Journal of Experimental Botany, Vol. 64, No. 15, pp. 4635–4649, 2013
doi:10.1093/jxb/ert255 Advance Access publication 24 September, 2013

Journal of Experimental Botany
www.jxb.oxfordjournals.org

REVIEW PAPER

Plant micro- and nanomechanics: experimental techniques for plant cell-wall analysis

Ingo Burgert* and Tobias Keplinger

ETH Zurich, Institute for Building Materials, Schafmattstrasse 6, 8093 Zurich & Empa, Applied Wood Materials Laboratory, Ueberlandstrasse 129, 8600 Duebendorf, Switzerland

* To whom correspondence should be addressed. Email: iburgert@ethz.ch

Received 18 March 2013; Revised 9 July 2013; Accepted 12 July 2013

Abstract

In the last few decades, micro- and nanomechanical methods have become increasingly important analytical techniques to gain deeper insight into the nanostructure and mechanical design of plant cell walls. The objective of this article is to review the most common micro- and nanomechanical approaches that are utilized to study primary and secondary cell walls from a biomechanics perspective. In light of their quite disparate functions, the common and opposing structural features of primary and secondary cell walls are reviewed briefly. A significant part of the article is devoted to an overview of the methodological aspects of the mechanical characterization techniques with a particular focus on new developments and advancements in the field of nanomechanics. This is followed and complemented by a review of numerous studies on the mechanical role of cellulose fibrils and the various matrix components as well as the polymer interactions in the context of primary and secondary cell-wall function.

Key words: Atomic force microscopy, fibre–matrix interactions, micromechanics, nanoindentation, plant biomechanics, primary cell walls, secondary cell walls.

Introduction

Plant micro- and nanomechanics are subdisciplines of plant biomechanics, which primarily address the cell and cell-wall level of the plant body (Niklas & Spatz, 2012). Due to the hierarchical structure of plants (Speck & Burgert, 2011; Gibson, 2012), research progress in this field has a great impact on plant biomechanics in general, as properties and features at this scale inevitably influence the macroscopic appearance and performance of the plant body (Fratzl and Weinkamer, 2007). In recent years, there has been a tremendous increase in mechanical characterization studies at the cell and cell-wall level dealing with various issues of structure–property and structure–function relationships, which have been reviewed by various authors (Geitmann, 2006; Burgert & Dunlop, 2011; Cosgrove & Jarvis, 2012; Eder *et al.*, 2013; Kasas *et al.*, 2013). On the one hand, this has been due to rapid methodological developments in this field that allow more precise determination of mechanical properties, in particular at the nanoscale of the biological systems. On the other hand, cell and cell-wall mechanics are increasingly considered to be highly relevant as we gain a deeper understanding of how mechanics affects growth processes and how mechanical properties are sensed, controlled, and tuned by the plant (Cosgrove, 1993, 2005; Burgert, 2006; Telewski, 2006; Niklas, 2009). This knowledge gain substantially affects matters of plant growth and morphology as well as closing a feedback loop in terms of the biosynthesis and structure of cell-wall components. In this context, cell and cell-wall mechanics have become an important characterization tool for evaluating the impact of genetic modifications regarding effects on plant structure and growth processes, material performance, and mechanical stability of crops (Ryden *et al.*, 2003; Pena *et al.*, 2004; Bjurhager *et al.*, 2008; Bjurhager *et al.*, 2010; Hoenicka *et al.*, 2012).

Besides a tight interplay of plant biomechanics and plant morphology, cell and cell-wall mechanics are highly relevant

Abbreviations: AFM, atomic force microscopy; MFA, microfibril angle.

© The Author 2013. Published by Oxford University Press on behalf of the Society for Experimental Biology. All rights reserved.
For permissions, please email: journals.permissions@oup.com

in various fields of plant material utilization. For instance, mechanical characterization techniques provide highly valuable information about material properties, such as fibre performance in pulp and paper (Jayne, 1959; Page *et al.*, 1971; Kersevage, 1973; Page & El-Hosseiny, 1983; Groom *et al.*, 2002*a*). Furthermore, the increased knowledge offers the potential to transfer principles and mechanisms evolved by nature to the design of bio-inspired materials (Sidorenko *et al.*, 2007; Dunlop & Fratzl, 2010; Martone *et al.*, 2010; Paris *et al.*, 2010).

Surprisingly, research on primary and secondary cell walls in recent decades has been conducted largely by separate communities that have rarely interacted. While primary cell-wall research has been closely related to physiological aspects due to the interplay with the living cell, research on secondary cell walls has focused on material aspects of predominately dead cells. In recent years, however, the research communities have come closer together, due in part to our increase in understanding of how genes regulate biosynthesis processes and determine the structure, chemistry, and properties of cell walls. Additionally, we see a bridging function by micro- and nanomechanical approaches, which help to identify common principles of cell-wall assembly and the resulting structure–function relationships (Burgert, 2006; Cosgrove & Jarvis, 2012). In this review, we aim to provide an overview of the basic principles of micro- and nanomechanical techniques that are applied to both primary and secondary cell-wall analysis and give examples of how these methods are used to unravel the underlying structure–property relationships of cells and cell walls in view of their specific functions.

While there has been great progress in the field of cell-wall modelling (Salmén & de Ruvo, 1985; Perez *et al.*, 1996; Yamamoto & Kojima, 2002; Besombes & Mazeau, 2005; Hofstetter *et al.*, 2005; Hanus & Mazeau, 2006; Bader *et al.*, 2011; de Borst *et al.*, 2012) and in combined approaches in which experimental data and modelling complement each other, here we focus on the experimental side and review the tremendous knowledge gain exclusively in this field. This comprises mechanical analysis of primary and secondary cell walls of various cell types, which are briefly introduced in terms of general mechanical constraints and specific functions.

Mechanical functions of primary and secondary cell walls

Micro- and nanomechanical characterization needs to thoroughly consider the functional context of the investigated plant material as this is crucial in order to gain insight into the underlying structure–function relationships. The main mechanical functions that primary cell walls need to fulfil are to provide sufficient stiffness and strength to the cell but at the same time to allow for cell growth, as well as enabling reversible changes of cell size and shape with regard to pre-stressing and organ movements (Cosgrove, 2005; Martone *et al.*, 2010). Primary cell walls have to be considered together with turgor pressure regulated by the living cell to attain stiffness in an 'inflated' state. With regard to the specific mechanical properties of the primary cell wall, it is fascinating to realize that the same wall has to fulfil two mutually exclusive mechanical requirements. In order to allow growth processes, primary cell walls need to be plastically deformable, whereas to provide mechanical stability or allow reversible movements such as stomata opening and closure, the primary cell wall needs to be entirely elastic (Williams & Bennett, 1982; Proseus *et al.*, 1999; Boyer, 2001; Cosgrove, 2001, 2005; Forterre *et al.*, 2005; Roelfsema & Hedrich, 2005; Moran, 2007). The mechanism by which the cell wall can 'switch' between elastic and plastic deformability by alterations of biomacromolecule interactions is still debated (Fry *et al.*, 1992; Cosgrove, 2000, 2005; Schopfer, 2001; Proseus & Boyer, 2006).

