Struct Chem (2009) 20:245–253
DOI 10.1007/s11224-009-9427-y

ORIGINAL RESEARCH

Plant cell walls: supramolecular assembly, signalling and stress

Michael C. Jarvis

Received: 22 January 2009 / Accepted: 1 February 2009 / Published online: 19 February 2009

Abstract The structure of the primary cell wall in non-graminaceous plants is briefly reviewed and its role in providing mechanical strength to the plant and protecting it from microbial infection are described. A variety of signalling mechanisms involve oligosaccharides released by glycanase enzymes from microbial pathogens, and some of the mechanisms may be implicated in the regulation of metabolism in ripening fruits. There is some evidence that cell walls are able to sense damage or loss of integrity and that signals can accordingly be passed back to the cytoplasm. Primary cell walls must combine the mechanical and other functions with the capacity to grow in a controlled way. A modification of the 'Molecular Velcro' model developed originally to describe deformation of wood is used to predict load-deformation curves like those described by the Lockhart equation for the relationship between turgor stress and growth. Predicting a stress threshold for growth does not require the assumption of enzyme activity, although in fact enzyme activity is indeed required to permit growth at the rates normally observed.

Keywords Plant growth · Morphogenesis · Cellulose · Hemicellulose · Pectin · Signalling

Introduction

The cell walls of plants provide protection for the cell against a wide variety of mechanical, microbial and other stresses. The protection that the cell walls give to the cell can be passive, predating the onset of the stress; or it can be active, entailing changes to the cell wall structure in response to and counteracting the stress. These protective functions have to be compatible with the capacity of primary cell walls to grow as the cells inside them grow, and with their capacity to control and orient that growth; constraints that are met in somewhat different ways in different families of higher and lower plants. This article will focus on the responses of the cell walls of higher plants to stress, with the emphasis on mechanical stress, and will explore the conflicting demands of growth and of stresses imposed by the plant's environment.

M. C. Jarvis (✉)
Chemistry Department, Glasgow University,
Glasgow G12 8QQ, Scotland, UK
e-mail: mikej@chem.gla.ac.uk

Primary cell walls: structure

The structure and biosynthesis of primary cell walls have been extensively reviewed [1–3]. In summary, they are hydrated biocomposite materials in which the fibre phase consists of individual cellulose microfibrils approximately 3 nm in diameter. Each microfibril is spun out as a unit from a terminal synthase complex moving in the plane of the cell membrane, so that the direction in which the terminal complex moves determines the orientation of the microfibril [2].

The microfibrils are embedded in a matrix of non-cellulosic polysaccharides and smaller quantities of protein, synthesised in the Golgi and secreted by exocytosis to the inner face of the cell wall [4, 5]. The complement of non-cellulosic polysaccharides varies between the plant taxa, from tissue to tissue and from one part of the cell surface to another [6]. Traditionally a distinction is made between the pectins and the hemicelluloses, although the difference between these two polymers classes is not easy to define. Pectins are the subject of a later article in this volume [7].

Descriptively, pectins contain the monosaccharide galacturonic acid or its methyl ester and are abundant in the primary cell walls of most of the higher plants except the grasses, cereals and some of their related species. While long linear segments of galacturonan are usually present, there are also highly branched structures including, in order of abundance, the highly variable rhamnogalacturonan I with α(1,5)arabinan and β(1,4)galactan side-chains; the complex, well-defined rhamnogalacturonan II; and simpler xylogalacturonan segments. The principal hemicelluloses accompanying these pectic polymers are xyloglucans, with glucomannans and small quantities of xylans also present [6].

The hemicelluloses all have some degree of structural similarity to cellulose and can adopt similar, twofold helical conformations. Formerly the ability to hydrogen bond to cellulose was considered to be a distinguishing feature of the hemicelluloses, and it was surmised that hemicellulose-cellulose bonding resembled the bonding between cellulose chains within a microfibril [8]. More recently it has been shown that the arabinan and galactan segments of pectic rhamnogalacturonan I are also capable of non-covalent binding to the cellulose [9]. An alternative distinction between pectic and hemicellulosic polymers may reside, however, in chain conformation and stiffness.

The most favoured conformation of a cellulose chain is a fully extended flat ribbon, formally a 2_1 helix, stabilised by two lines of intramolecular O3H–O5 and O2H–O6 hydrogen bonds that run along the edges of the ribbon [10]. This internal hydrogen bonding prevents the chain from bending. It also fixes the dimer repeat distance within the chain at 1.03 nm, so that any favoured external hydrogen bonding arrangement between two such cellulose chains will automatically be repeated along the interface between the chains. Cellulose does not have any more capacity for interchain hydrogen bonding than other (1,4)-linked hexose polymers, so the stiffness and regularity of its chain conformation, with the ensuing coordinated formation of long runs of hydrogen bonds between two chains, may be suggested as the basis for the cohesion of cellulose microfibrils. The stiffly extended conformation of each chain within a microfibril may also explain the stability of cellulose to hydrolysis, as the necessary protonation of the glycosidic oxygen requires an increase in the bridging angle and hence some bending of the chain [11].

The polymers traditionally classed as hemicelluloses all resemble cellulose in having β(1,4)-linked monomers in the pyranose ring form. Thus, they are all capable of adopting the same conformation as cellulose. However, each glycosidic linkage is flanked only by one intramolecular hydrogen bond and not two. In the xyloglucans most of the glucose units forming the cellulose-like main chain carry a xylose substituent on O-6. In the xylans O-6 is absent. In the mannose residues of glucomannans the O2H group points in the wrong direction to permit a hydrogen bond to the next residue in the chain. We can therefore predict that hemicellulose chains are less stiff than cellulose chains and less stable in the cellulose-like conformation that is argued above to provide cellulose microfibrils with their mechanical and chemical stability. These properties of hemicelluloses are consistent with their ability to hydrogen bond onto the cellulose microfibrils but not to form microfibrils on their own.

