Planta (2002) 215: 989–996
DOI 10.1007/s00425-002-0783-8

ORIGINAL ARTICLE

Elisabeth Chanliaud · Kathleen M. Burrows
George Jeronimidis · Michael J. Gidley

Mechanical properties of primary plant cell wall analogues

Received: 20 December 2001 / Accepted: 6 March 2002 / Published online: 18 June 2002

Abstract Mechanical effects of turgor pressure on cell walls were simulated by deforming cell wall analogues based on *Acetobacter xylinus* cellulose under equi-biaxial tension. This experimental set-up, with associated modelling, allowed quantitative information to be obtained on cellulose alone and in composites with pectin and/or xyloglucan. Cellulose was the main load-bearing component, pectin and xyloglucan leading to a decrease in modulus when incorporated. The cellulose-only system could be regarded as an essentially linear elastic material with a modulus ranging from 200 to 500 MPa. Pectin incorporation modified extensibility properties of the system by topology/architecture changes of cellulose fibril assemblies, but the cellulose/pectin composites could still be described as a linear elastic material with a modulus ranging from 120 to 250 MPa. The xyloglucan/cellulose composite could not be modelled as a linear elastic material. Introducing xyloglucan into a cellulose network or a cellulose/pectin composite led to very compliant materials characterised by time-dependent creep behaviour. Modulus values obtained for the composite materials were compared with mechanical data found for plant-derived systems. After comparing bi-axial and uni-axial behaviour of the different composites, structural models were proposed to explain the role of each polysaccharide in determining the mechanical properties of these plant primary cell wall analogues.

Keywords *Acetobacter* · Bi-axial tensile testing · Cellulose · Pectin · Xyloglucan

E. Chanliaud · K.M. Burrows · M.J. Gidley (✉)
Unilever Research, Colworth House,
Sharnbrook, Bedford MK44 1LQ, UK
E-mail: mike.gidley@unilever.com
Fax: +44-1234-222552

G. Jeronimidis
University of Reading, Department of Engineering,
Whitenights, Reading RG6 2AY, UK

Present address: E. Chanliaud
ULICE, ZAC "Les Portes de Riom",
BP 173, 63204 Riom Cedex, France

Abbreviation CDTA: *trans*-1,2-diaminocyclohexane-N,N,N′,N′-tetraacetic acid

Introduction

Mechanical properties of plant tissues are mainly dependent on cell walls, i.e. their intrinsic mechanical characteristics, their interactive response to turgor pressure, and the way they are attached to their neighbours. The primary (growing) plant cell wall is a heterogeneous and dynamic polymeric structure composed of a three-dimensional interwoven network of cellulose microfibrils embedded in a complex matrix of pectins, heterogeneous hemicelluloses (e.g. xylans, mannans, xyloglucans), and structural proteins. The exact nature of the interactions and bonds that hold the wall together is still uncertain, but the networks need to accommodate a variety of mechanical requirements. The wall has to be capable of bearing high tensile stresses, allowing reversible (elastic) deformation in response to turgor pressure changes, or alternatively be sufficiently extensible to allow elongation.

Quantitative mechanical information on primary walls can now be obtained in situ by micro-mechanical techniques (Hiller et al. 1996; Davies et al. 1998) but the role of each polymer family in determining these characteristics is still largely unknown, as is the distribution of stress between the various wall components. The few hypotheses found in the literature mainly extrapolate from the mechanical properties of the isolated polymers. Cellulose is traditionally quoted as the main cell-wall load-bearing component (Probine and Preston 1962). It is well established that middle-lamella pectins play a role in adhesion of adjacent cells (Van-Buren 1979; Ng et al. 1998); however, the mechanical role of primary-wall pectins is less clear. Pectin is thought to form an independent, co-extensive but synergistic network with the cellulose/hemicellulose structure (McCann and Roberts 1991; Talbott and Ray 1992; Carpita and Gibeaut 1993) but it is still debatable whether or not this network has

any load-bearing capacity (Virk and Cleland 1988; Rayle 1989). Hemicelluloses constitute a diverse group of β-(1→4)-linked backbone polysaccharides. Xyloglucans, the major hemicellulose of the growing dicotyledon cell wall, have in particular the ability to cross-link cellulose and may play a role in keeping cellulose fibrils apart (McCann et al. 1990). Studies of cellulose/xyloglucan composites under uni-axial tension show that cross-linking reduces the stiffness and strength of cellulose networks and increases their extensibility (Whitney et al. 1999).

In order to characterise mechanical properties due to individual polymers, the approach taken here is to synthesise network structures containing polymers typical of primary plant cell walls. These composite materials, based on *Acetobacter xylinus* cellulose, serve as a model system for cell wall deposition and assembly processes (Whitney et al. 1995, 1998; Chanliaud and Gidley 1999). Studying the intrinsic material properties of these cell wall analogues can give valuable information on the physics of the wall in the absence of biological interference.

In this study the mechanical role of polymers under stress conditions encountered in vivo through the effects of turgor on a cell wall were simulated by deforming the composites under equi-biaxial tension using a pressure method. This experimental set-up, combined with modelling, allowed quantitative mechanical information to be obtained. Comparison of bi-axial and uni-axial behaviour of the different composites allows conclusions about the specific role of each polymer to be drawn.