At a first glance, secondary cell walls appear to be by far the more pre-determined in their functionality. The rigid network of parallel aligned cellulose fibrils and matrix substances provides mechanical stability, even to dead cells (Cave, 1968; Mark & Gillis, 1970, 1973). Beyond this fundamental requirement, one can see a wide variability in structure and composition of secondary cell walls that mirrors the different functions of cell and tissue types accompanied by different mechanical property profiles (Donaldson, 2001; Wegst & Ashby, 2004; Donaldson, 2008; Eder *et al.*, 2009; Eder & Burgert, 2010). These profiles include a vast variability in terms of material stiffness, toughness and strength as well as the capability to generate both tensile and compressive mechanical stresses (Yamamoto, 1998; Lichtenegger *et al.*, 1999; Reiterer *et al.*, 1999; Farber *et al.*, 2001; Burgert *et al.*, 2007; Goswami *et al.*, 2008; Burgert & Fratzl, 2009; Clair *et al.*, 2011; Eder *et al.*, 2013). The important mechanical requirements that need to be fulfilled by primary and secondary cell walls are illustrated in Fig. 1.

Brief overview of structure and chemistry in relation to biomechanics

In view of the wide range of various cell types and cell-wall compositions, it is impossible to treat the topic comprehensively. Therefore, we will focus on a simplified illustration of the underlying principles and concepts of primary and secondary cell walls. Generally speaking, both primary and secondary cell walls can be described by means of natural fibre composites consisting of a stiff fibrous phase made of cellulose fibrils composed of crystalline and amorphous regions as well as a pliant amorphous matrix comprising various biopolymers with hydrogen bonding between the two phases (Kerstens *et al.*, 2001; Fratzl *et al.*, 2004*a*). According to their specific functions, both cell-wall types can be distinguished by means of structural and chemical parameters. This applies to cell-wall thickness, cellulose orientation, degree of crystallization, volume fractions of cell-wall components, composition of the matrix, chemical bonding patterns, and water content (McCann & Roberts, 1991; Pauly *et al.*, 1999; Brändström, 2001; Donaldson, 2001; Somerville *et al.*, 2004; Burgert, 2006; Donaldson, 2008; Jarvis, 2009; Cosgrove & Jarvis, 2012). It is important to note that all these parameters are also highly varied within primary and secondary

cell walls, meaning that plants manifest a vast potential to regulate properties at the nano-and microscale level of the cell walls. In Table 1, crucial parameters of primary and secondary cell walls, predominantly from a biomechanical perspective, are listed. Such a tabular comparison inevitably results in a simplification, which we assume to be tolerable for the benefit of clarity.

Plant materials and characterization techniques

In this review, we intend to discuss plant materials that are examined intensively in micro- and nanomechanical analyses, as well as to introduce the related characterization techniques for biomechanical studies. In principle, any plant organ could be studied with at least one of the below-mentioned methods. However, screening of the relevant literature shows that particular plant materials have been more prominently examined either because of being used as model systems or because of having important mechanical properties for the plant body or in application (Fig. 2).

In terms of primary cell walls, basic investigations on cell-wall structure and properties as well as growth processes have been conducted mainly on hypocotyls of various plant species (Nakahori *et al.*, 1991; Cosgrove, 1993, 2011; Kutschera & Kohler, 1994; Ryden *et al.*, 2003; Refregier *et al.*, 2004). In recent years, with the technological developments in nanomechanical studies, even meristems have become a matter of intensive studies as very local cell-wall stiffness alterations can be monitored (Peaucelle *et al.*, 2011). More applied research activities are related to the mechanical characterization of fruit parenchyma or fruit peels (Bargel *et al.*, 2004; Landahl *et al.*, 2004; Matas *et al.*, 2004; Bargel & Neinhuis, 2005). Micro- and nanomechanical studies on secondary cell walls at the level of basic research are aimed at unravelling general principles and mechanisms of strength and deformation behaviour, including how plants achieve and control certain property profiles.

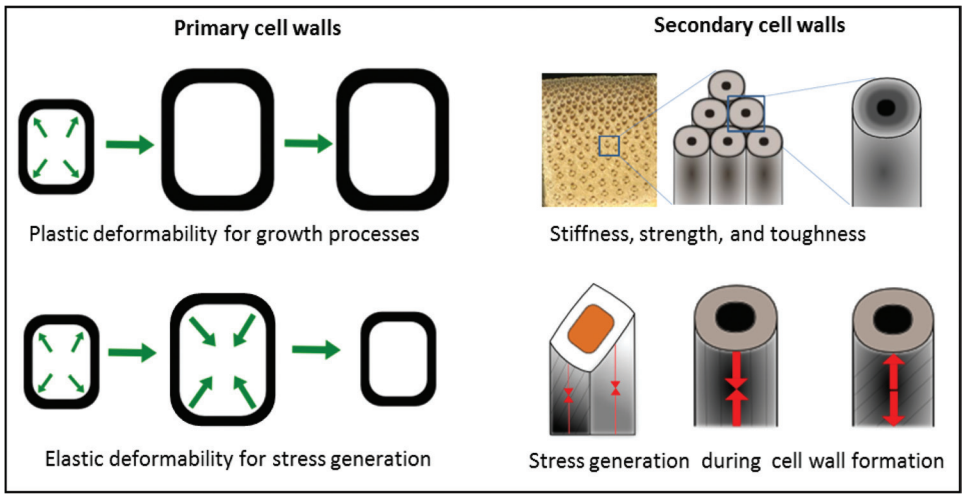
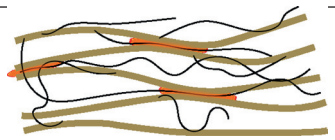
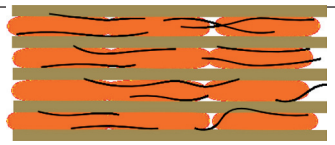


Fig. 1. Main functions and mechanical requirements of primary and secondary cell walls.