Fully extended chain conformations of the pectic polysaccharides, on the other hand, are not stabilised in this way by intramolecular hydrogen bonding. These polymers are therefore normally rather flexible and in hydrated cell walls they exist in loose and random-coil conformations [12]. Using ^{13}C-NMR methods it is possible to show that the thermal mobility of the pectic arabinans and galactans is greater than that of any other components of the hydrated cell wall and that their time-averaged conformations are similar to those of the same polysaccharides in aqueous solution, so that in their native state they may be described as 'tethered liquids' [13]. A semi-quantitative measure of the chain flexibility of a polymer is its persistence length in solution, which in simple terms means the length over which the chain stays nearly straight. Pectic arabinans and galactans have typical persistence lengths of 3–4 nm [12] whereas hemicelluloses have persistence lengths of the order of 8 nm [14]. Persistence lengths of pectic galacturonans vary greatly according to charge density and the pH and ionic strength of the environment, because electrostatic repulsion between successive free carboxyl groups extends the chain [12].

These differences in chain flexibility may be assumed to affect the way in which the polymers concerned can contribute to the mechanical properties of the cell wall. A chain that is long enough and flexible enough to coil can show entropic (rubber) elasticity when stretched. This behaviour is less likely for the relatively stiff hemicellulose chains, which are also incapable of being stretched by the alternative enthalpic mechanism involving ring deformation by the axial glycosidic linkages operating as molecular levers [15]. The opportunities for elasticity of the cell wall polymers are difficult to evaluate in detail when we have incomplete knowledge of which chains are covalently attached to which.

Thus, whilst for many years there has been some vagueness as to how the pectic and hemicellulosic polymers should be defined, their properties differ enough to justify maintaining the distinction between them.

The cellulose microfibrils are spaced about 10–20 nm apart in the plane of the cell wall [16]. This means that any object of larger dimensions, such as an aphid stylet, a bacterium or the appressorium of a fungal pathogen, must

break, degrade or dislodge cellulose microfibrils before it can penetrate into the cell. Significant forces [17, 18] or an array of hydrolytic enzymes are implicated when this happens [19, 20]. Smaller invasive objects that can pass between the microfibrils must nevertheless penetrate a network of non-cellulosic polymers, principally the pectins. There is a considerable evidence that the pore size of the cell wall is limited by the pectic network and is of the order of several nm, sufficient to allow low-MW molecules and peptides up to about 15 kD to pass through them [21–23].

The cell wall therefore filters potential signalling molecules, allowing only those under the MW cut-off to reach the cell membrane. The implications are profound. Plant hormones such as indole acetic acid are small molecules that can pass freely through the pectic network. Other forms of intercellular signalling that are necessary for plant morphogenesis tend to use the symplastic route through plasmadesmata (membrane-lined pores through the cell wall that link the cytoplasm of one cell to that of the next) instead of the apoplastic route across the cell wall itself [24]. When cells of a foreign organism succeed in making intimate contact with a plant cell, they do so by first degrading their way through the plant cell wall and then producing haustorial structures that invaginate the host cytoplasm: examples are biotrophic pathogens such as late blight on potatoes [25] and parasitic higher plants like mistletoe [26]. In these cases a close association cannot be established until the signalling barrier of the cell wall has been locally removed. The limited pore size of the cell wall also restricts access for enzymes produced by pathogens to degrade the cell wall itself, and the first stage of pathogenic invasion can involve pectin-degrading enzymes of low enough molecular mass to move relatively freely within the pectic network, depolymerise pectins and thus increasing the pore size [20, 25, 27].

Primary cell walls in pathogenesis

The pectic network is a battleground between plants and their microbial pathogens. Where fungi or bacteria invade living plant tissues their growth, with the exception of haustoria, is confined to the apoplast, i.e. the extracellular region comprising cell walls, intercellular spaces and in some cases xylem vessels. The ability to grow within the cell wall is therefore normally essential and the pathogen synthesises a battery of polysaccharide-degrading enzymes, with polygalacturonases commonly appearing first [27]. The plant host can then activate a number of forms of defence. Galacturonan fragments of approximate DP 10, released by microbial galacturonan-degrading enzymes, act as signalling agents called ‘elicitors’ that initiate defence reactions [28–30]. Polygalacturonase inhibitor proteins of plant origin reduce the degradation of the pectic network and, more importantly, alter the balance of products in favour of the chain lengths active as elicitors [31]. In cereal species where arabinoxylans replace pectins as the most abundant group of cell wall polysaccharides, these arabinoxylans are degraded by fungal xylanases which in turn can be inhibited by specific proteins synthesised by the host plant [32]. During the pathogenic interactions involving certain fungi the host cell also synthesises β-glucanases that depolymerise structural polymers of the fungal cell wall and can be inhibited by fungal proteins [33]. Again specific oligosaccharide products function as signalling molecules, eliciting a cascade of events that lead to the hypersensitive death of the plant cell concerned and the synthesis of toxins and barrier polymers by neighbouring cells to confine the site of infection.

Oligomeric fragments of cell wall polysaccharides function as signalling molecules in a number of other situations where the parent polysaccharide undergoes depolymerisation, and the term ‘oligosaccharins’ is sometimes used to describe these molecules. Depolymerisation of pectic polysaccharides is a common feature of the softening cell walls of ripening fruit, and in some species oligomeric galacturonan fragments contribute to positive feedback modulation of the ripening process [28]. There is also evidence for some degree of pectin turnover during the remodelling of the primary cell wall that accompanies growth, but in this case pectic fragments do not appear to play a signalling role [28]. However, morphogenetic activity has been demonstrated for oligomeric xyloglucan fragments, whether these function as signalling molecules in the conventional sense or as analogues, too short to be functional, of the native xyloglucans that bridge between cellulose microfibrils in growing cell walls as these xyloglucans are cleaved and rejoined by xyloglucan endotransglycosylase during cell expansion [34].