Materials and methods

Composite preparation

Acetobacter xylinus (ATCC 53524) was cultured in Hestrin Schramm medium pH 6.2 (Hestrin and Schramm 1954) containing 2% glucose. Xyloglucan/cellulose composites were obtained by dissolving 0.5% tamarind xyloglucan (purified Glyloid 3S; Dainippon Pharmaceutical Co., Osaka, Japan) in this medium. Pectin/cellulose composites were obtained by adding 0.5% apple pectin (30% esterified; prepared as described by Chanliaud and Gidley 1999) and 12.5 mM $CaCl_2$ to the medium. Three-component composites (cellulose/pectin/xyloglucan) were obtained by dissolving 0.25% xyloglucan and 0.25% apple pectin (30% esterified) in 6 mM $CaCl_2$ Hestrin Schramm medium. Incubations were performed at 30 °C. After 72 h incubation, composite material of several centimetres in diameter was collected and soluble components were washed out at room temperature by gentle agitation in excess water, or $CaCl_2$ solutions in the case of pectin-containing composites.

Composition analysis

Composite compositions were calculated from individual sugar contents on the basis of dry weights. The neutral sugars were analysed by gas chromatography after methanolysis (Quemener and Thibault 1990) and silylation (Kamerling and Vliegenthart 1982). They were separated on a capillary column (CpSil 5CB Chrompack; injection at 150 °C; 15 min to reach 210 °C; 10 min at 210 °C). Uronic acids were quantified by the *meta*-hydroxybiphenyl assay (Blumenkrantz and Hasboe-Hansen 1973) after a prehydrolysis for 15 min in 72% H_2SO_4 at 4 °C.

Mechanical tests

All tests were carried out on highly hydrated composites, which varied between 10 and 12% dry matter. The geometry of the bulge-testing device is shown in Fig. 1. It was composed of a cylindrical chamber (internal dimensions: 2 cm × 7 cm) whose upper part was closed by a lid. A circular orifice present in the lid matched the internal dimensions of the chamber (2 cm diameter). The sample (typically 0.35 mm thick) was placed between the top of the chamber and the lid and was clamped between two rubber O-rings to prevent damage. The chamber was filled with a viscous silicone oil (200/1,000 cs fluid; Dow Corning) purposely chosen not to permeate the composite structure. Bi-axial tensile deformations were obtained by applying uniform pressure to the lower side of the sample using the pressurised oil. The pressure was increased in a constant manner. The composite distended as a spherical bulge and uniform bi-axial deformation occurred in the central portion. The pressure was measured through a pressure gauge (LM20, TDCR; RDP Group, Wolverhampton, UK) and the resulting maximum deflection was recorded by a Linear Variable Displacement Transducer (LVDT, AC type, D5 300HK/L25; RDP Group, Wolverhampton, UK). Creep tests were carried out by applying a constant pressure (7 kPa, unless otherwise quoted) to the sample and recording the maximum deflection occurring as a function of time.

Uni-axial tensile deformations were performed on a miniature material-testing device, the Minimat mark II (Polymer Laboratories, Loughborough, UK). The extensions were carried out on 1 mm × 2 mm × 20 mm samples at 10 mm/min and the resulting load recorded. The strain was calculated as the percentage deformation undergone by the sample compared to its initial length. The conventional stress (Pa) was used and was calculated as the load divided by the cross-section of the sample.

Modelling

Finite element modelling (Zienkiewicz 1979) of the bulge-test geometry outlined in Fig. 1 was carried out using the MARC finite element package from MSC software, Camberley, UK. The planar geometry of the specimen under test was modelled using doubly curved thick shell 4-node quadrilateral elements, MARC element type 75. The model for the 2-cm-diameter circular specimen consisted of 328 elements, generated using the MARC automatic mesh-generation facility. The nodes at the edge of the specimen were confined in both x and y directions, so as to represent the clamping of the specimen. Pressurization by silicone oil was represented by a normal load on the elements, the load being increased incrementally. The samples were modelled using linear elastic material properties, with the calculated z displacement at the central node being compared to the measured displacement at the centre of the test piece. Gravity loading was not considered. Each individual

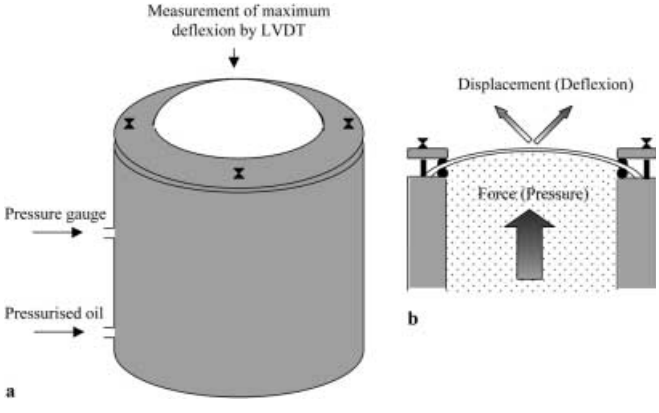

Fig. 1a, b. Schematic of the bulge-testing device. **a** Side view. **b** Section view. *LVDT* Linear variable displacement transducer

experiment was modelled, with the specimen thickness being taken from measurements on each test piece. Modelling of linear elastic material behaviour requires knowledge of two material properties, the Young's modulus and the Poisson ratio. For an assumed value of Poisson's ratio, Young's modulus can be estimated. A value for Poisson's ratio of 0.3 was used, similar to that used previously for cell wall material (Pitt and Davis 1984) and consistent with a partially compressible solid as observed experimentally. It has recently been demonstrated that calculated moduli show minimal sensitivity to Poisson's ratio for values ranging from 0.30 to 0.49 (Hiller et al. 1996).

