fibres of poplar.
	RG I (?–8%)	Structure similar to that in P-layers, but with a higher degree of branching, longer side-chains of [→4)-β-D-Gal*p*-(1→] and minor frequencies of other types of Gal linkages.
	Mannan (≈2%)	[→4)-β-D-Man*p*-(1→], no structural details; may be similar to S-layer glucomannan. The content of mannose is lower than in S-layers.
	Type II arabinogalactans (≈2%)	Highly variable structure based on staining with Yariv reagent or antibodies. Biochemically demonstrated by linkage analysis only (Nishikubo *et al.*, 2007).

* Contents per dry weight of alcohol insoluble residue (d.w. AIR) in each layer; for the P-layer, approximate content is given before (BL) and after (AL) lignification.
† Lignin content based on semiquantitative estimations of its abundance in the compound middle lamella (P-layer and middle lamella).

S-layers, are reportedly the major non-cellulosic polymers of G-layers: xyloglucan in poplar (Nishikubo *et al.*, 2007; Kaku *et al.*, 2009) and pectic galactan in flax (Gurjanov *et al.*, 2008). Xylan, the main non-cellulosic polysaccharide of S-layers, has been shown to be absent in G-layers of gelatinous fibres (in several plant species) by immunocytochemical analysis, using anti-xylan antibodies that heavily label S-layers but do not reportedly bind to G-layers (Bowling and Vaughn, 2008; Decou *et al.*, 2009). Xylose is present in G-layers isolated from poplar tension wood, but in xyloglucan rather than xylan, as demonstrated using several approaches, including linkage analysis, immunolabelling, cleavage with specific

enzymes, and the presence of active xyloglucan-endo-transglycosylase (XET), a xyloglucan-specific enzyme (Nishikubo *et al.* 2007; Mellerowicz *et al.* 2008; Baba *et al.*, 2009; Kaku *et al.*, 2009). In accordance with these findings, a sharp decline in xylan biosynthesis and processing during tension wood development, in association with G-layer initiation, is suggested by transcriptomal changes (Andersson-Gunnerås *et al.*, 2006). Observed proportions of sugars indicate that xyloglucan is by far the most abundant non-cellulosic component of G-layers in *Populus alba*, comprising 10–15% of their dry mass (Nishikubo *et al.*, 2007; Mellerowicz *et al.*, 2008; Kaku *et al.*, 2009).

In an extensive analysis of tension wood fibres in sweetgum (*Liquidambar styraciflua* L., Hamamelidaceae) and the taxonomically distinct hackberry (*Celtisoccidentalis* L., Ulmaceae), none of a wide set of antibodies raised against non-cellulosic polymers labelled both the S- and the G-layers (Bowling and Vaughn, 2008). Hence, the polysaccharide components of these layers appear to be mutually exclusive. A probable exception to this rule is that mannan has been detected among the monomers of polysaccharides in isolated G-layers (Furuya *et al.*, 1970; Nishikubo *et al.*, 2007), with (1→4)-type links between Man residues (Nishikubo *et al.*, 2007). However, proportions of both Man and 1,4-β-mannan are ≈2–3-fold lower in tension wood with G-fibres than in normal wood with S-fibres in aspen (Hederström *et al.*, 2009). In this species, levels of mannan biosynthesis-related transcripts are reportedly significantly, and consistently, lower in differentiating G-fibres than in S-fibres (Andersson-Gunnerås *et al.*, 2006).

The matrix polysaccharides of the G-layer surprisingly include pectins, which are typical components of primary cell walls. Indications of the presence of some acidic polymers in G-layers of tension wood have been obtained from simple histochemical staining, initially by Wardrop and Dadswell (1948) in eucalyptus, and subsequently in several species (Furuya *et al.*, 1970; Scurfield, 1972; Bowling and Vaughn, 2008). Homogalacturonan-specific antibodies (JIM5, JIM7) do not apparently bind to G-layers; however, rhamnogalacturonan I (RG I) backbone and de-arabinosylated RG I-specific antibodies (CCRC-M10 and CCRC-M22, respectively), heavily label, and thus indicate the presence of, RG I in G-layers of sweetgum and hackberry tension wood fibres (Bowling and Vaughn, 2008). Further, a member of the PL4 family of RG I lyases is consistently and highly up-regulated during G-fibre differentiation according to Andersson-Gunnerås *et al.* (2006), indicating that the substrate for this enzyme is present in these fibres. However, rhamnose contents of isolated poplar G-layers amount to only 0.2% of the total cell-wall sugars, according to Nishikubo *et al.* (2007), and identification of galacturonic acid has not been reported.