There is evidence that signalling oligosaccharides involved in pathogenesis, acting through the ethylene, jasmonate and salicylic acid pathways, can influence the plant development even in the absence of any pathogen [28, 29, 35]. Conversely non-pathogenic damage to cell walls, resulting for example from defective cellulose synthesis, leads in some circumstances to developmental changes that augment resistance to pathogens [36, 37]. It has been suggested [36, 38] that this is evidence for a mechanism to perceive disruption or locally reduced capacity to withstand mechanical stress within the cell wall, together with a signalling route into the cytoplasm via a receptor-like kinase. Wall-associated kinases have also been suggested as components of a signalling mechanism from cell wall to cytoplasm [39]. Developmental responses to external mechanical stress are well established [40],

although the mechanisms are not as well understood in higher plants as in yeasts [41]. An intriguing example is the induction of the complete developmental process leading to a new leaf by local application of expansin protein to cause local cell wall loosening [42].

There is thus a wide range of ways in which the primary cell walls of plants are adapted to participate in defence reactions, and some of the mechanisms overlap with the machinery by which plant cells grow and plant morphology emerges. The most conspicuous function of the plant cell wall is to withstand mechanical loads, and its behaviour in the face of externally imposed mechanical stresses from wind and gravity is particularly difficult to disentangle from its response to the internal stress of turgor pressure, a phenomenon that lies at the heart of plant growth and morphogenesis.

Growth of cell walls

Plants do not grow by accretion of cells. Instead, plant cells expand inside their primary walls and divide as they expand, remaining firmly attached to their neighbouring cells throughout. Cell expansion is driven by turgor (osmotic) pressure within the cell membrane, which normally is pressed tightly against the inside of the cell wall. But it is the cell wall's capacity for controlled stretching that modulates the rate and direction of cell expansion: turgor pressure is not normally the limiting factor [43, 44]. The gradual, long-term cell expansion characteristic of growth requires enzyme activity [45]. Enzymatically inactive cell walls show a range of viscoelastic responses to external stresses but do not grow. The enzymes required for growth include expansins, which are difficult to characterise in vitro but may act at the hydrogen bonded interface between cellulose and xyloglucan [45]. A strong case has also been made for the involvement of xyloglucan-specific glucanases [46] and endotransglycosylases, and a wide range of further enzymes have been implicated [47].

To understand the mechanism by which cell walls expand during growth it is clearly necessary to understand first how they respond non-enzymatically to external mechanical stress, since the two phenomena are superimposed [45]. Primary cell walls are normally loaded in tension, the cell within being inflated by turgor. Thus, when a non-woody plant bends in the wind, the tensile stress on the windward side is added to the axial component of the existing turgor stress, while on the side away from the wind the compressive stress is carried by the turgor pressure itself. This mechanical strategy puts a limit on the height of non-woody plants: they cannot grow to a height beyond that at which compressive stresses due to wind and gravitational loading would exceed the maximum practicable turgor pressure.

The relationship between turgor pressure Π and growth rate in one or more dimensions is given by the Lockhart equation [48]:

$$\text{Growth rate } = K(\Pi - \Pi_0)$$

where K is a constant and Π_0 is a threshold turgor pressure below which growth does not occur. Many growing plant systems conform to this rule but the reason for the existence of a threshold turgor pressure has not been satisfactorily explained.

It should be noted that the Lockhart equation describes only steady-state, irreversible growth of the kind that is known to be enzymatically mediated. Below the threshold turgor pressure the steady-state growth rate is zero but the cell walls still show reversible, non-enzymatic, and viscoelastic deformation behaviour in short-term experiments or under fluctuating external stresses like those imposed by wind.

The load-deformation behaviour described by the Lockhart equation is not unique. It is characteristic of other biological materials that comprise microfibrils, oriented at an angle to the direction of stress, embedded in a hydrated matrix. Wood [49] and tendon [50], for example, show similar load-deformation curves although the upper portion, beyond the yield point, describes irreversible extension that is not enzymatically assisted.

Because wood is a major engineering material, the modelling of its mechanical properties is further advanced than the modelling of most other plant and mammalian materials. While much of the effort has been devoted to constitutive, 'top-down' models that take little account of the structure of wood at the molecular level [51–53], a recent group of very simple models start at this level and work upwards towards macroscopic properties. The first of these was the 'Molecular Velcro' model [49]. We have recently published a modification of this model based on a more realistic mechanism for cohesion between cellulose microfibrils [54], while an alternative formulation in terms of localised damage has also been suggested [53]. In the remainder of this article the extension of the original and modified 'Molecular Velcro' models to growing primary cell walls will be addressed.

The mechanical properties of wood are dominated by the S_2 layer of the secondary cell wall, in which the cellulose microfibrils, aggregated into bundles 10–20 nm thick, are wound around the cell in a rather regular right-handed helix so that their angle to the cell axis, the microfibril angle, varies from about 5° to 45° under developmental control [55]. Variation in microfibril angle makes little difference to the strength of wood but changes its longitudinal stiffness over an order of magnitude, and this variation in stiffness is fully exploited in the adaptation of trees to their environment [54].

The 'Molecular Velcro' model [49] applies to wood with a microfibril angle large enough (>15° approximately) to ensure that stretching of the microfibrils can be neglected and that deformation is confined to the matrix between them. In these circumstances shear deformation in the matrix leads to right-handed twisting of the wood cell. If the cell cannot twist because it is bonded to its neighbours, shear must be balanced by an increase in the distance between microfibrils which on its own would twist the cell to the left [49, 56]. The linear, elastic region of the load-deformation curve can be explained in this way with reasonable assumptions about the shear and tensile moduli of the hemicellulose–lignin complex forming the matrix [57]. In the original version of the 'Molecular Velcro' model it is assumed that at the yield point, the shear stress in the matrix reaches a point where entanglement interactions between hemicelluloses attached to adjacent microfibrils are unravelled and the shear modulus falls to zero [56]. In the modified model the assumption is that below the yield point shear is resisted mainly by hemicellulose chains that bridge between microfibrils, and that these chains are detached from the microfibrils by the tensile stress component to reduce the shear modulus at the yield point [54]. The two models differ, therefore, in the trigger for the loss of shear stiffness at the yield point but with suitable parameterisation they lead to quite similar predicted load-deformation curves and to predictions about the variation of microfibril angle with strain that match well with experiment [49]. There are also different assumptions about microfibril aggregation but these do not affect the geometrical basis of either model.