Results

Cellulose composites

Pure cellulose material (Fig. 2a) was a randomly oriented network of well defined fibrils of 24–86 nm diameter (Whitney et al. 1995, 1998; Chanliaud and Gidley 1999). When produced into a pectin gel, an interpenetrating network is formed (Chanliaud and Gidley 1999) with pectin chains aggregated in the vicinity of cellulose fibrils (Fig. 2b). On average, 39% pectin was incorporated in the cellulose composite. Xyloglucan on the other hand formed an intimate interaction with the cellulose, with the average composite composition being 78% cellulose and 22% xyloglucan. Segments of xyloglucan molecules bound to cellulose, with other segments constituting cross-bridges between fibres (Fig. 2c; Whitney et al. 1995). As a more complete model of plant cell wall polysaccharide structure, composites based on cellulose, xyloglucan and pectin were synthesised. On average, they consisted of 63% cellulose, 22% pectin and 15% xyloglucan, a composition similar to type-I primary cell walls (McCann and Roberts 1991). Structural characteristics of both cellulose/pectin and cellulose/xyloglucan composites were found in the three-component composite. A pectin layer was seen to cover the cellulose/xyloglucan structure (Fig. 2d), but the presence of xyloglucan bridges between cellulose fibres was still visible by transmission electron microscopy and was especially apparent after pectin extraction by *trans*-1,2-diaminocyclohexane-N,N,N′,N′-tetraacetic acid (CDTA; result not shown).

Bi-axial tensile testing

If the assumption of a spherical cell is made, the force applied on the cell wall by the cell contents and cytoplasmic membrane causes equal stretches in the two directions orthogonal to the cell radius. Its effect on the wall can then be described as a bi-axial deformation of a thin material. In order to mimic this effect, a bi-axial tensile device commonly called a ''bulge testing'' device (Fig. 1) was constructed. A uniform pressure was applied to one side of a circular sample. The resulting deflection was measured at the centre of the sample up to the rupture point.

Pressure/deflection curves were recorded for different composites (Fig. 3). Reasonably reproducible results were obtained for each composite, with major differences being observed between them. The cellulose composite behaved as a relatively stiff and strong material, i.e. substantial pressure was required for deformation, with rupture occurring at high pressure.

When pectin was incorporated into the cellulose network, the failure deformation was only slightly reduced (about 1.75 mm displacement) but the rupture pressure was greatly reduced, indicating an appreciably weaker structure. Removal of pectin by CDTA

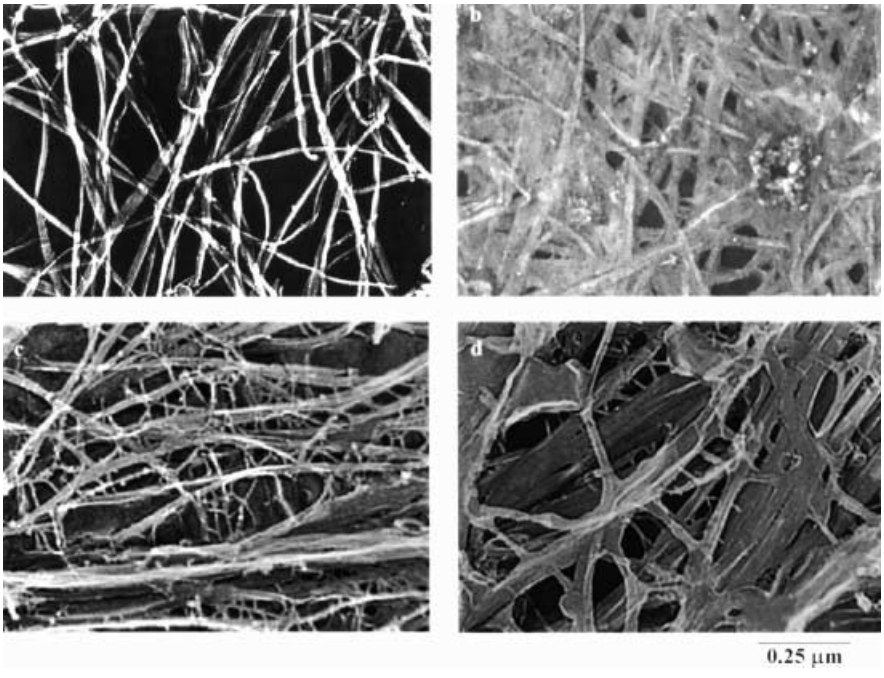

Fig. 2. Transmission electron micrographs of tungsten/tantalum/carbon replicas of cellulose (**a**), cellulose/pectin (**b**), cellulose/xyloglucan (**c**) and cellulose/pectin/xyloglucan (**d**) composites

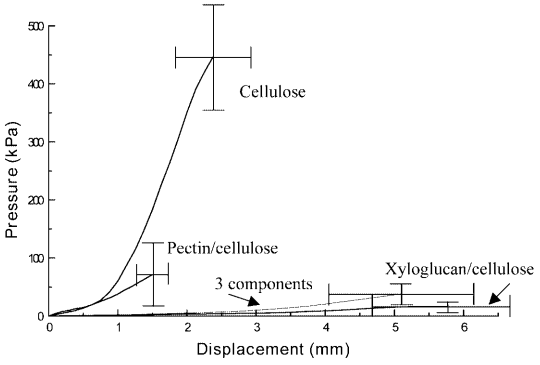

Fig. 3. Typical pressure/displacement curves obtained for bi-axial tensile testing of *Acetobacter xylinus* cellulose composites. Average of 10 measurements ± SE