G-layers can also be labelled by antibodies specific for neutral polysaccharides usually present as side chains of RG I. For instance, Arend (2008) detected β-(1→4)-galactan in hybrid poplar (*Populus trichocarpa*×*Populus koreana*) using the LM5 antibody, while Bowling and Vaughn (2008) found indications that arabinogalactan may be present in sweetgum and hackberry using CCRC-M7, which binds to RG I/arabinogalactan proteins (AGPs), but they observed no LM5 labelling. Galactans of complex structure were long considered to be among the distinctive polysaccharides of G-fibres in tension wood. Tension wood is known to contain 2–4-fold more galactose than normal wood in many species, including *Eucalyptus goniocalyx* (7.5 versus 2.5%; Schwerin, 1958), *Betula pubescens* and *Betula verrucosa* (11.6 versus 2.6% and 8.0 versus 2.3%, respectively; Gustafsson *et al.*, 1952), and *Fagus sylvatica* (4.9 versus 1.3%; Meier, 1962). In American beech (*Fagus grandifolia* Ehrl.) galactose constitutes 6.0% of tension wood, compared to only 1.6% of normal wood. High galactose content has even been suggested as an indicator of G-fibre content (Ruel and Barnoud, 1978). This galactose is present in a unique galactan, characterized by high structural complexity and degree of branching (Meier, 1962; Kuo and Timell, 1969; Azuma *et al.*, 1983). The structure of the G-fibre galactan in American beech has been partially characterized (Kuo and Timell 1969), and shown to consist largely of β-(1→4)- and β-(1→6)-linked galactopyranosidic residues (Meier, 1962; Kuo and Timell, 1969; Azuma *et al.*, 1983), with some galacturonic acid, rhamnose, and the disaccharide 2-*O*-α-D-GalAp-L-Rha, indicating the presence of RG I. However it is not known if the examined fraction contained a single galactan polymer or a mixture (Meier, 1962). In Japanese beech (*Fagus crenata* Blume) one fraction of alkaline cell-wall extracts, separated by Azuma *et al.* (1983) using a Sepharose 4B column, was found to have higher galactose and rhamnose contents in tension wood than in normal wood. In G-fibres of the phloem, galactans with RG I backbones are the major non-cellulosic polysaccharides and play a prominent role in determining fibre mechanical properties (Gorshkova *et al.*, 2010; Roach *et al.*, 2011). However, in tension wood the presence of RG I has not been fully proven biochemically.

Matrix polymers of another class, AGPs, are also characteristically present in G-layers, and have a highly variable structure. Their backbone consists of protein, but up to 95% can be comprised of carbohydrate, which is sometimes referred to as type II arabinogalactan. This is because the glycan component of AGPs has chains of [→3)-β-D-Gal*p*-(1→] and [→6)-β-D-Gal*p*-(1→] units, often decorated by terminal arabinose residues and connected to each other by (1→3,1→6)-linked branch points, which are indicative of AGPs. Another characteristic of all AGPs is their ability to bind the Yariv reagent, a β-D-Glc derivative of phloroglucinol. AGPs are highly water soluble, but they are sometimes tightly fixed within cell walls, indicating that they are covalently linked with other cell-wall constituents. AGPs are present in all types of plant tissues in all stages of their development, but no roles of any arabinogalactan proteins have been elucidated.

The presence of AGPs in G-layers has been demonstrated by analysis of monosaccharide linkages in isolated G-layers (Nishikubo *et al.*, 2007; Kaku *et al.*, 2009), and immunochemically by probing with antibodies (Lafarguette *et al.*, 2004; Bowling and Vaughn, 2008), in all species investigated

so far. The content of this polymer in isolated G-layers was estimated to be around 2% by Mellerowicz *et al.* (2008), but this may be an underestimate due to the high water solubility of AGPs. Rocket electrophoresis using agarose gels containing β-glycosyl Yariv reagent has shown that substantial levels of AGP accumulate in poplar tension wood (Lafarguette *et al.*, 2004). Western blot analysis of these proteins with the JIM14 monoclonal antibody has revealed the presence of polypeptides with apparent molecular masses of 100 and 200 kDa in both tension wood and opposite wood, but much more abundantly in the former. In addition, gene-expression analyses have identified classes of highly expressed AGPs that are specifically and very strongly up-regulated during tension wood formation in both poplar (Déjardin *et al.*, 2004; Lafarguette *et al.*, 2004; Andersson-Gunneras *et al.*, 2006) and eucalyptus (Qiu *et al.*, 2008).