The key assumptions of the two models can be extended with minor modifications to primary cell walls. First, in most primary cell walls the microfibrils are not arranged in a regular helix. In the walls of isotropically growing cells the distribution of microfibril orientations is essentially random [16]. Where growth is uniaxial the distribution is centred around 90° to the axis of growth, but varies considerably from layer to layer without, in general, a consistent bias to right or left [58–61]. In a cell that has already grown uniaxially there is normally a trend towards smaller microfibril angles in the older, outer microfibril layers [60, 62]. Twisting is not likely to be an issue, therefore, but modelling needs to be restricted to individual cell wall domains within which the microfibril orientation is approximately uniform. Microfibril angles are in general much larger than in wood [55] so the assumption that cellulose does not extend is certainly valid for primary cell walls. However, the primary wall microfibrils are altogether different in diameter and spacing from the microfibril aggregates of wood cell walls, and the geometrical parameters need to be established anew.

The cellulose microfibrils of the primary cell wall are approximately 3 nm in diameter and typically make up about one-third of the dry mass of the cell wall, but the percentage of its volume that they occupy in vivo is smaller and less certain because much of the matrix between the microfibrils consists of water. A typical water content of 80% is suggested by the small number of experiments that have been carried out on primary cell walls [63–66]. These figures lead to a mean centre-to-centre microfibril spacing of 11 nm. The spaces between the microfibrils are thus similar in width to the spaces between the microfibril aggregates in water-saturated wood [67], although the primary wall microfibrils themselves are much thinner. The persistence length of the xyloglucans in primary cell walls is similar to that of the glucomannan and glucuronoxylan hemicelluloses in wood, of the order of 8 nm [14, 54], so that they are likely to form slanting bridges from one microfibril to the next, rather than to coil and entangle between the microfibrils. Such bridges have indeed been observed by electron microscopy [16] and have generally been assumed to comprise xyloglucan chains. The arabinan and galactan side-chains of pectin, on the other hand, are much more flexible with persistence lengths of only 2–3 nm and are more likely to adopt random-coil conformations in the space available to them [12].

Slanting xyloglucan bridges of the type suggested are very well positioned to resist shear—sliding forces—between the microfibrils to which they are attached. However tension, pulling the microfibrils directly apart, will readily strip the bound xyloglucan segments from the microfibril surface [54]. A 10 nm segment of bound xyloglucan will be bound to the microfibril by 20–30 hydrogen bonds, all of which must be broken almost simultaneously to overcome the resistance to shear. In contrast, microfibril separation will peel off the xyloglucan chain in such a way that only one or two monomer units are in course of detachment, and 2–3 hydrogen bonds in course of cleavage, at any one time. Using a value of 2.8×10^{-20} J for the energy of formation of a single hydrogen bond [68] and assuming that it can be considered broken when stretched by 0.1 nm, it can be calculated that a tensile force of about 1 nN would be needed to peel off a 10 nm xyloglucan segment breaking hydrogen bonds three at a time, or a 10 nN shear force breaking all the hydrogen bonds at once. These forces are probably overestimated because new bonds to water will form as soon as an intermolecular hydrogen bond is broken, but the association clearly resists shear much more than tension.

Quantifying the geometry involved as for wood [54], we consider a primary walled cell with its microfibril orientation at a large angle μ to the cell axis, sufficient to ensure that if the turgor pressure exceeds the Lockhart threshold then growth will be essentially axial and radial growth can be neglected. The axial stress on the cell wall $\sigma_x = \Pi A_L / A_W$, where Π is the turgor pressure and A_L/A_W is the ratio

of lumen area to wall area in the cross-section of the cell. Alternatively σ_x may represent an external, axial stress applied to the cell.

The nanoscale geometry envisaged is shown in Fig. 1. The macroscopic axial stress σ_x is aligned parallel to the cell axis at an angle μ to the local microfibril orientation,