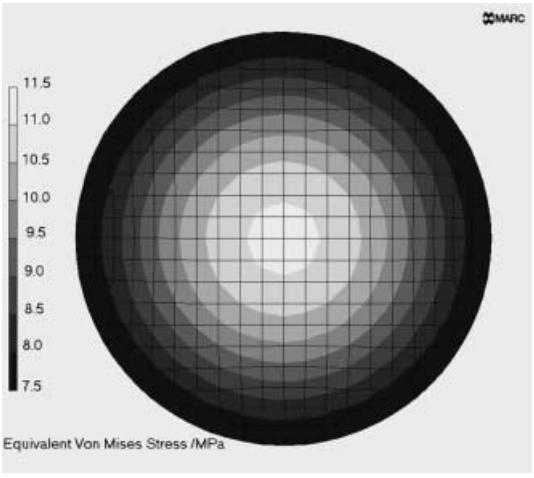

Fig. 4. Calculated stress distribution in the *Acetobacter* cellulose material with $E = 500$ MPa. At an applied pressure of 200 kPa, the maximum calculated stress is 11 MPa

treatment did not modify the mechanical properties of the sample (result not shown). The observed weakening effect of pectin on cellulose was therefore not directly due to pectin present in the composite, but must reflect a modification of the cellulose network during its deposition, as inferred previously from uni-axial tensile tests (Chanliaud and Gidley 1999).

The incorporation of xyloglucan into the cellulose network caused a major increase in extensibility, up to three times higher than cellulose, together with a very low stiffness. A change in mechanical response of this magnitude suggests a qualitative change in the way load-bearing components interact. Mechanical behaviour of the three-component composite was intermediate between pectin and xyloglucan composites and somewhat closer to the latter with characteristic high extensibility and low resistance to pressure.

Modelling of bi-axial tensile testing

The geometry of the bulge-type bi-axial tensile test leads to a non-linear force vs. displacement curve, even for linear elastic materials. However, simulation of pressure/displacement data can be used to test hypotheses of general materials behaviour, and to obtain modulus values by quantitative comparison between simulation and experiment. The simplest assumption is that the material behaves as a linear elastic solid until it fails. If the experimental curves are comparable to the calculated curves, then this assumption is consistent with the observed behaviour.

The finite element method used allows the local stress (force applied/original area) and strain (extension/original length) to be calculated, and subsequently visualised. Figure 4 shows that the maximum calculated stress occurred at the centre of the sample. It is reassuring that the majority of samples failed in the centre of the test-piece. This suggests that the samples were relatively homogeneous, and that the test was not suffering from edge effects, which would be expected to lead to failure where the sample is held. The experimental pressure vs. displacement curves obtained are shown in Fig. 3. In order to estimate the material properties of the various composites, calculated curves were produced for each of the composites, assuming linear elastic behaviour.

For cellulose alone, the modelling showed that the material could be regarded as linear elastic (Fig. 5a) up to a total strain of 3.1% for $E = 500$ MPa (that is, a strain of 1.55% in each of two orthogonal directions). Above this strain, the material became 'strain softening' – that is, the material extends more for the same increase in pressure, suggesting possible disruption of the fibrous network. The calculated stress at 3.1% strain (the apparent elastic limit) was approximately 11 MPa (again for $E = 500$ MPa). Failure occurred outside the apparent linear elastic region, so it was not strictly possible to use the comparison of model data and experimental curves to obtain the strain at failure. Modelling of the region up to the apparent elastic limit may be used to obtain an estimate of the material modulus, by comparison of calculated pressure/displacement curves with experimental ones. Calculations on extremes of 10 samples suggested that the modulus of the cellulose material varied between 200 and 500 MPa.

Generally, the cellulose/pectin composites behaved as linear elastic materials until they failed (Fig. 5b). The model could therefore be used to gain an estimate of both stress and strain at failure. The elastic modulus was found to vary between 120 and 250 MPa, for experimental extremes. At failure, the material with a calculated modulus of 120 MPa was under a calculated stress of 4 MPa and at a total strain of 4.8% (2.4% strain in each of two orthogonal directions).

The cellulose/xyloglucan system (Fig. 5c) could not be modelled as a linear elastic material. The composite could be exhibiting non-linear elasticity, plasticity or visco-elasticity. The three-component system (Fig. 5d) gave a better fit to linear elastic behaviour, but this was not as convincing as that seen for cellulose and cellulose/pectin. The rupture strains for these systems