Table 1. *Tabular comparison of general features of primary and secondary cell walls accompanied by cell-wall illustrations*

The schematic drawing of the primary cell wall is adapted from a new cell-wall model by Park & Cosgrove (2012b)

Feature	Primary cell walls	Secondary cell walls
Illustration		
Cell wall	Thin, ongoing property alterations by the cell	Thick, multi-lamellar, fully differentiated structure
Polymer network	Flexible network, ongoing modification processes	Rigid network, interlocked status
Cellulose	Low content, reorientation possible, orientation more variable	High content, densely packed, strictly parallel orientation
Matrix	Predominately hemicelluloses, pectin, and structural proteins	Predominately hemicelluloses and lignin
Water interactions	Highly hydrophilic, hydrogel character	More hydrophobic when lignified
Fibre–matrix interactions—uncertainties	Widely accepted tethered cellulose–xyloglucan model recently challenged. Illustration above shows a new cell-wall model suggested by Park and Cosgrove (2012*b*)	Mechanical function and spatial orientation in particular of hemicellulose composition and lignin not fully understood

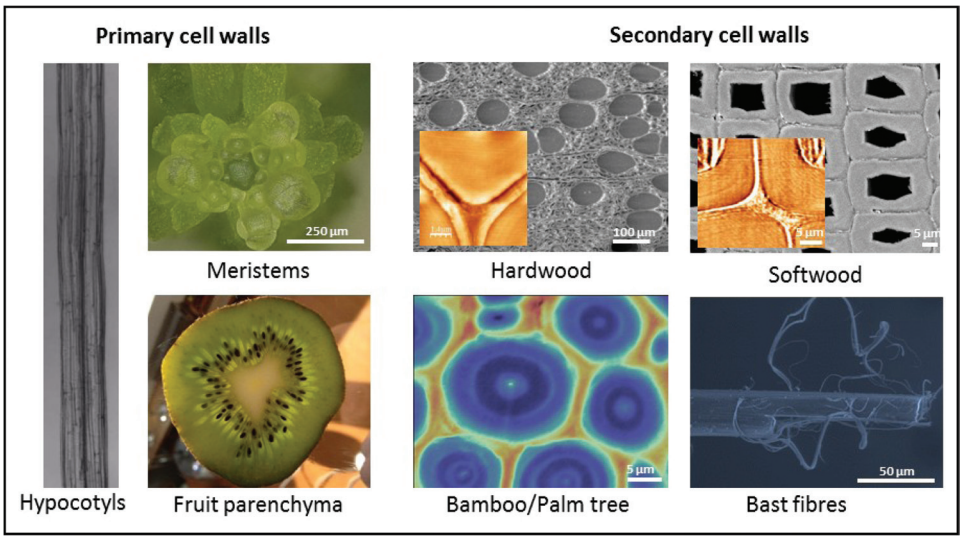


Fig. 2. Common plant materials in mechanical analysis visualized by light microscopy, scanning electron microscopy, Raman imaging and/or scanning near-field optical microscopy. Meristem image with kind permission from S. Braybrook.

In addition to establishing a general relationship between cellulose orientation and mechanical performance, understanding cellulose–matrix interactions has developed into a major focus in recent years (Reiterer *et al.*, 1999; Spatz *et al.*, 1999; Köhler & Spatz, 2002; Keckes *et al.*, 2003; Fratzl *et al.*, 2004*b*; Altaner & Jarvis, 2008). More applied research approaches aim at determining local mechanical properties of industrially relevant crops. A major interest is on wood fibre properties of wood species with regard to pulp and paper as well as on annually harvested bast fibres (hemp, flax) for utilization in natural fibre composites (Page & El-Hosseiny, 1983; Eichhorn *et al.*, 2001*a*; Bos *et al.*, 2002; Groom *et al.*, 2002*a*,*b*; Peetla *et al.*, 2006; Thygesen *et al.*, 2007). Due to the excellent mechanical performance and fast-growing capacities of bamboo and other grasses, several micro- and nanomechanical studies have been conducted in order to unravel the underlying structure–function relationships and derive mechanical characteristics for utilization purposes (Ruggeberg *et al.*, 2008; Shao *et al.*, 2010; Yu *et al.*, 2011; Wang *et al.*, 2012).

Microtensile tests

Micromechanical testing techniques address the plant level of tissues and individual cells. This includes tests on entire organs such as *Arabidopsis* hypocotyls, small tissue samples, and fibre bundles (e.g. wood segments, and fibre bundles of bamboo, palms, flax, and hemp), as well as individual cells mainly in the form of fibres and tracheids. The size and dimensions of the samples as set by plant tissue structure and variability limit the kind of loading conditions that can be applied. Accordingly, at the micromechanical level, predominantly uniaxial tensile tests are conducted, comprising common standard tests to gain information on stiffness, strength, and toughness of the plant material and time-dependent investigations to study relaxation and creep phenomena. When deriving information on cell-wall properties from mechanical tests at the tissue level, one needs to consider that a multitude of additional parameters affect the obtained data, such as tissue density, variability of the tissue, cell length, cell–cell interactions, and turgor pressure.

For primary cell walls, the most widely utilized micromechanical test is a creep test in which a defined weight is attached to the tissue and the elongation is recorded over time. Based on creep tests, important information on primary cell-wall architecture and cell-wall loosening mechanisms during cell growth have been gained (Cleland, 1971; Richmond *et al.*, 1980; Suslov & Verbelen, 2006; Cosgrove, 2011). However, it needs to be emphasized that there are various constraints on what can be inferred from creep experiments and uniaxial tensile tests concerning growth processes and turgor-driven expansion, respectively (Cosgrove, 1993). Rather recently, standard tensile tests and cyclic loading tests have been applied to study the impact of genetic modification treatments on mechanical properties, in particular of dark-grown hypocotyls of *Arabidopsis thaliana* (Ryden *et al.*, 2003; Pena *et al.*, 2004; Cavalier *et al.*, 2008; Abasolo *et al.*, 2009). The hypocotyls are fixed in a microtensile tester and a uniaxial tensile stress is applied until final rupture of the specimen occurs. Methodologically, besides maintaining a sufficient level of humidity of the specimen, fixation of the hypocotyls is crucial, as the fragile and turgorized samples do not allow classical clamping, which makes a gluing process more favourable. The applied forces are recorded with load cells of small capacity, and the elongation can be derived from the machine path. However, it is always recommendable and sometimes mandatory to use optical systems such as video extensometry to record the strain more precisely and avoid measuring errors, for instance due to sample slippage in the clamps. In order to calculate the stress based on the force measurement, the cross-sectional area of the hypocotyl needs to be considered. A rather fast method is to calculate it from the diameter of the almost circular hypocotyl. However, one has to be aware that this procedure results in a stress–strain diagram for the entire hypocotyl and rules out structural differences at the tissue level such as cell size and shape, as well as cell-wall thickness.