The distribution of AGPs within cell walls has also been studied, using the JIM14 antibody, in sweetgum and hackberry (Bowling and Vaughn, 2008), and hybrid poplar (*Populus tremula*×*P. alba*) (Lafarguette *et al.*, 2004). The results of these analyses indicate that AGPs are present throughout the entire G-layer, but while they are most abundant in its outer layers in sweetgum and hackberry they are apparently mainly present in its inner part in hybrid poplar. However, secondary antibodies labelled with 15 nm gold particles were used in the cited poplar study, hence the difference in findings may reflect differences in the accessibility of AGP epitopes within the layer for such large antibody–gold complexes. Although the genes encoding the protein backbone of tension wood AGPs are well characterized, to our knowledge structural details of their glycan constituents have not been reported to date.

Porosity of the G-layer

Structural studies of the G-layer are difficult because the G-fibres are in a metastable state in the living plant, and thus their structure is examined after stress has occurred. Analysis of supercritically dried wood, in which the ultrastructure of cell walls is preserved in a relaxed state, by nitrogen adsorption-desorption have revealed that tension wood has much higher porosity (total pore area per unit volume) than normal wood (Clair *et al.*, 2008). Tension wood pores typically have reported diameters of 2–50 nm in chestnut (*Castanea sativa* Mill.) (Clair *et al.*, 2008) and 6–12 nm in several tropical species (Chang *et al.*, 2009). Further, the thicker the G-layer the higher the observed porosity, suggesting that high porosity is an attribute of the G-layer itself.

Tensile stress generation in G-fibres

Most data on tensional stress development in G-fibres have been obtained for those in tension wood because in many species formation of tension wood can be simply induced by tilting stems from the vertical position, providing a convenient, easily controlled system to study them.

Extent and timing of stem bending

The formation of G-fibres in tilted stems induces strong tensional stresses unilaterally in the stem tissues, which are manifested in the stem righting reaction. The kinetics of stem righting in relation to the differentiation of G-fibres have been studied in young poplar stems. Typically, the response can be initially seen during the first week of tilting and is strongest after 2–3 weeks (Coutand *et al.*, 2007). However, the first gelatinous fibres can be observed after 48 h of gravitropic stimulation, preceding stem righting by at least 2 days (Jourez and Avella-Shaw, 2003). The substantially higher than normal tensional stress generated by the gelatinous fibres can be visualized in the strain (shrinkage) that occurs when wood segments are isolated from a stem by transversal cuts. Typically, between −0.1 and −0.5% strain is recorded in tension wood segments, up to 10 times more than in normal wood fibres (Sugiyama *et al.*, 1993; Yoshida *et al.*, 2002). Accordingly, changes in the strain at the stem surface of inclined poplar trees are reportedly detectable after 2 days of tilting (Baba *et al.*, 2009).

Further, in atomic force microscopy analyses of cut surfaces of tension wood (kept under water to avoid drying effects) Clair and Thibault (2001) observed pronounced longitudinal shrinkage of the G-layer relative to S-layers, corresponding to a −4.7% strain, which is much greater than the recorded strain of the tissue. These findings imply that the G-layer is a key source of tension in the G-fibres. The importance of the G-layer for creating pulling force is also supported by observations that its enzymatic removal leads to S-layer elongation by 1.6% (Goswami *et al.*, 2008).

In aerial roots, gelatinous fibres distributed in concentric rings create tensional stress uniformly within the entire organ, in contrast to its unilateral distribution in tension wood. However, the strain values observed in aerial roots of *Ficus* are of the same order of magnitude as those observed in its tension wood (Abasolo *et al.*, 2009).