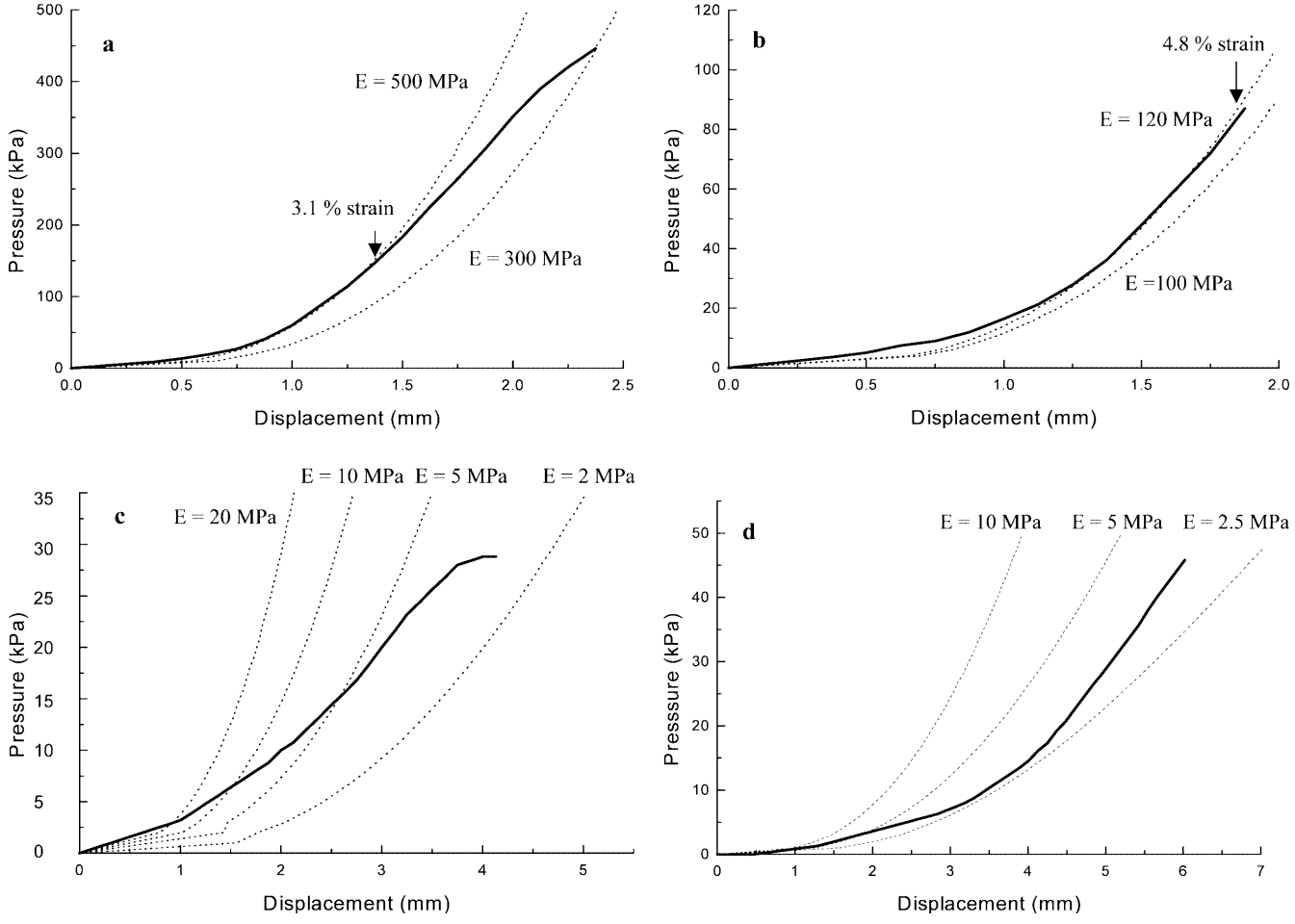

Fig. 5a–d. Experimental (*bold line*) and calculated (*dotted line*) curves for *Acetobacter* cellulose and cellulose composites. **a** Cellulose, sample thickness = 0.34 mm; **b** cellulose/pectin, sample thickness = 0.34 mm; **c** cellulose/xyloglucan, sample thickness = 0.55 mm; **d** cellulose/pectin/xyloglucan, sample thickness = 0.29 mm. Calculations based on Poisson's ratio = 0.3. The *arrows* show the calculated total strain at the apparent elastic limit for the cellulose and cellulose/pectin composites

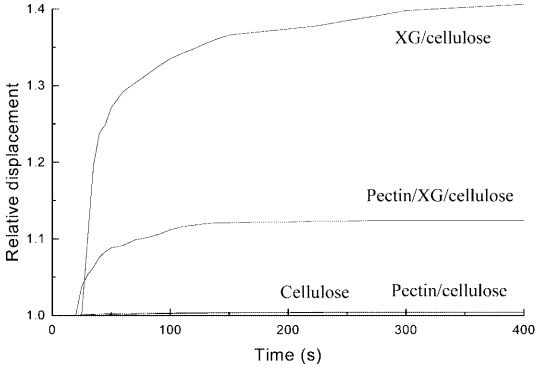

Fig. 6. Relative displacement (deflection/deflection when the chosen pressure is first applied) as function of time after application of 7 kPa pressure on *Acetobacter*-cellulose-based composites. The apparent time lag reflects the time required to achieve the desired pressure. *XG* Xyloglucan

were calculated using the measured displacement. Cellulose/xyloglucan and three-component composites failed on average at about 21 and 17% strain, respectively (Fig. 3). The high failure strain for these composites is also indicative of non-elastic behaviour.

Creep experiment

In order to confirm the modelling results and to distinguish possible hypotheses for cellulose/xyloglucan and cellulose/xyloglucan/pectin composite behaviour, a creep test was conducted on the composites. Creep tests measure the time-dependent extension of a sample under constant load. A range of pressures was employed: the example results shown in Fig. 6 were all obtained at 7 kPa. This particular pressure was chosen because it fell within the linear strain/stress region of cellulose and cellulose/pectin composites and was remote from rupture conditions for the other composites. The graph shows relative displacement vs. time. Relative displacement (displacement at t_i/displacement when the pressure first reached its set value) was used in order to compare samples with different tensile properties.

No apparent time-dependent response was observed for the cellulose composite up to an applied pressure of

120 kPa. Cellulose/pectin composites also exhibited a non-time-dependent behaviour up to an applied pressure of 20 kPa. Cellulose/xyloglucan composites, however, did show time-dependent deformation after the initial pressure application. This was also the case for the three-component composites. The creep observed for this sample was again intermediate between that observed for either cellulose or cellulose/pectin composites, and that observed for cellulose/xyloglucan composite.