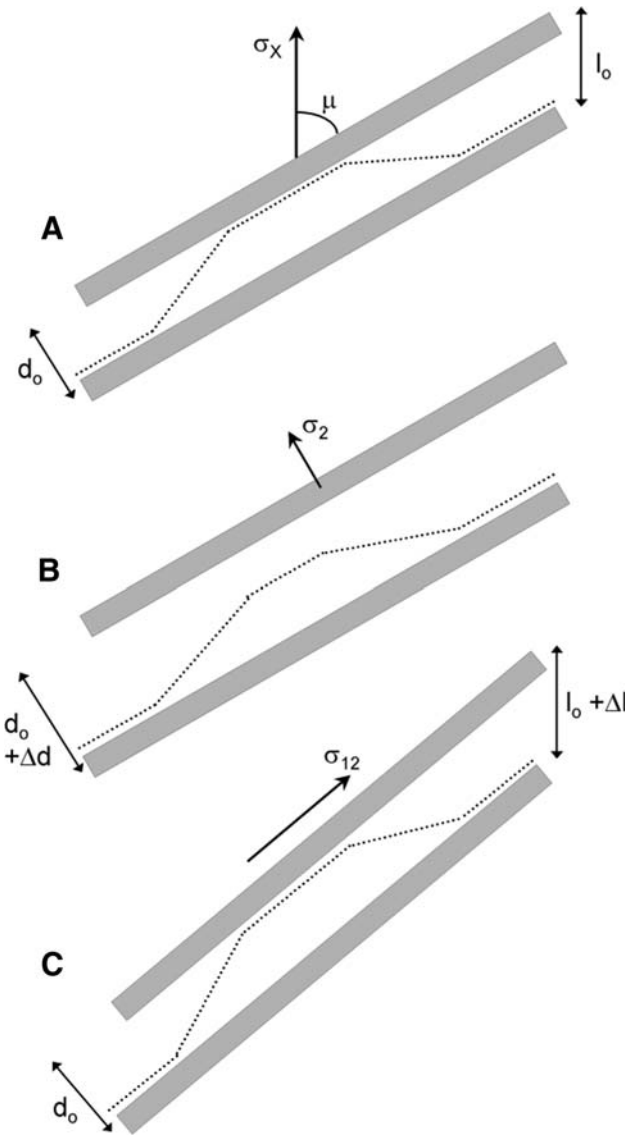

Fig. 1 Stages in the elongation of a primary cell wall according to the modified Molecular Velcro model. **a** Initial state of the system with a xyloglucan chain (*dotted*) bridging between two cellulose microfibrils aligned at an angle μ to the cell axis. The axial stress σ_x can be resolved into two components, σ_{12} parallel to the microfibrils and σ_2 normal to the microfibrils. **b** The microfibrils have separated under the influence of σ_2 and a bound xyloglucan segment has been peeled off the upper microfibril. The whole domain is skewed slightly to the left. **c** The microfibrils have rotated so that the domain is no longer skewed and the microfibril spacing has returned to its original value, restoring contact between the xyloglucan segment and the upper microfibril but in a different position from before. The whole process has increased the axial length of the domain by Δl

and may be divided into two components: the local shear stress σ_{12} parallel to the microfibrils and the local tensile stress σ_2 at 90° to the microfibrils. The corresponding components ε_{12} and ε_2 of the overall axial strain ε_x within the matrix between the microfibrils are related to σ_{12} and σ_2 by the shear and tensile moduli G and E, respectively.

If the nanoscale structure shown is embedded within a network of cell walls so that each cell is prevented from twisting, then ε_{12} and ε_2 must be balanced in such a way that the overall strain ε_x remains vertical, as shown in Fig. 1. Denoting $m = \cos\ \mu$, $n = \sin\ \mu$ with the suffix o denoting the initial state prior to application of tensile stress along the cell axis, then the axial strain $\varepsilon_x = m/m_0 - 1$ and:

Tensile strain in matrix between fibrils $\varepsilon_2 = (mn - m_0 n_0)/m_0 n_0$

Shear strain in matrix between fibrils $\varepsilon_{12} = (m_2 - m_0^2)/m_0 n_0$

It would be anticipated that application of the initial stress σ_x would lead to separation of the microfibrils (ε_2) in preference to shear (ε_{12}), for two reasons: because the microfibrils are oriented at a very large angle to the stress, and because slanting xyloglucan cross-bridges of the kind shown are well placed to resist shear but easily lifted off the microfibril by tensile forces. More precisely this will happen when the ratio of local shear to tensile stress σ_{12}/σ_x does not exceed $L/2$ where L is the number of cellulose-bound monomer residues in the loop. For example if $L = 10$ it can be shown that this condition is satisfied whenever the microfibril angle μ is greater than 11°, a threshold value far below the angles normally encountered in elongating cells.

Thus, only microfibril separation occurs until xyloglucan segments begin to detach from the cellulose surface. When a xyloglucan segment is detached in this way by the tensile stress (σ_2) component, it can no longer resist shear deformation (ε_{12}) in the same way as before. This point corresponds to a macroscopic yield stress, and beyond it shear between microfibrils occurs much more freely within the limited domain concerned. The residual resistance to shear may depend on the pectic polysaccharides.

Eventually the resulting shear between the fibrils cancels the skew of the domain and relieves any twisting strains that have been set up at its boundaries, leaving it straight but elongated. When the local axial stress is sufficiently reduced the microfibril spacing can contract and the hemicellulose loops regain contact with their adjacent fibrils, restoring the mechanical properties of the material to approximately what they were at the start.

At the end of this sequence of events, then, each xyloglucan segment is attached as strongly as before but in a different position along the fibril. The process can now

begin again. The global deformation process is then the sum of many local, repeated stick-slip events each involving detachment and re-attachment of bridging xyloglucan chains within one small domain.

Following the analysis presented for wood cell walls:

The shear strain in the matrix: $\varepsilon_{12} = (m_2 - m_0^2)/m_0 n_0$
And the corresponding shear stress in the matrix: $\sigma_{12} = G\varepsilon_{12}$
The tensile strain in the matrix: $\varepsilon_2 = (mn - m_0 n_0)/m_0 n_0$
And the corresponding tensile stress in the matrix: $\sigma_2 = E\varepsilon 2$

When the tensile strain in the matrix reaches a critical local value C, the detachment of hemicellulose loops from the fibrils triggers the reduction in the shear modulus of the matrix from G to G'.

In the stress region up to the yield point, with $\varepsilon_2 < C$, the relationships above lead to the following expression for the axial stress:

$$\sigma_x = 2m/n \cdot ((m^2 - m_0^2/m_0 n_0) \cdot G - ((m^2 - n^2)/n^2) \cdot ((mn - m_0 n_0)/m_0 n_0) \cdot E$$

Beyond the yield point, with $\varepsilon_2 > C$, the expression for the axial stress is identical except that G is replaced by G'.