Further parameters that influence the testing protocol and can lead to a large scatter of data and prevent direct comparisons between different studies are the influence of hypocotyl age, the germination conditions, the culture medium and the segment of the hypocotyl that has been tested.

In terms of secondary cell walls, one can state that the microtensile testing is a bit more determined than for primary cell walls, because the specimens are less fragile and usually dead, which rules out the influence of turgor pressure. However, in particular, specimen size can have an underestimated effect on the mechanical response of the tested plant material (Navi *et al.*, 1995). The standard tensile testing setups are similar to the one described for primary cell walls, although certain fibre bundles can be sufficiently rigid to allow fast clamping in the machine. Single fibres are usually fixed by gluing, which can be achieved either by gluing them on supporting frames that are fixed in the tensile tester or by glue droplets that are directly mounted at the fibre ends for a ball and socket setup (Kersevage, 1973; Groom *et al.*, 2002b; Burgert *et al.*, 2003; Sedighi-Gilani *et al.*, 2005). In any case, the fibres or tissue samples need to be aligned precisely, because even small deviations from the uniaxial loading condition have a large influence on the recorded mechanical behaviour due to the occurrence of shear stresses. The cross-sectional area of the sections in terms of tissues/fibres or cell walls can be determined by light or electron microscopy studies on samples that are cut transversally after testing, or cutting can be avoided when confocal light microscopy is applied (Groom *et al.*, 2002*a*; Burgert *et al.*, 2005). Usually, the strain required to fracture fibres and small tissue sheets containing cells with secondary cell walls is rather small, which makes a coupling of the testing setup to an optical system for high-resolution strain measurements such as video extensometry essential. Figure 3 shows in simple illustrations the most utilized micromechanical testing setups for biomechanical studies on primary and secondary cell walls.

Unique micromechanical testing approaches are so-called combined (*in situ*) methods in which external loading is combined with simultaneous observation of nano- and microstructural deformation. The material response at the nano- and microstructural level reveals insights into specific deformation mechanisms and crack propagation events in the specimen. In plant biomechanics in particular, uniaxial tensile tests have been combined with various load-monitoring techniques to examine deformation patterns of plants at different levels of hierarchy. Detailed structural information can be obtained at the micro- and mesoscale by combining mechanical loading with light microscopy and for higher resolution with scanning electron microscopy (Mott *et al.*, 1995; Bodner *et al.*, 1996; Badel & Perré, 1999; Fruhmann *et al.*, 2003; Thygesen *et al.*, 2007; Eder *et al.*, 2008). An *in situ* approach based on computed tomography allows the three-dimensional monitoring of deformation and fracture events at the level of cells and cell-wall layers (Nazarian *et al.*, 2005; Zauner *et al.*, 2012). For an even closer look into nanostructural deformation mechanisms at the level of polymer interactions of cell-wall components, spectroscopy and X-ray scattering techniques are utilized. *In situ* infra-red and Raman spectroscopy approaches can provide information on specific load-bearing capacities of cell-wall components, as well as collective mechanical responses of polymer assemblies (Salmén & Olsson, 1998; Eichhorn *et al.*, 2000; Akerholm & Salmén, 2001; Eichhorn *et al.*, 2001b; Akerholm & Salmén, 2003; Gierlinger *et al.*, 2006; Sturcova *et al.*, 2006; Salmén & Bergstrom, 2009). *In situ* X-ray measurements at synchrotron facilities allow detailed observations of cellulose fibril–matrix interactions, including monitoring of cellulose fibril reorientation during loading and the impact of water (Keckes *et al.*, 2003; Kamiyama *et al.*, 2005; Kölln *et al.*, 2005; Zabler *et al.*, 2010).

Nanoindentation

For many decades, traditional hardness tests (pushing a tip with a defined geometry into the surface of the sample)

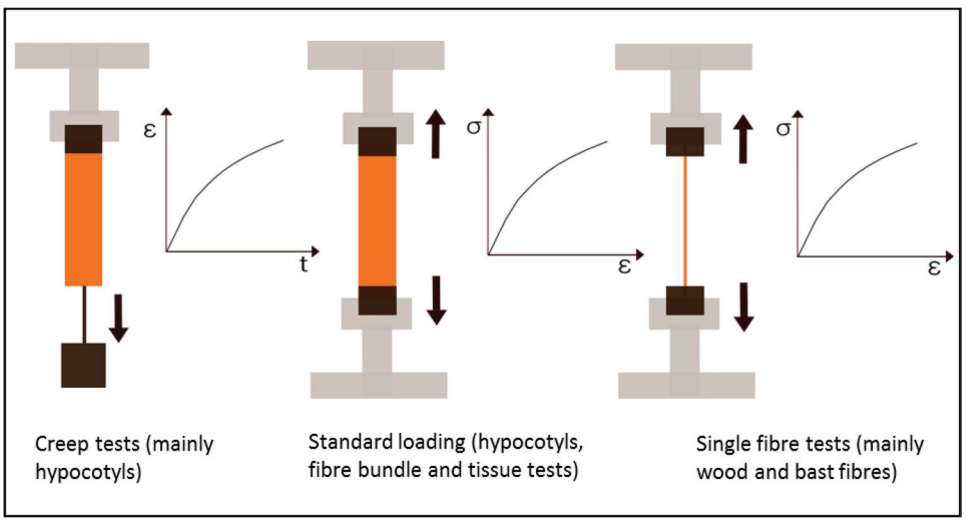


Fig. 3. Most applied micromechanical testing techniques for the characterization of primary and secondary cell walls illustrated by schematic drawings of the setup as well as of strain–time and stress–strain diagrams, respectively. (This figure is available in colour at *JXB* online.)

have been used for mechanical characterization in structural biology, including testing of cartilage, bones, soft tissues, plants, and wood in which hardness is defined as the ratio of a maximum load and the indent area (Kempson *et al.*, 1971*a*,*b*; Doyle & Walker, 1985). Nanoindentation is based on the same principle but is performed at a smaller length sale and is advanced by exactly monitoring the displacement and the loading of the indenter during the measurement. The displacement is controlled via inductance or capacitance, and for the force actuation, normally piezo elements or magnetic coils are used (Ebenstein & Pruitt, 2006; Fischer-Cripps, 2011; Oyen, 2011).