Controlled unloading of the sample was not possible with the bulge-testing device but for all of these samples, completely reversible mechanical behaviour was observed up to 1.5% strain in uni-axial stress-relaxation tests (results not shown). At higher strains, non-elastic (plastic or viscoelastic) behaviour was found consistent with many previous studies on cellular plant material and walls. All the deformations measured during the creep test were well within the elastic limit of 1.5% (unidirectional) strain. Indeed for the most compliant composite (xyloglucan/cellulose), the maximum relative displacement shown in Fig. 6 corresponds to 0.6 ± 0.2 mm maximum deflection which can be translated by modelling to 0.25% unidirectional strain. The creep-test results are consistent with the cellulose and cellulose/pectin composites behaving as elastic materials for most of their bi-axial tensile extension.

Uni-axial tensile testing

Both pectin and xyloglucan have a strong effect on cellulose mechanical properties under bi-axial tension. In order to clarify their modes of action, tensile tests were also carried out under uni-axial conditions. Figure 7 gives examples of typical behaviour for four different composites.

On its own, the hydrated bacterial cellulose behaved, as in the bulge test, as a relatively stiff material, with deformation leading to brittle fracture. However, for the pectin composite, large uni-axial tensile deformations resulted in a more compliant material behaviour (Chanliaud and Gidley 1999). In xyloglucan/cellulose composites the presence of cross-links between fibres is accompanied by decreased stiffness and increased extensibility compared with cellulose alone (Whitney et al. 1999). When cellulose was deposited in the presence of both polysaccharides the mechanical behaviour of the resulting composites was once again intermediate between that of cellulose/pectin and that of cellulose/xyloglucan. A very compliant structure was obtained, characterised by a rupture strain of about 55% and a rupture stress of 0.4 MPa.

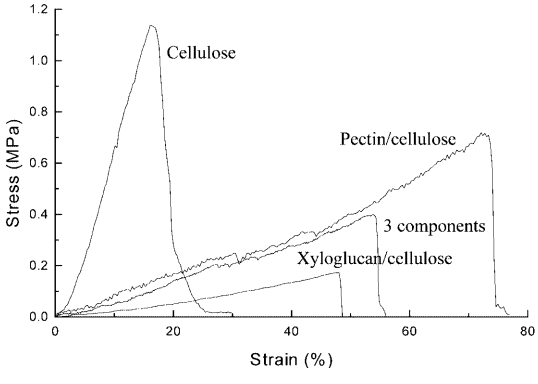

Fig. 7. Strain/stress curves obtained by uni-axial tensile testing of 11% (w/w) dry matter composites. Deformation rate = 10 mm/min

Discussion

Comparison of the *Acetobacter* model with plant tissues

In all composites, cellulose was the main load-bearing component, pectin and xyloglucan causing a decrease of modulus when incorporated. The values obtained for the Young's Modulus of the composites varied between 120 and 500 MPa depending on composition. Cellulose/xyloglucan and the three-component composite, which could not be fitted by a linear elastic model, would however have much lower stiffness values. Failure strains calculated under biaxial conditions for composites modelled as linear elastic (i.e. cellulose and cellulose/pectin) were typically of the order of 2–2.5%, which is comparable to the typical failure strain of a fibre-glass network, a classic example of an elastic fibrous system. Matrix polysaccharides appeared to change not only the stiffness of the composites but also their mechanical behaviour. In particular, time-dependent deformations were observed for xyloglucan-containing composites in biaxial tension whereas cellulose and cellulose/pectin composites did not exhibit any.

The modulus of individual cellulose microfibrils has been both measured and calculated (Marhöfer et al. 1996; Ishikawa et al. 1997) giving a modulus range of 0.7–3.5 GPa. This provides an upper limit of cellulose-composite modulus, taking into account effects of hydration and random alignment of cellulose fibres (Vincent 1990). These calculated values are close to those obtained from measurements made on cellulosic networks under uni-axial tensile conditions, e.g. moduli of 2.7 GPa for softwood fibres at a relative humidity of 50% (Hamad 1998) or 1–3 GPa for a cast network of sugar beet cellulose microfibrils depending on hydration (Dufresne et al. 1997). All these values give upper limits for the mechanics of cellulose composites.

Experiments have been conducted on a small number of cells that are large enough for samples of cell wall to be cut from the cell and then tested. An example of such a system is *Nitella*. When tested in uni-axial tension, values obtained range from 0.5 to 4 GPa (Probine and Preston 1962). However, in this study, the samples were dried and subsequently rehydrated before the experiments were conducted. Recent measurements (Toole et al. 2001) on never-dried walls of the related alga

Chara corallina gave modulus values ranging from 400 to 700 MPa, increasing with sample age (and cell wall thickness). Although these systems differ from many plant walls in terms of thickness and an external location, the values obtained are encouragingly similar to those obtained for our composites. Values for the modulus of cell walls 'in vivo' have also been estimated for potato parenchyma. Micro-penetration techniques have been used to obtain force-displacement curves for individual cell walls with results interpreted with the aid of computer modelling (Hiller et al. 1996; Davies et al. 1998). Values for the modulus of the wall between $E = 100$ MPa and $E = 600$ MPa were obtained, depending to some extent on the model employed. These values span the range obtained for our composite materials. All these values were obtained through modelling of very different experimental results and the compatibility of the different approaches helps confirm the validity of moduli obtained from *Acetobacter* cellulose composites.

Values obtained previously for failure stress (= strength) lie in the range of 1–5 MPa for non-woody plant tissue and ca. 50 MPa for *Chara* cell walls (Toole et al. 2001). These compare reasonably with estimated values in this work of 4 MPa for pectin/cellulose composites and 11 MPa for cellulose.