Figure 2 shows computed stress–strain relationships for initial microfibril orientations at 60°, 70° and 80° to the cell axis. The parameters used were:

Tensile modulus of matrix: $E = 74$ MPa
Initial shear modulus of matrix: $G = 330$ MPa
Post-yield shear modulus of matrix: $G' = 10$ MPa
Critical local tensile strain in matrix: $C = 0.15$

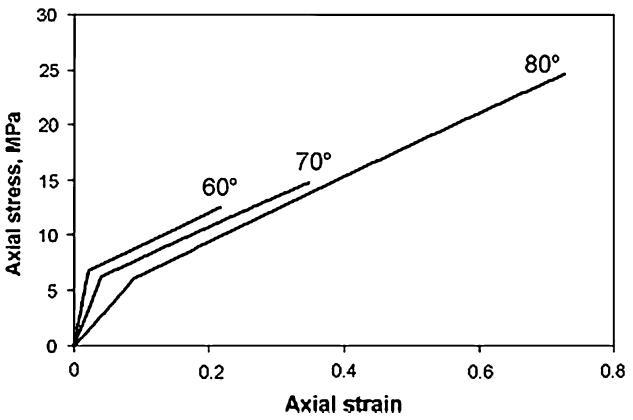

Fig. 2 Modelled load-deformation curve based on the modified Molecular Velcro model with microfibrils initially oriented at 60, 70 and 80 degrees to the cell axis. The break in slope corresponds to the transition from reversible (elastic) extension to irreversible extension, at the stress where xyloglucan segments become detached from cellulose microfibrils as shown in Fig. 1

This analysis shows that even without taking enzymatic action into account, it is possible to explain the two-sage nature of deformation in the primary cell wall where, at relatively small stresses, it is quite stiff and deforms elastically (or viscoelastically: the viscous component is not considered here but may be attributable to pectins): while at stresses above a threshold corresponding to the Lockhart value, the cell wall becomes more readily deformed and the deformation is irreversible. The latter stage corresponds to elongation growth and is presumably facilitated by enzymes, while diverse further enzyme activities are required to integrate new material into the cell wall as it elongates.

Acknowledgements Parts of the work described here were supported by grants from EPSRC, BBSRC and the Scottish Funding Council.

References

1. Zhong RQ, Ye ZH (2007) Regulation of cell wall biosynthesis. Curr Opin Plant Biol 10:564–572. doi:10.1016/j.pbi.2007.09.001
2. Somerville C, Bauer S, Brininstool G, Facette M, Hamann T, Milne J, Osborne E, Paredez A, Persson S, Raab T, Vorwerk S, Youngs H (2004) Toward a systems approach to understanding plant cell walls. Science 306:2206–2211. doi:10.1126/science.1102765
3. Taylor NG (2008) Cellulose biosynthesis and deposition in higher plants. New Phytol 178:239–252. doi:10.1111/j.1469-8137.2008.02385.x
4. Fry SC (2004) Primary cell wall metabolism: tracking the careers of wall polymers in living plant cells. New Phytol 161:641–675. doi:10.1111/j.1469-8137.2004.00980.x
5. Scheller HV, Jensen JK, Sorensen SO, Harholt J, Geshi N (2007) Biosynthesis of pectin. Physiol Plant 129:283–295. doi:10.1111/j.1399-3054.2006.00834.x
6. Popper ZA, Fry SC (2004) Primary cell wall composition of pteridophytes and spermatophytes. New Phytol 164:165–174. doi:10.1111/j.1469-8137.2004.01146.x
7. Voragen AGJ (2009) Struct Chem (this issue)
8. Levy S, Maclachlan G, Staehelin LA (1997) Xyloglucan sidechains modulate binding to cellulose during in vitro binding assays as predicted by conformational dynamics simulations. Plant J 11:373–386. doi:10.1046/j.1365-313X.1997.11030373.x
9. Ralet MC, Crepeau MJ, Lefebvre J, Mouille G, Hofte H, Thibault JF (2008) Reduced number of homogalacturonan domains in pectins of an Arabidopsis mutant enhances the flexibility of the polymer. Biomacromolecules 9:1454–1460. doi:10.1021/bm701321g
10. Jarvis M (2003) Chemistry: cellulose stacks up. Nature 426:611–612. doi:10.1038/426611a
11. Jarvis MC (2000) Interconversion of the I alpha and I beta crystalline forms of cellulose by bending. Carbohydr Res 325:150–154. doi:10.1016/S0008-6215(99)00316-X
12. Cros S, Garnier C, Axelos MAV, Imberty A, Perez S (1996) Solution conformations of pectin polysaccharides: determination of chain characteristics by small angle neutron scattering, viscometry, and molecular modeling. Biopolymers 39:339–352. doi:10.1002/(SICI)1097-0282(199609)39:3<339::AID-BIP6>3.0.CO;2-P
13. Ha MA, Vietor RJ, Jardine GD, Apperley DC, Jarvis MC (2005) Conformation and mobility of the arabinan and galactan side-