Role of G-layer cellulose

Among the fibre and wood properties most highly and consistently correlated with tensional stress are high cellulose content and crystallinity (Sugiyama *et al.*, 1993; Washusen and Evans, 2001a; Washusen *et al.*, 2005; Yang *et al.*, 2006), large crystallite size (Ruelle *et al.*, 2007*b*), and low MFA (Yang *et al.*, 2006; Donaldson, 2008). Effective tension development requires MFAs of less than 10 ° (Wahyudi *et al.*, 2000). These findings raise questions about mechanisms whereby cellulose networks could generate tensile forces in the G-layers that could be transmitted to longitudinal tensile stresses with macroscopic, organ-level effects, especially in time frames of a couple of days (including the time required for G-layer deposition).

Considering the honeycomb cellulose structure model, the longitudinal strain could involve lateral swelling of the porous network, longitudinal shrinkage of fibrils, or both (Fig. 2). The possibility that lateral swelling of G-layers

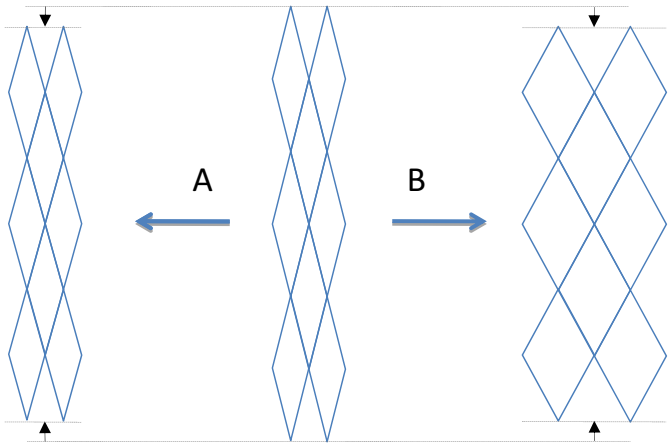

Fig. 2. Longitudinal shrinkage of the cellulose network in the G-layer, schematically shown as a honeycomb-like net of merging and splitting fibrils, may involve two processes that are not mutually exclusive: (A) longitudinal fibril shrinkage due to a reduction in the glucan chain repeat value, and/or (B) lateral swelling of the network, resulting in longitudinal shrinkage.

(pictured in Fig. 2B) may be involved is supported by observations of residual lateral strain in G-fibres after removal of their G-layers by cellulase (Goswami *et al.*, 2008). The cited authors showed that remaining S wall layers shrunk by 1% in the tangential direction when the G-layer was removed, indicating that the G-layer exerts lateral pressure *in situ* on S-layers. It has not been shown whether the layer laterally expands; however, it is worth noting that due to its very low MFA a lateral strain close to 25% (25 times larger than that observed) is needed to accommodate a longitudinal strain of 0.2%.

The longitudinal shrinkage of cellulose fibrils (pictured in Fig. 2A) is supported by observations of extended lattice spacing in the fibril direction *in situ* (repeat value), and its contraction after tension release (Clair *et al.*, 2006, 2011). Assuming an MFA of 5 °, the recorded level of lattice shrinkage (0.19%) is sufficient to induce a stem strain of −0.18%, within the range of observed values. Moreover, increases in lattice spacing were observed during or very soon after the deposition of the G-layer in developing tension wood, but not during S-layer deposition in normal wood, and they coincided with the tensional stress development. These findings indicate that tension develops in cellulose fibrils when their lattice spacing increases. Key issues that remain to address are the mechanisms that generate this tension.

Roles of XET and xyloglucan

Very high XET activities, visualized by incorporation of fluorescently labelled acceptor xyloglucan or its oligomers to cell wall, have been detected *in situ* in developing G-layers of *Populus* tension wood (Nishikubo *et al.*, 2007; Baba *et al.*, 2009). Accordingly, transcript levels of several xyloglucan endotransglucosylase/hydrolase (*XTH*) genes (including *PtxtXTH14*, *PtxtXTH21*, and *PtxtXTH36*) are reportedly higher during the development of tension wood than during normal wood development (Nishikubo *et al.*, 2007). Moreover, analyses with XET16A antibodies indicate that the enzyme is also present in the mature G-layers. However, XET activity was no longer detected in the mature G-layers but it was located just outside the G-layer, in adjacent S-layers. This activity is restricted to mature G-fibres, and is surprisingly long lived as it can be detected several years after cell death (Nishikubo *et al.*, 2007). High levels of mixed-link XET (MXE) have been also found in mature stems of *Equisetum* spp., which are known to form gelatinous fibres (Fry *et al.*, 2008).