Mechanical roles of specific polysaccharides

Comparing composite bi-axial tensile behaviour with that obtained under uni-axial conditions (Chanliaud and Gidley 1999; Whitney et al. 1999) allows some conclusions to be drawn on likely structural origins for the mechanical effects of the different polysaccharides. In uni-axial extension, the hydrated bacterial cellulose behaved, as in the bulge testing, like a relatively stiff material, with deformation leading to brittle fracture. Its behaviour was characteristic of a mostly elastic network of fibres, very efficient in terms of strength but with modest extensibility properties. In cellulose and cellulose/pectin composites, cellulose fibres were the main load-bearing components. Indeed removal of the pectin from cellulose/pectin composite by CDTA treatment did not cause apparent changes in (uni- or bi-axial) tensile behaviour, indicating that the mechanical characteristics of this composite were entirely due to its cellulose component (Chanliaud and Gidley 1999).

However, the modulus calculated for cellulose deposited into a pectin gel was not the same as the modulus for cellulose alone. The change in extensibility properties between cellulose alone and cellulose deposited into a pectin gel is assigned to topology/architecture changes of cellulose fibril assemblies. Comparison between uni-axial and bi-axial tensile behaviour of the two composites gives more evidence for this assignment. Bi-axial tensile testing revealed similar rupture strains for both composites, suggesting no drastic organisational changes of cellulose fibres. Under large uni-axial tensile deformations, however, cellulose/pectin composite had a much more compliant material behaviour (75% rupture strain) than cellulose alone (15% rupture strain) (Chanliaud and Gidley 1999). Differences in test geometries can explain these apparently contradictory results. The uni-axial tensile stress allows alignment of components (especially microfibrils) to occur when stress is applied (analogous to "fibre pull-out"). This mechanism cannot occur under conditions of multi-axial stress due to geometrical constraints. It is proposed that pectin causes changes in architectural organisation of cellulose microfibrils during deposition (Chanliaud and Gidley 1999). These changes result in increased inter-fibrillar freedom, resulting in a small decrease in (bi-axial) modulus but a major increase in uni-axial extensibility. This uni-axial extensibility is non-elastic in nature above ca. 1.5% strain, i.e. for the majority of the test (Fig. 7), consistent with irreversible alignment of components during extension.

Addition of xyloglucan to cellulose or cellulose/pectin structures modifies drastically the observed mechanical behaviours. In both test geometries the xyloglucan-containing composites were characterised by a large extensibility (i.e. 50% rupture strain for cellulose/xyloglucan composites under uni-axial tensile deformation, Whitney et al. 1999). The composite strength was much reduced and the mechanical behaviour characterised by a time-dependent component. Cellulose/xyloglucan composites are characterised by the presence of inter-fibrillar cross-links leading to local cellulose alignment. It is suggested that, due to cellulose alignment, the load is not only transmitted through the fibres, but also through the xyloglucan cross-bridges. This hypothesis could explain why the modulus of xyloglucan-containing composites (Fig. 5) is so low compared with those of the other composites. It would also explain why a time-dependent component was observed in their mechanical behaviour, as the thin cross-linking strands of xyloglucan exhibit hydrodynamic mobility as detected by nuclear magnetic resonance (Whitney et al. 1995).

The strikingly large effect of matrix polysaccharides on the mechanical properties of the cellulose network might have implications at two levels in planta. Many walls show localisation of matrix molecules to particular zones within the wall and to different regions of the cell surface (McCann and Roberts 1991; Parr et al. 1996). Localised compositions are likely to have profound effects on the mechanical properties of the wall and have repercussions at the tissue level. Also components of the matrix are secreted into the cell wall by Golgi-derived vesicles beginning at cell plate formation and continuing throughout the growth of the wall. Different components may be incorporated into the wall at different times during its subsequent growth and differentiation (Moore and Staehelin 1988; Dolan et al. 1997). Changes in composition might have a direct impact on elongation. Indeed perfectly isotropic cellulose on its own cannot allow significant elongation. In this case, failure would be the only elongation mechanism because of the strong fibre interactions and the elastic like behaviour of

the network. During plant growth, it is highly likely that cortical microtubules play a role in directing cellulose deposition (Baskin et al. 1999) resulting in composite wall structures with demonstrably anisotropic mechanical properties (Kerstens et al. 2001). Preferential incorporation of matrix polysaccharides, either by tailoring cellulose orientation (pectin) or building in alternative load-bearing cross-links (xyloglucan), can also assist elongation by causing increased compliance. In the case of xyloglucan, elongation may also be aided by introducing a reversible time-dependent mechanical component in the wall. This is, however, a large extrapolation from the relatively rapid strain rates used in this study compared with growth rates in vivo. It is also relevant to note that a cucumber expansin has been shown to catalyse the extension of cellulose/xyloglucan composites much more effectively than cellulose alone (Whitney et al. 2000), and that elongation phenomena have been linked to changes in cell-wall viscoelasticity (Cleland 1971; Nolte and Schopfer 1997).

Acknowledgements We thank Jennie Brigham for producing electron micrographs and Trevor Orpin for constructing the bulge tester and for his invaluable technical help and advice.