In nanoindentation, it is highly important to obtain perfectly flat surfaces in order not to distort the measurement due to surface roughness. Thus, to fulfil this requirement, an embedding material is needed (e.g. epoxy resin), which gives mechanical support during microtoming and nanoindentation. The question of penetration of the embedding material into the samples and its effect on the measurement remains unresolved. However, there is currently no conclusive evidence suggesting infiltration of the embedding material into the cell wall. For more details about sample preparation, see Konnerth *et al.* (2008) and Meng *et al.* (2013). A related limitation in nanoindentation studies towards plant biomechanics is that it is very difficult to retain the natural wet condition of the samples due to the embedding procedure. Furthermore, one has to keep in mind that only a small area/volume is tested, which is a problem in view of the vast heterogeneity of plant cell walls (de Borst *et al.*, 2012).

The main principle in nanoindentation is to calculate hardness and elastic modulus from a load-displacement (compliance) curve recorded during a local indentation. Unlike in conventional hardness tests (e.g. Vickers hardness), the size of the indent is too small for measurements with optical methods. Therefore, the area is indirectly determined from the penetration depth together with the known geometry of the indenter (e.g. Berkovich, spherical indenter, power law indenters) (Fischer-Cripps, 2011; Oyen, 2011). Fig. 4 illustrates the testing principle and shows a typical load-displacement curve for an elastic–plastic solid.

Based on the approach by Oliver and Pharr (1992), called compliance method, the reduced modulus and the hardness are determined by analysing the load-displacement curve. For further details on the analysis of the data and the calculation of the various parameters, see Eder *et al.* (2013).

In recent last years, a tremendous effort has gone into improving the technique in order to extract further parameters such as creep compliance functions, storage modulus, and loss modulus (VanLandingham, 2003; Oyen, 2005; Fischer-Cripps, 2011; Oyen, 2011).

For data interpretation, it has to be considered that the compliance method is based on the assumption of a homogeneous and isotropic half space (Oliver & Pharr, 1992). A general restriction for biological materials is their anisotropic non-homogeneous nature. Hence, there have been huge efforts towards developing methods to reflect these constraints, such as the introduction of a holding period at peak load, continuous stiffness measurements, and multi-load indentation experiments (Ebenstein & Pruitt, 2006; Tze *et al.*, 2007; Oyen, 2011). An anisotropic indentation theory was introduced by the work of Vlassak *et al.* (2003) and applied for wood cell-wall characterization by Jager *et al.* (2011*a*,*b*). They showed that it is necessary to perform nanoindentation experiments with at least five different indention angles (compared with the orientation of the cellulose fibrils) to determine the elastic constants with the help of an error minimization procedure. As a result of the complex behaviour of the sample under the indenter, the absolute stiffness values are smaller than expected for the longitudinal modulus (Eder *et al.*, 2013). For a comprehensive review of the mechanical modelling of cell-wall indentation, see Milani *et al.* (2013) in this issue.

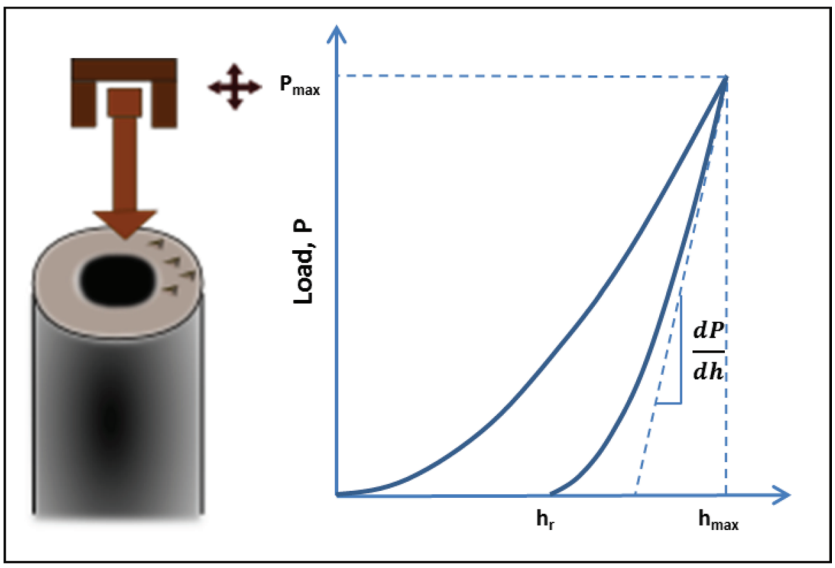


Fig. 4. Schematic of a nanoindentation test on secondary cell walls with a typical load–displacement curve for an elastic–plastic solid. (This figure is available in colour at *JXB* online.)

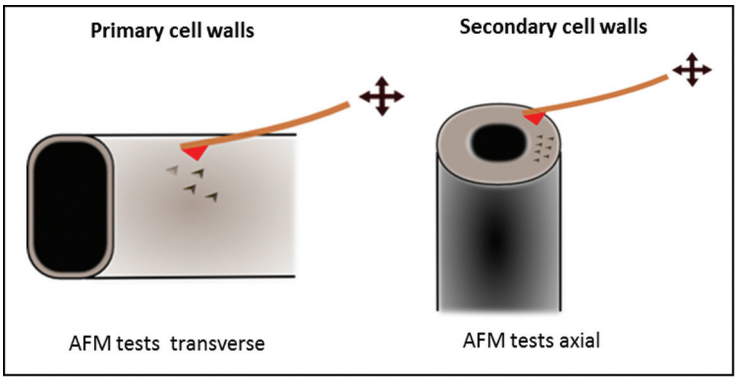


Fig. 5. Schematics of AFM in a transverse direction on primary cell walls and in the longitudinal direction on secondary cell walls. (This figure is available in colour at *JXB* online.)

Atomic force microscopy (AFM)

For measurements of mechanical properties at a smaller scale than in nanoindentation (for instance for primary cell walls), AFM has to be used. Figure 5 schematically illustrates the common loading setups for primary and secondary cell walls.