The functions of XET and MXE in gelatinous fibres are not known, but they may hypothetically reinforce cell walls. XET localization in mature G-fibres indicates that the enzyme may act between G and S2 wall layers (Mellerowicz *et al.*, 2008), where abundant xyloglucan deposition has been observed (Sandquist *et al.*, 2010). Its longevity suggests that it may be involved in repairing the xyloglucan cross-links that connect the G-layer to adjacent S-layers, which may locally break during fibre shrinkage. Such cross-links would be immediately repaired by XET and after subsequent G-layer shrinkage they would be under stress until they locally failed again, initiating a repetition of the cycle. In accordance with this XET repair hypothesis, when tension wood is mechanically stressed by pulling, the stress is released in many small steps over an extended time (Goswami *et al.*, 2008).

The role of XET in developing G-layers, which cannot be related to cell expansion as the fibres have ceased growing when these layers develop, is more puzzling. One possibility is that XET trims nascent xyloglucan attached to cellulose microfibrils, thus allowing aggregation of the microfibrils that enclose the remaining short chains of xyloglucan inside macrofibril cores (Mellerowicz *et al.*, 2008). This hypothesis is consistent with the transient detection of xyloglucan by antibodies in developing G-layers, but not in mature G-layers, although xyloglucan-diagnostic sugars and linkages have been detected in mature G-layers by gas chromatography-mass spectrometry (Nishikubo *et al.*, 2007; Bowling and Vaughn, 2008; Baba *et al.*, 2009), and XET has transient ability to incorporate labelled xyloglucan oligosaccharides or xyloglucan into the xyloglucan network of the G-layer (Nishikubo *et al.*, 2007; Baba *et al.*, 2009).

Thus, in both phases of fibre development, XET appears to play essential putative roles in tension generation. In accordance with these proposals, Baba *et al.* (2009) found that removing xyloglucan from cell walls by a fungal xyloglucanase expressed in transgenic poplars did not affect G-layer formation, although it completely abolished stem righting. This shows that in *Populus*, at least, the mechanism of tensile stress development in G-fibres is dependent on the presence of xyloglucan.

Roles of RG I deposition and in muro *modification*

G-layer deposition in flax fibres is accompanied by the formation of tissue-specific pectic galactan that has an RG I

backbone and a sophisticated set of side chains, mainly composed of β(1→4)-linked galactose. The mechanical properties of flax fibres are dependent on the *in muro* trimming of RG I galactan side chains by tissue-specific β-galactosidase (Roach *et al.*, 2011), and the corresponding gene is among the most strongly up-regulated gene during the initiation of gelatinous layer deposition in flax fibres (Roach and Deyholos, 2008; Snegireva *et al.*, 2010). Major proportions of pectic galactan cannot be extracted from cell walls of mature fibres by conventional methods, indicating that this polymer is tightly entrapped by cellulose macrofibrils (Gurjanov *et al.*, 2008).

Role of moisture content

Overall, tension wood is less hygroscopic than normal wood (Wardrop and Dadswell, 1955; Tarmian *et al.*, 2009). However, in addition to the absence of hydrophobic lignin and high content of hydrophilic cellulose, the G-layer contains hydrophilic pectins, hemicelluloses, and arabinogalactans (Nishikubo *et al.*, 2007; Arend 2008; Bowling and Vaughn, 2008), thus it is thought to be hygroscopically active. In contrast, the adjacent P- and S-layers are lignified. *In situ* Raman spectroscopy has demonstrated that G-layers contain more water than the lignified adjacent S-layers (Gierlinger and Schwanninger, 2006; Schreiber *et al.*, 2010), prompting the hypothesis that hygroscopic swelling of the former might drive G-fibre shrinkage (Goswami *et al.*, 2008; Burgert and Fratzl, 2009). However, simple deposition of a hydrophilic cell-wall layer cannot be the cause of tension since the swelled material would be correspondingly packed within the cell wall. Thus, the radial swelling due to water uptake must, presumably, occur in portions of the G-layer that have already been deposited. If so, this would be expected to occur during S-layer lignification, when lignin polymerization starting from the middle lamella displaces water towards the cell lumen. To participate in stress generation, water displacement following G-layer biosynthesis would need to occur within 2 days when tensional stress develops in the G-fibres. It is not known if there is any change in moisture content in the G-layer within this time frame; hence the role of the high moisture content of G-fibres in tensional stress generation is presently unclear. Moreover, radial swelling of G-layers due to water uptake has not yet been demonstrated, although it is well established that drying induces severe longitudinal shrinking of G-layers (Clair *et al.*, 2008) and results in both tight adhesion of G-layers to adjacent S-layers and their radial shrinking (Fang *et al.*, 2007). However, G-layers are very unlikely to dry sufficiently quickly after their deposition to account for the developing stress, although this might contribute to stress development as an accessory mechanism during later maturation stages.