References

Baskin TI, Meekes HTHM, Liang BM, Sharp RE (1999) Regulation of growth anisotropy in well-watered and water-stressed maize roots. II. Role of cortical microtubules and cellulose microfibrils. Plant Physiol 119:681–692

Blumenkrantz N, Hasboe-Hansen G (1973) New method for quantitative determination of uronic acids. Anal Biochem 54:484–489

Carpita NC, Gibeaut DM (1993) Structural models of primary cell walls in flowering plants: consistency of molecular structure with the physical properties of the walls during growth. Plant J 3:1–30

Chanliaud E, Gidley MJ (1999) In vitro synthesis and properties of pectin/*Acetobacter xylinus* cellulose composites. Plant J 20:25–35

Cleland R (1971) The mechanical behavior of isolated *Avena* coleoptile walls subjected to constant stress. Plant Physiol 47:805–811

Davies GC, Hiller S, Bruce DM (1998) A membrane model for elastic deflexion of individual plant cell walls. J Texture Stud 29:645–667

Dolan L, Linstead P, Roberts K (1997) Developmental regulation of pectic polysaccharides in root meristem of *Arabidopsis*. J Exp Bot 48:713–720

Dufresne A, CavaillJ J-Y, Vignon MR (1997) Mechanical behavior of sheets prepared from sugar beet cellulose microfibrils. J Appl Polym Sci 64:1185–1194

Hamad WY (1998) On the mechanisms of cumulative damage and fracture in native cellulose fibres. J Mater Sci Lett 17:433–436

Hestrin S, Schramm M (1954) Synthesis of cellulose by *Acetobacter xylinus*. Biochem J 58:345–352

Hiller S, Bruce DM, Jeronimidis G (1996) A micro-penetration technique for mechanical testing of plant cell walls. J Texture Stud 27:559–587

Ishikawa A, Okano T, Sugiyama J (1997) Fine structure and tensile properties of ramie fibres in the crystalline form of cellulose I, II, III_I and IV_I. Polymer 38:463–468

Kamerling JP, Vliegenthart JFG (1982) Gas-liquid chromatography and mass spectrometry of sialic acids. Cell Biol Monogr 10:95–125

Kerstens S, Decraemer WF, Verbelen JP (2001) Cell walls at the plant surface behave mechanically like fiber-reinforced composite materials. Plant Physiol 127:381–385

Marhöfer RJ, Reiling S, Brickmann J (1996) Computer simulations of crystal structures and elastic properties of cellulose. Ber Bunsenges Phys Chem 100:1350–1354

McCann MC, Roberts K (1991) Architecture of the primary cell wall. In: CW Lloyd (ed) The cytoskeletal basis of plant growth and form. Academic Press, London, pp 109–129

McCann MC, Wells B, Roberts K (1990) Direct visualisation of cross-links in the primary plant cell wall. J Cell Sci 96:323–334

Moore PJ, Staehelin LA (1988) Immunogold localization of the cell-wall-matrix polysaccharides rhamnogalacturonan I and xyloglucan during cell expansion and cytokinesis in *Trifolium pratense* L.; implication for secretory pathways. Planta 174:433–445

Ng A, Harvey AJ, Parker ML, Smith AC, Waldron KW (1998) Effect of oxidative coupling on the thermal stability of texture and cell wall chemistry of beet root (*Beta vulgaris*). J Agric Food Chem 46:3365–3370

Nolte T, Schopfer P (1997) Viscoelastic versus plastic cell wall extensibility in growing seedling organs: a contribution to avoid some misconceptions. J Exp Bot 48:2103–2107

Parr AJ, Waldron KW, Ng A, Parker ML (1996) The wall-bound phenolics of chinese water chestnut (*Eleocharis dulcis*). J Sci Food Agric 71:501–507

Pitt RE, Davis DC (1984) Finite element analysis of fluid-filled cell response to external loading. Trans ASAE 27:1976–1983

Probine MC, Preston RD (1962) Cell growth and the structure and mechanical properties of the wall in internodal cells of *Nitella opaca*. J Exp Bot 13:111–127

Quemener B, Thibault J-F (1990) Assessment of methanolysis for the determination of sugars in pectins. Carbohydr Res 206:277–287

Rayle DL (1989) Calcium bridges are not load-bearing cell-wall bonds in *Avena* coleoptiles. Planta 178:92–95

Talbott LD, Ray PM (1992) Molecular size and separability features of pea cell wall polysaccharides. Plant Physiol 98:357–368

Toole GA, Gunning PA, Parker ML, Smith AC, Waldron KW (2001) Fracture mechanics of the cell wall of *Chara corallina*. Planta 212:606–611

Van-Buren JPS (1979) The Chemistry of texture in fruits and vegetables. J Text Stud 10:1–23

Vincent J (1990) Structural biomaterials. Princeton University Press, London

Virk SS, Cleland RE (1988) Calcium and mechanical properties of soybean hypocotyl cell walls: possible role of calcium and protons in cell-wall loosening. Planta 176:60–67

Whitney SEC, Brigham JE, Darke AH, Reid JSG, Gidley MJ (1995) In vitro assembly of cellulose/xyloglucan networks: ultrastructural and molecular aspects. Plant J 8:491–504

Whitney SEC, Brigham JE, Darke AH, Reid JSG, Gidley MJ (1998) The interaction of mannan-based polysaccharides with bacterial cellulose. Carbohydr Res 307:299–309

Whitney SEC, Gothard MGE, Mitchell JT, Gidley MJ (1999) Roles of cellulose and xyloglucan in determining the mechanical properties of primary plant cell walls. Plant Physiol 121:657–663

Whitney SEC, Gidley MJ, McQueen-Mason SJ (2000) Probing expansin action using cellulose/hemicellulose composites. Plant J 22:327–334

Zienkiewicz OC (1979) The finite element method. McGraw-Hill Book Company (UK), Maidenhead