chains of pectin. Phytochemistry 66:1817–1824. doi:10.1016/j.phytochem.2005.06.001
14. Picout DR, Ross-Murphy SB, Errington N, Harding SE (2003) Pressure cell assisted solubilization of xyloglucans: tamarind seed polysaccharide and detarium gum. Biomacromolecules 4:799–807. doi:10.1021/bm0257659
15. Williams MAK, Marshall AT, Anjukandi P, Haverkamp RG (2007) Investigation of the effects of fine structure on the nanomechanical properties of pectin. Phys Rev E Stat Nonlin Soft Matter Phys 76:021927
16. McCann MC, Wells B, Roberts K (1990) Direct visualization of cross-links in the primary plant-cell wall. J Cell Sci 96:323–334
17. Bechinger C, Giebel KF, Schnell M, Leiderer P, Deising HB, Bastmeyer M (1999) Optical measurements of invasive forces exerted by appressoria of a plant pathogenic fungus. Science 285:1896–1899. doi:10.1126/science.285.5435.1896
18. Howard RJ, Ferrari MA, Roach DH, Money NP (1991) Penetration of hard substrates by a fungus employing enormous turgor pressures. Proc Natl Acad Sci USA 88:11281–11284. doi:10.1073/pnas.88.24.11281
19. Herbert C, O'Connell R, Gaulin E, Salesses V, Esquerre-Tugaye MT, Dumas B (2004) Production of a cell wall-associated endopolygalacturonase by *Colletotrichum lindemuthianum* and pectin degradation during bean infection. Fungal Genet Biol 41:140–147. doi:10.1016/j.fgb.2003.09.008
20. Deising HB, Werner S, Wernitz M (2000) The role of fungal appressoria in plant infection. Microbes Infect 2:1631–1641. doi:10.1016/S1286-4579(00)01319-8
21. Carpita N, Sabularse D, Montezinos D, Delmer DP (1979) Determination of the pore size of cell walls of living plant cells. Science 205:1144–1147. doi:10.1126/science.205.4411.1144
22. Baron-Epel O, Gharyal PK, Schindler M (1988) Pectins as mediators of wall porosity in soybean cells. Planta 175:389–395. doi:10.1007/BF00396345
23. Rondeau-Mouro C, Defer D, Leboeuf E, Lahaye M (2008) Assessment of cell wall porosity in Arabidopsis thaliana by NMR spectroscopy. Int J Biol Macromol 42:83–92. doi:10.1016/j.ijbiomac.2007.09.020
24. Lucas WJ, Lee JY (2004) Plant cell biology: plasmodesmata as a supracellular control network in plants. Nat Rev Mol Cell Biol 5:712–726. doi:10.1038/nrm1470
25. Hardham AR (2001) The cell biology behind Phytophthora pathogenicity. Australas Plant Pathol 30:91–98. doi:10.1071/AP01006
26. Pate JS (2001) Haustoria in action: case studies of nitrogen acquisition by woody xylem-tapping hemiparasites from their hosts. Protoplasma 215:204–217. doi:10.1007/BF01280315
27. Jones TM, Albersheim P, Anderson AJ (1972) Host–pathogen interactions. 4. Studies on polysaccharide-degrading enzymes secreted by *Fusarium oxysporum* f. sp. *lycopersici*. Physiol Plant Pathol 2:153. doi:10.1016/0048-4059(72)90023-9
28. Dumville JC, Fry SC (2000) Uronic acid-containing oligosaccharins: their biosynthesis, degradation and signalling roles in non-diseased plant tissues. Plant Physiol Biochem 38:125–140. doi:10.1016/S0981-9428(00)00163-7
29. Simpson SD, Ashford DA, Harvey DJ, Bowles DJ (1998) Short chain oligogalacturonides induce ethylene production and expression of the gene encoding aminocyclopropane 1-carboxylic acid oxidase in tomato plants. Glycobiology 8:579–583. doi:10.1093/glycob/8.6.579
30. Humphrey TV, Bonetta DT, Goring DR (2007) Sentinels at the wall: cell wall receptors and sensors. New Phytol 176:7–21. doi:10.1111/j.1469-8137.2007.02192.x
31. Di CX, Zhang MX, Xu SJ, Cheng T, An LZ (2006) Role of polygalacturonase-inhibiting protein in plant defense. Crit Rev Microbiol 32:91–100. doi:10.1080/10408410600709834
32. Sansen S, De Ranter CJ, Gebruers K, Brijs K, Courtin CM, Delcour JA, Rabijns A (2004) Structural basis for inhibition of *Aspergillus niger* xylanase by *Triticum aestivum* xylanase inhibitor-I. J Biol Chem 279:36022–36028. doi:10.1074/jbc.M404212200
33. York WS, Qin QA, Rose JKC (2004) Proteinaceous inhibitors of endo-beta-glucanases. Biochim Et Biophys Acta-Proteins Proteomics 1696:223–233. doi:10.1016/j.bbapap.2003.07.003
34. Takeda T, Furuta Y, Awano T, Mizuno K, Mitsuishi Y, Hayashi T (2002) Suppression and acceleration of cell elongation by integration of xyloglucans in pea stem segments. Proc Natl Acad Sci USA 99:9055–9060. doi:10.1073/pnas.132080299
35. Norman C, Vidal S, Palva ET (1999) Oligogalacturonide-mediated induction of a gene involved in jasmonic acid synthesis in response to the cell-wall-degrading enzymes of the plant pathogen *Erwinia carotovora*. Mol Plant Microbe Interact 12:640–644. doi:10.1094/MPMI.1999.12.7.640
36. Hematy K, Sado PE, Van Tuinen A, Rochange S, Desnos T, Balzergue S, Pelletier S, Renou JP, Hofte H (2007) A receptor-like kinase mediates the response of Arabidopsis cells to the inhibition of cellulose synthesis. Curr Biol 17:922–931. doi:10.1016/j.cub.2007.05.018
37. Cano-Delgado A, Penfield S, Smith C, Catley M, Bevan M (2003) Reduced cellulose synthesis invokes lignification and defense responses in Arabidopsis thaliana. Plant J 34:351–362. doi:10.1046/j.1365-313X.2003.01729.x
38. Turner SR (2007) Cell walls: monitoring integrity with THE kinase. Curr Biol 17:R541–R542. doi:10.1016/j.cub.2007.05.033
39. Wagner TA, Kohorn BD (2001) Wall-associated kinases are expressed throughout plant development and are required for cell expansion. Plant Cell 13:303–318
40. Zhou J, Wang BC, Li Y, Wang YC, Zhu LQ (2007) Responses of chrysanthemum cells to mechanical stimulation require intact microtubules and plasma membrane–cell wall adhesion. J Plant Growth Regul 26:55–68. doi:10.1007/s00344-006-0029-2
41. Philip B, Levin DE (2001) Wsc1 and Mid2 are cell surface sensors for cell wall integrity signaling that act through Rom2, a guanine nucleotide exchange factor for Rho1. Mol Cell Biol 21:271–280. doi:10.1128/MCB.21.1.271-280.2001
42. Pien S, Wyrzykowska J, McQueen-Mason S, Smart C, Fleming A (2001) Local expression of expansin induces the entire process of leaf development and modifies leaf shape. Proc Natl Acad Sci USA 98:11812–11817. doi:10.1073/pnas.191380498
43. Passioura JB, Fry SC (1992) Turgor and cell expansion: beyond the Lockhart equation. Aust J Plant Physiol 19:565–576
44. Passioura JB, Boyer JS (2003) Tissue stresses and resistance to water flow conspire to uncouple the water potential of the epidermis from that of the xylem in elongating plant stems. Funct Plant Biol 30:325–334. doi:10.1071/FP02202
45. Cosgrove DJ (2005) Growth of the plant cell wall. Nat Rev Mol Cell Biol 6:850–861. doi:10.1038/nrm1746
46. Van Sandt VST, Suslov D, Verbelen JP, Vissenberg K (2007) Xyloglucan endotransglucosylase activity loosens a plant cell wall. Ann Bot (Lond) 100:1467–1473. doi:10.1093/aob/mcm248
47. Jamet E, Albenne C, Boudart G, Irshad M, Canut H, Pont-Lezica R (2008) Recent advances in plant cell wall proteomics. Proteomics 8:893–908. doi:10.1002/pmic.200700938
48. Lockhart JA (1965) An analysis of irreversible plant cell elongation. J Theor Biol 8:264. doi:10.1016/0022-5193(65)90077-9
49. Keckes J, Burgert I, Fruhmann K, Muller M, Kolln K, Hamilton M, Burghammer M, Roth SV, Stanzl-Tschegg S, Fratzl P (2003) Cell-wall recovery after irreversible deformation of wood. Nat Mater 2:810–814. doi:10.1038/nmat1019
50. Puxkandl R, Zizak I, Paris O, Keckes J, Tesch W, Bernstorff S, Purslow P, Fratzl P (2002) Viscoelastic properties of collagen: synchrotron radiation investigations and structural model. Philos