Models explaining tension stress generation

Although the mechanism of tension stress generation in G-fibres remains unknown, recent progress towards elucidating G-fibre functions allows us to revisit the two main theories: the G-layer swelling (or pressure) hypothesis (Goswami *et al.*, 2008; Burgert and Fratzl, 2009) and the G-layer tension hypothesis (Sugiyama *et al.*, 1993; Okuyama *et al.*, 1994; Clair *et al.*, 2006) the latter incorporating the recently proposed 'matrix entrapment' mechanism (Mellerowicz *et al.*, 2008). Finally, we present a new unifying hypothesis.

The G-layer swelling (or G-layer pressure) hypothesis

Originally proposed by Münch (1938), this theory postulates that the G-layer generates outward pressure on the S-layers, which induces their circumferential expansion (Goswami *et al.*, 2008; Burgert and Fratzl, 2009) (Fig. 3A). Since the MFA of S-layers is high, even a small circumferential expansion is likely to cause relatively large longitudinal shrinkage of the S-layers, which was proposed to drive the tissue shrinkage.

Evidence that the G-layer exerts pressure on the S-layers has been obtained by observations of both residual lateral strain (Goswami *et al.*, 2008) and G-layer behaviour during drying (Fang *et al.*, 2007). Since the thickness of the G-layer decreases during drying when it exerts radial outward pressure on S-layers, whereas swelling of the G-layer remains to be demonstrated, the 'swelling hypothesis' does not seems to be an accurate term for the proposed mechanism and it should be replaced by 'pressure hypothesis'. Causes of the postulated outward pressure exerted by the G-layer are unclear.

Cellulose tension (or matrix entrapment) hypothesis

This theory postulates that tensional stress develops in the G-layer, which then drives shrinkage of the S-layers. There is evidence that considerable tensional stress develops in the G-layer in the green condition (without any drying), for example the high strain of the G-layer observed after fibres are transversally cut (Clair and Thibaut, 2001). Since the cellulose fibrils forming the mechanical skeletons of G-layers run parallel to the fibre axis, they must shrink to approximately the same degree as the G-layer, as discussed above (Fig. 2). There is also evidence from X-ray diffraction analyses that stress of expected magnitude resides in cellulose fibrils (Clair *et al.*, 2006) and that it develops during or very soon after G-layer deposition (Clair *et al.*, 2011), when tensional stress is known to develop. This provides strong indications that the tensional strain in cellulose is a primary source of tensional stress in the G-layers.

High correlations of stress with the diameter of cellulose fibrils (Ruelle *et al.*, 2007*b*) suggest that the mechanism depends on cellulose macrofibril formation. The mechanism responsible is unknown, but it has been proposed that it involves entrapment of matrix polysaccharides, such as xyloglucan, inside nascent cellulose macrofibrils, which induces strain within the glucan chains that are wrapped around the entrapped polysaccharides (Fig. 3B), thus forming a 'molecular muscle' (Mellerowicz *et al.*, 2008). The model has been proposed for *Populus* tension wood