In AFM studies, a tip mounted on a cantilever spring is scanned over a sample, and the deflection of the cantilever, based on the force between the tip and the sample, is measured with the help of a photodiode. With this configuration, topography images are obtained. However, to receive mechanical properties, so-called 'force measurements' are necessary, where the tip is moved towards the sample in the normal direction and force–distance curves are recorded (Green *et al.*, 2002; Butt *et al.*, 2005). In contrast to nanoindentation, in AFM the results of a force measurement—cantilever deflection and piezo position—have to be converted into force and distance. First, the deflection of the photodiode has to be converted to vertical displacement and the spring constant of the cantilever is needed to calculate a force based on Hooke's law. Additionally, a conversion for the height (change in piezo) is necessary, as it must be corrected for the deflection of the cantilever. For a detailed description of the conversion methods, see Hutter & Bechhoefer (1993), Hinterdorfer *et al.* (1996), and Butt *et al.* (2005). The main objective in using AFM for mechanical characterization is not to take force–distance curves on selected points of the sample but rather to generate images that are based on mechanical properties (force–distance curve for every pixel). Various modes for the imaging of mechanical properties are available. However, there is still much potential for improvement and new techniques. Two possible modes are resonant contact AFM and pulsed force mode. In resonant contact AFM, the tip is used as a resonator whose frequency depends on the interactions between the sample and the tip, which allows measurement of the elastic properties of the sample (Clair *et al.*, 2003). In pulsed force mode, the Z-piezo of the AFM is modulated with a sinusoidal voltage (oscillation amplitudes between 20 and 50 nm). At the lowest point of the oscillation, the tip is out of contact, and at the highest point, it reaches a deflection maximum. The outcome of pulsed force mode is force curves at high frequencies that are recorded in real time, and high-resolution images of the mechanical properties are obtained (Krotil *et al.*, 1999).

AFM has been used intensively to characterize the molecular architecture of cell walls, such as the orientation and size of microfibrils or pore size distributions (Kirby *et al.*, 1996; Fahlen & Salmén, 2003, 2005; Yarbrough *et al.*, 2009). When interpreting the obtained data, one has to consider the fact that the stiffness values extracted from force–distance curves do not exclusively reflect the elastic properties of the cell wall (Routier-Kierzkowska *et al.*, 2012), but that indenter geometry (Bolduc *et al.*, 2006), turgor pressure (Wang *et al.*, 2004), and internal stresses also have an impact (Zamir & Taber, 2004). Another limiting factor in AFM examinations of primary cell walls is that the force that can be applied is too small to stretch the cell walls of turgid tissues. For this purpose, a so-called cellular force microscopy with the potential of applying of up to 1 mN has been developed, which allows one to indent cells until their rupture and to measure the release of the turgor pressure by changes in local stresses (Routier-Kierzkowska *et al.*, 2012).

The AFM methods for mechanical characterization introduced above are based on examining force–distance curves, but it should be also mentioned that alternative methods exist for determining mechanical properties at the nanoscale. Clair *et al.* (2003) developed resonance contact AFM for determining elastic properties of wood cells. Tetard *et al.* (2010*a*,*b*) used the so-called mode synthesizing AFM, which is based on exerting a multi-harmonic force on the substrate on the probe, which creates multiple orders coupling in frequency and allows the deduction of mechanical properties within the plant cell-wall structure. Furthermore, single-molecule force spectroscopy has been used to characterize interactions between xyloglucan molecules and a cellulose substrate (Morris *et al.*, 2004). There is ongoing research into developing new techniques for measuring mechanical properties, and many methods have simply not been utilized in plant cell-wall characterization yet (Krotil *et al.*, 1999).

Biomechanics of plant cell walls

In the following, we intend to discuss micro and nanostructural examinations on primary and secondary cell walls with

regard to the specific mechanical role of the cell-wall components, and their interaction in cell-wall assembly.

Secondary cell walls

In terms of biomechanical studies on secondary cell walls, we intend to restrict ourselves to the basic structure–property relationships, which exclude the principles of the stress-generation mechanisms mentioned and referenced above. For approaches aiming at unravelling the specific mechanical role of a cell-wall component, cellulose is probably the most easily accessible cell-wall biomacromolecule. Structure–property relationships can be derived directly due to the parallel arrangement and the partly crystalline nature of the cellulose fibrils, which allows the acquisition of detailed information on cellulose structure and formation via various methodical techniques. Hence, its crucial role in cell-wall mechanics has been shown in various studies at the tissue and fibre levels in which the tensile stiffness of the material has been related to the cellulose orientation [microfibril angle (MFA)]. The larger the MFA, the lower the stiffness of the cell wall, which enables the cell to control and adjust mechanical performance in the cellulose spinning process. Using micromechanical tests on tissue and fibres, this relationship has been shown in various studies (Page & El-Hosseiny, 1983; Lichtenegger *et al.*, 1999; Reiterer *et al.*, 1999; Saren *et al.*, 2001; Salmén & Burgert, 2009; Eder & Burgert, 2010; Eder *et al.*, 2013). *In situ* tests combining tensile straining of single wood fibres with Raman spectroscopy have directly revealed the load-bearing capacity of the cellulose fibrils, by showing a strong correlation between applied stress/strain and the nanodeformation of covalent bonds in the cellulose fibrils (Gierlinger *et al.*, 2006). In recent years, nanoindentation has also been increasingly utilized to work on secondary cell walls (Wimmer *et al.*, 1997; Gindl & Schoberl, 2004; Gindl *et al.*, 2004; Konnerth *et al.*, 2009; Wu *et al.*, 2009; Adusumalli *et al.*, 2010) and in particular on the mechanical impact of cellulose orientation. Figure 6 shows the correlation between MFA and cell-wall stiffness measured by nanoindentation as well as microtensile tests based on values reported in literature. Data obtained by both methods show the same trend following an increase in MFA, but in particular the decrease in stiffness measured by nanoindentation above 15° MFA is less pronounced compared with the microtensile studies. This can be explained by the different mechanical loading and testing conditions, which result in discrepancies between the methods, predominately for samples with larger MFAs, when the influence of the matrix properties becomes more relevant.

The mechanical role of cell-wall matrix components is more difficult to examine than that of cellulose, because of a less-ordered assembly and their amorphous nature. In consequence, this excludes almost entirely an unravelling of direct structure–property relationships. Hence, a common procedure is specifically to alter matrix components by chemical, enzymatic, or genetic treatments and to conduct mechanical tests to discriminate between the modified and the reference sample. The influence of individual cell-wall components in secondary cell walls has been shown by tensile tests on delignified, and cellulose or hemicellulose extracted samples (Köhler & Spatz, 2002; Konnerth *et al.*, 2010; Takeichi *et al.*, 2013). For interpretation of the obtained data, it has to be considered that the mechanical influence of the matrix components is largely affected by the cellulose orientation and the loading condition, as the influence of the matrix becomes more prominent with increasing MFA (see also Fig. 6). How this further relates to the loading condition can be seen in studies on the mechanical role of lignin. While genetic alteration in aspen trees only marginally influenced the tensile stiffness (Bjurhager *et al.*, 2010), nanoindentation tests on hardness revealed a positive correlation with the lignin content (Gindl *et al.*, 2004; de Borst *et al.*, 2012).