Trans R Soc Lond B Biol Sci 357:191–197. doi:10.1098/rstb.2001.1033
51. Navi P, Rastogi PK, Gresse V, Tolou A (1995) Micromechanics of wood subjected to axial tension. Wood Sci Technol 29:411–429. doi:10.1007/BF00194199
52. Vidal-Salle E, Chassagne P (2007) Constitutive equations for orthotropic nonlinear viscoelastic behaviour using a generalized Maxwell model application to wood material. Mech Time-Depend Mater 11:127–142. doi:10.1007/s11043-007-9037-2
53. Sedighi-Gilani M, Navi P (2007) Experimental observations and micromechanical modeling of successive-damaging phenomenon in wood cells' tensile behavior. Wood Sci Technol 41:69–85. doi:10.1007/s00226-006-0094-5
54. Altaner CM, Jarvis MC (2008) Modelling polymer interactions of the 'molecular Velcro' type in wood under mechanical stress. J Theor Biol 253:434–445. doi:10.1016/j.jtbi.2008.03.010
55. Barnett JR, Bonham VA (2004) Cellulose microfibril angle in the cell wall of wood fibres. Biol Rev Camb Philos Soc 79:461–472. doi:10.1017/S1464793103006377
56. Fratzl P, Burgert I, Keckes J (2004) Mechanical model for the deformation of the wood cell wall. Z Metallk 95:579–584
57. Salmen L (2004) Micromechanical understanding of the cell-wall structure. C R Biol 327:873–880. doi:10.1016/j.crvi.2004.03.010
58. McCann MC, Stacey NJ, Wilson R, Roberts K (1993) Orientation of macromolecules in the walls of elongating carrot cells. J Cell Sci 106:1347–1356
59. Kutschera U (2008) The growing outer epidermal wall: design and physiological role of a composite structure. Ann Bot (Lond) 101:615–621. doi:10.1093/aob/mcn015
60. MacKinnon IM, Sturcova A, Sugimoto-Shirasu K, His I, McCann MC, Jarvis MC (2006) Cell-wall structure and anisotropy in *procuste*, a cellulose synthase mutant of *Arabidopsis thaliana*. Planta 224:438–448. doi:10.1007/s00425-005-0208-6
61. Sugimoto K, Himmelspach R, Williamson RE, Wasteneys GO (2003) Mutation or drug-dependent microtubule disruption causes radial swelling without altering parallel cellulose microfibril deposition in Arabidopsis root cells. Plant Cell 15:1414–1429. doi:10.1105/tpc.011593
62. Roland JC, Reis D, Mosiniak M, Vian B (1982) Cell-wall texture along the growth gradient of the mung bean hypocotyl: ordered assembly and dissipative processes. J Cell Sci 56:303–318
63. Jarvis MC (1992) Control of thickness of collenchyma cell walls by pectins. Planta 187:218–220. doi:10.1007/BF00201941
64. Redgwell RJ, MacRae E, Hallett I, Fischer M, Perry J, Harker R (1997) In vivo and in vitro swelling of cell walls during fruit ripening. Planta 203:162–173. doi:10.1007/s004250050178
65. Ishii T, Matsunaga T, Hayashi N (2001) Formation of rhamnogalacturonan II-borate dimer in pectin determines cell wall thickness of pumpkin tissue. Plant Physiol 126:1698–1705. doi:10.1104/pp.126.4.1698
66. Fleischer A, O'Neill MA, Ehwald R (1999) The pore size of non-graminaceous plant cell walls is rapidly decreased by borate ester cross-linking of the pectic polysaccharide rhamnogalacturonan II. Plant Physiol 121:829–838. doi:10.1104/pp.121.3.829
67. Fahlen J, Salmen L (2005) Pore and matrix distribution in the fiber wall revealed by atomic force microscopy and image analysis. Biomacromolecules 6:433–438. doi:10.1021/bm040068x
68. Henry M (2002) Thermodynamics of hydrogen bond patterns in supramolecular assemblies of water molecules. ChemPhysChem 3:607–616. doi:10.1002/1439-7641(20020715)3:7<607::AID-CPHC607>3.0.CO;2-A