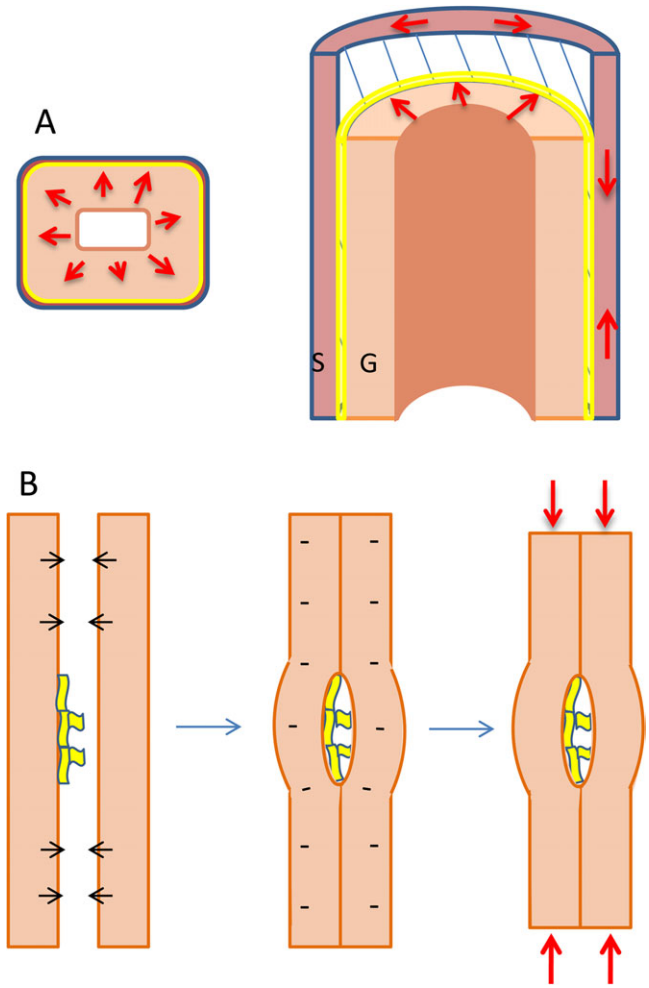

Fig. 3. Hypotheses regarding the mechanism of tensile stress generation in gelatinous fibres. (A) According to the G-layer pressure (swelling) hypothesis (modified from Burgert and Fratzl, 2009), the G-layer exerts pressure on the outer cell-wall layers, causing their longitudinal shrinkage due to their high MFA. (B) According to the G-layer longitudinal shrinkage hypothesis (modified from Mellerowicz *et al*., 2008), tensile stress is generated by the crystallization of adjacent microfibrils with short stretches of matrix polysaccharides trapped between them, causing strain in the cellulose lattice (marked by dashes). The fibrils with extended lattice spacing are in a metastable state. The tensional stress in the G-layer fibrils is passed to the entire fibre due to the contact of the G-layers with the adjacent S-layers, maintained by xyloglucan cross-links and long-lived XET activity. Shortening of fibrils is accompanied by a return to the normal (relaxed) lattice spacing. (C) Proposed combined mechanism of tension generation in G-fibres by longitudinal shrinkage of the G-layer, leading to radial pressure on the S-layers. The top row presents side views and the bottom row views of cell walls from outside, showing S- and G-layers. The longitudinal tensional stress arising within the G-layer by mechanism B leads to more pronounced shortening of the inner G-layer strata, distant from its attachment to the S-layer. The differential shrinkage of inner and outer strata in the G-layer leads in turn to pressure on adjacent S-layers, which contributes to shortening of the S-layers in step 2 by a mechanism proposed in (A).