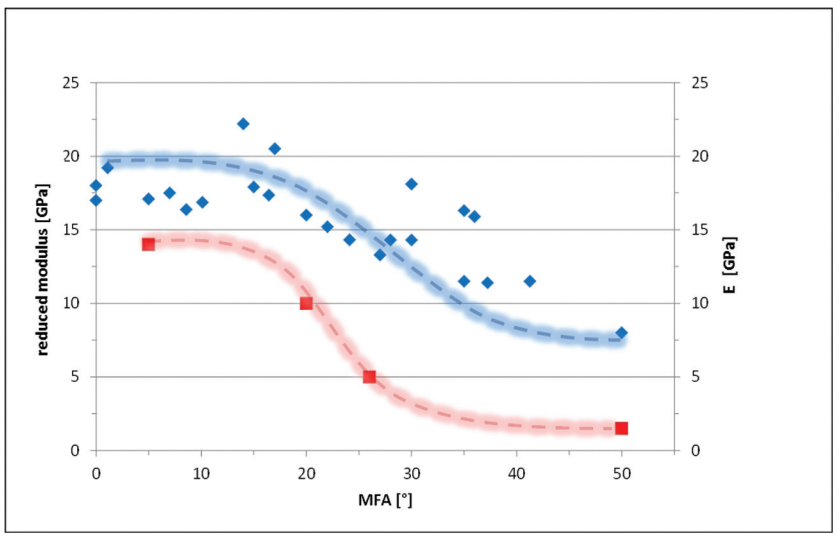


Fig. 6. Reduced moduli measured by nanoindentation plotted against microfibril angle (MFA) based on data in the literature (blue dots) of softwood cell walls (Gindl *et al.*, 2004; Tze *et al.*, 2007; Jager *et al.*, 2011*a*,*b*) and cell-wall stiffness calculated from microtensile tests plotted against MFA based on data in the literature (red dots) of spruce (Reiterer *et al.*, 1999). Dashed lines have been inserted to accent the different decreases in stiffness measured with both methods following an increase in cellulose MFA.

These interdependencies in characterizing the mechanical impact of cell-wall components underpin the fact that it is very important to gain further insight into the fibril–matrix interactions. This applies in particular to the hemicelluloses, as they are the dominating matrix components at the interface with cellulose fibrils and have a mediating function between cellulose and the other matrix components such as lignin. In this matter, *in situ* methods have contributed significantly to an advanced understanding of cell-wall deformation processes. X-ray measurements on plant tissues under external stresses showed that the orientation of cellulose fibrils can change upon external loading, in particular in wet secondary cell walls with a high MFA in the range of 30–50°. Analysis of cyclic loading behaviour indicated a passive movement or reorientation of cellulose fibrils (Köhler & Spatz, 2002; Keckes *et al.*, 2003). It is supposed that a multitude of hydrogen bonds that attach the hemicellulose chains to the cellulose surface can be opened and closed and thereby facilitate this 'Velcro' mechanism, which leads to a tight but highly flexible interface (Keckes *et al.*, 2003; Altaner & Jarvis, 2008). However, it needs to be mentioned that the required strains are beyond tensile deformations that can appear in woody tissues under natural conditions in living plants. Dynamic mechanical tests in combination with Fourier transform infra-red spectroscopy have allowed the division of hemicelluloses into two categories: those in close affinity to cellulose and those coupled to lignin. Additionally, this technique has provided information on the structural orientation of lignin and has thereby contributed to a more detailed model of secondary cell-wall architecture (Salmén & Olsson, 1998; Akerholm & Salmén, 2001, 2003).

Primary cell walls

In biomechanical studies on primary cell walls, the cellulose is less easily accessible due to the low thickness of the expanding primary cell wall and the rather low cellulose content. In primary cell walls, a tilting of cellulose is supposed to be a consequence of the axial expansion of the cell walls during cell growth (Preston, 1974, 1982; Baskin, 2005). However, we currently have no *in situ* technology at hand that allows the monitoring of reorientation of cellulose fibrils during growth processes. Studies that compare the cellulose orientation before and after expansion indicate that the tilting could be less pronounced than expected (Marga *et al.*, 2005).

Mechanical tests mainly aim at unravelling the specific function of pectin and hemicelluloses in the cell-wall network, as well as of assembly-modifying substances in the growth process (e.g. expansins, enzymes). Likewise, in secondary cell walls, the mechanical function of matrix components is addressed mainly by an alteration/modification of the targeted polymer. In primary cell-wall research, genetic modifications are further advanced and enzyme treatments are more favourable due to the better accessibility of the cell-wall structure. In addition to investigations on the natural cell wall, cell-wall analogues can also be utilized in micromechanical studies. Here, the cell-wall assembly is mimicked by merging bacterial cellulose with matrix polymers obtained from plant sources. Volume fractions and the composition

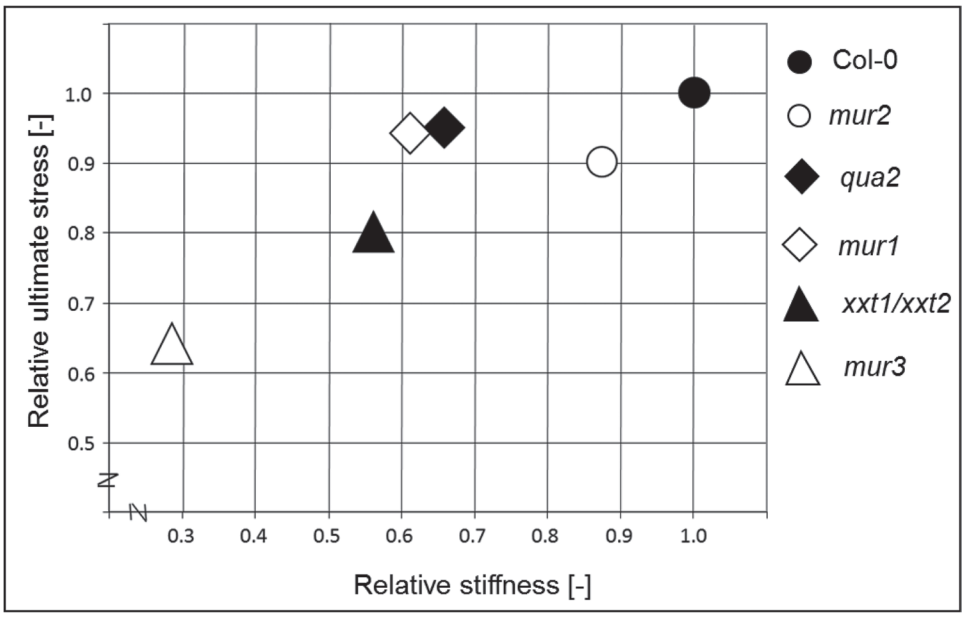


Fig. 7. Data plot of mean values of relative stiffness against relative ultimate stress of *Arabidopsis* hypocotyls to compare wild-type properties with xyloglucan and pectin mutants (4 and 6 d old); the arithmetic means are given as a percentage of the wild-type (Col-0=1.0). Detailed information on the mechanical properties (arithmetic means, standard deviation) of Col-0, *mur1*, *mur2*, and *qua2* is given in Abasolo *et al.* (2009), of Col-0 and *mur3* in Burgert (2006), and of Col-0 and *xxt1/xxt2* in Cavalier *et al.* (2008). Image from *Mechanical integration of plant cells and plants*, 2011, 27–52, Micromechanics of cell walls, Burgert I, Dunlop JWC. © Springer-Verlag Berlin Heidelberg 2011. With kind permission of Springer Science+Business Media.