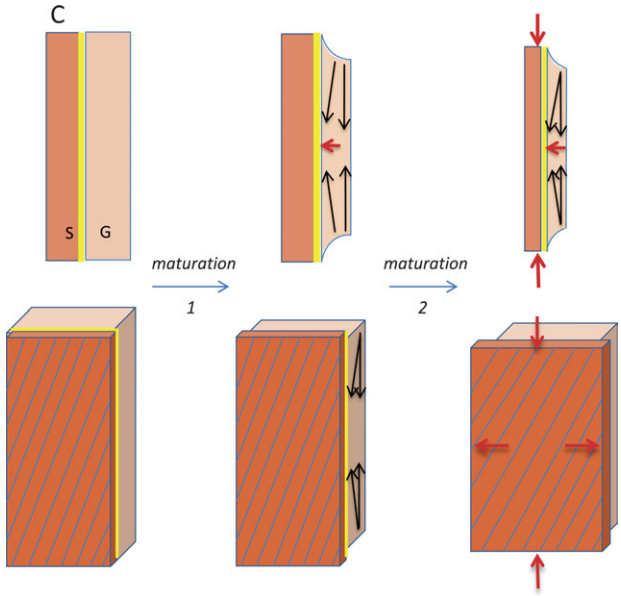

Fig. 3. Continued

and is consistent with the known requirement for xyloglucan for stress generation in this genus (Baba *et al.*, 2009). Theoretically, any other compact polysaccharide might play such a role (Gorshkova *et al*, 2010). Indeed, recent findings suggest that galactan hydrolysis in flax fibres promotes crystallinity and the formation of macrofibrils (Roach *et al.*, 2011), suggesting that this polymer could also participate in tension generation by entrapment.

Tensional stress in the G-layer can only be transmitted to adjacent layers if it is attached to them, and accordingly tight attachment can be seen, at distances of ≈100 μm from the cut faces of G-fibres (Clair *et al.* 2005). The attachment between layers with different cellulose MFAs, which are longitudinally shrinking, cannot be permanent and must involve re-making of broken contacts between cellulose fibrils as the fibrils of G- and adjacent S-layers slide past each other. The only known mechanism for this is transglycosylation. Hence, it has been proposed that the long-lived XET activity residing between the layers in *Populus* G-fibres is involved in the maintenance of cross-links (Nishikubo *et al.*, 2007; Mellerowicz *et al.*, 2008) using xyloglucan accumulated in this compartment (Sandquist *et al.*, 2010).

A unifying model

The models discussed above agree that the G-layer is essential for stress generation in G-fibres, but they propose different mechanisms for its action; either radial outward pressure or longitudinal tension. The presence of both pressure and tension have been experimentally confirmed, thus they co-exist in the G-fibres. Is one a consequence of the other? We propose that the outward pressure of the G-layer is a direct consequence of the longitudinal tension and is due to differential shrinkage of the inner and outer regions of the G-layer (Fig. 3c), arising from attachment of G-layer outer strata to the S-layer, which restricts the potential shrinkage

of these strata. The differential shrinkage of inner and outer strata in the G-layer may lead, in turn, to pressure on the adjacent S-layer. Thus the cell-wall sandwich behaves analogously to a bimetallic strip, which curves when its layers differentially shrink. In the case of G-fibres, the longitudinal shrinkage of the G-layer would exert horizontal force outwards (as indicated by the red horizontal arrow in Fig. 3c) on the S-layer. Thus, the G-layer shrinkage could be a key step in generation of the outward pressure of the G-layer on the S-layer, as observed by Goswami *et al.* (2008). This pressure would result in S-layer shortening by a previously proposed mechanism (Goswami *et al.*, 2008). Such differential shrinkage of the inner and outer regions of the G-layer has been indeed observed in some samples by electron microscopy (Clair and Tibault, 2001).

Concluding remarks and perspectives

The validity of the presented unified tension-pressure hypothesis could be explored using transgenic plants, for example xyloglucan-deficient plants (Baba *et al.*, 2009) and XET-deficient plants, to test whether pressure develops in the G-layer in the absence of proposed key agents for tension development. Xyloglucan is proposed to play a role in G-layer tension, and indirectly the generation of G-layer pressure, in the unifying tension-pressure hypothesis, but its presence is not predicted to affect the G-layer pressure arising by an independent mechanism. Lignin-deficient plants could be used to determine whether lignification is involved in tensional stress development in G-fibres, as hypothesized if lignification-dependent water displacement plays a role in pressure build-up, as proposed in the swelling theory. Abundant gelatinous fibres have been observed in such plants, but their mechanical properties have not been described (Kitin *et al.*, 2010). In some phloem fibres with extremely well-developed G-layers, such as those in flax, S-layers are very scarce (Gorshkova *et al.*, 2010), and it would be useful to assess the tension parameters in such fibres, especially as there are no indications that xyloglucan is present in their S- and G-wall layers. The possibility that RG I galactan modification may be essential for tension development in flax could also be addressed by studying tension development in flax lines deficient in β-galactosidase activity. This work could be further developed by deploying biomechanical models to test some of the ideas proposed here and integrating the acquired knowledge into robust models of plant organ movements (Moulia and Fournier, 2009).

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