of these artificial cell walls can be varied and the mechanical performance analysed (Chanliaud *et al.*, 2002). Generally, the addition of matrix substances leads to a reduction in cell-wall stiffness in comparison with a pure cellulose network whereupon the type of hemicellulose has a crucial impact on the mechanical performance (Whitney *et al.*, 1995, 1999).

AFM studies on local growth zones in meristems for organ initiation have revealed the prominent influence of pectin on cell-wall stiffness, as it was shown that tissue stiffness decreased with pectin demethylesterification (Peaucelle *et al.*, 2008, 2011). AFM stiffness tomography has also been used to map the mechanical properties of *Arabidopsis* during growth. The stiffness was higher in the exponential growth phase compared with the beginning and end of the growth process (Radotic *et al.*, 2012). Creep tests in particular on hypocotyls were utilized to investigate cell-wall extension in the presence of auxin, acid conditions, and cellulose- and hemicellulose-specific enzymes as well as expansins (Kutschera & Schopfer, 1986*a*,*b*; Cleland *et al.*, 1987; Cosgrove, 1988, 1989, 1993, 1999, 2011). These investigations have largely contributed to the understanding of primary cell-wall structure and composition, as well as the mechanisms of cell-wall loosening that allow cell expansion. More recently, standard tensile and cycling loading tests on genetically modified *Arabidopsis* hypocotyls further revealed the mechanical relevance of xyloglucan composition and pectin components, as well as the binding characteristics within and between the macromolecules. It was shown that hypocotyl stiffness and strength is highly influenced by the cellulose–xyloglucan network and by pectin in terms of rhamnogalacturonan II–borate complexes (Ryden *et al.*, 2003; Pena *et al.*, 2004). A severe reduction in the pectin homogalacturonan (*qua2*) largely affected the stiffness but only marginally affected the strength of the cell walls (Abasolo *et al.*, 2009). Hypocotyls with an altered xyloglucan structure (*mur2*, *mur3*, *xxt1*/*xxt2*) showed a loss in stiffness and strength to different extents. Interestingly, *mur1*, which possessed a more severe alteration in pectin than in xyloglucan, closely matched the *qua2* mutant (Fig. 7).

In particular, the remaining mechanical performance of the *xxt1*/*xxt2* mutant could not fully be explained on the basis of the widely accepted tethered cellulose–xyloglucan model, in spite of a severe xyloglucan alteration (Cavalier *et al.*, 2008). Park & Cosgrove (2012b) suggested recently a new cell-wall model with very local connections of adjacent cellulose fibrils based on observations of creep and relaxation behaviour of the same mutant (Park & Cosgrove, 2012*a*) and following treatments with cellulose- and hemicellulose-specific enzymes (see also the primary cell-wall illustration in Table 1).

Conclusion and outlook

Besides structural and (bio)chemical studies, micro- and nanomechanical techniques have become increasingly important tools to gain a deeper insight into structure–function relationships in plant materials. The obtained mechanical data contributes in a highly valuable manner to the development of new cell-wall models, as well as a better understanding of control and adjustment of mechanical properties and cell-expansion processes during cell growth. However, one always needs to be aware of the intrinsic constraints for a comprehensive and precise experimental characterization resulting from the inhomogeneity and anisotropy of the investigated plant material as well as polymer–water interactions and pre-stresses. In consequence, the present specific limitations in the mechanical characterization techniques have to be considered when interpreting the obtained data, and complementary modelling approaches are required to gain insight into the underlying principles of plant cell-wall structure and function.

Although primary and secondary cell walls have to fulfil different mechanical functions, they are both fibre composite structures governed by the general principles and mechanisms of biomacromolecular interactions. Hence, micro- and nanomechanical characterization techniques can not only reveal basic structure–function relationships of both cell-wall types but can also build a bridge between the rather independently acting research communities. New possibilities to gain a more advanced understanding of plant cell-wall structure and biomechanics arise both from the material and the methodology side. In terms of the material, the ongoing development in particular in the field of genetic modifications will lead to a vast pool of plants with highly specific alterations of cell-wall components and bonding patterns, for both primary and secondary cell walls. In terms of methodology, we expect further progress with *in situ* techniques making them also applicable at the primary cell-wall level. An even greater impact may arise from AFM and its combination with other methods, such as tip-enhanced Raman spectroscopy and scanning near-field optical microscopy, as they have the potential to provide new insights into the basic structure–function relationships with nanoscale resolution by simultaneously collecting the topography with AFM technology and chemical information with nano-optical methods. However, these methods are still highly challenging due to a lack of fully integrated instruments, problems with introducing artefacts, and data interpretation.

Acknowledgements

The authors thank Dr Matthew Harrington, MPI-KG, Potsdam, Germany, for proofreading this article. The financial support of the research group *Wood Materials Science* by the Bundesamt für Umwelt (BAFU) and Lignum, Switzerland, is gratefully acknowledged.

References

Abasolo W, Eder M, Yamauchi K, *et al.* 2009. Pectin may hinder the unfolding of xyloglucan chains during cell deformation: implications of the mechanical performance of *Arabidopsis* hypocotyls with pectin alterations. *Molecular Plant* **2,** 990–999.

Adusumalli RB, Mook WM, Passas R, Schwaller P, Michler J. 2010. Nanoindentation of single pulp fibre cell walls. *Journal of Materials Science* **45,** 2558–2563.

Akerholm M, Salmén L. 2001. Interactions between wood polymers studied by dynamic FT-IR spectroscopy. *Polymer* **42,** 963–969.

Akerholm M, Salmén L. 2003. The oriented structure of lignin and its viscoelastic properties studied by static and dynamic FT-IR spectroscopy. *Holzforschung* **57,** 459–465.

Altaner CM, Jarvis MC. 2008. Modelling polymer interactions of the 'molecular Velcro' type in wood under mechanical stress. *Journal of Theoretical Biology* **253,** 434–445.

Badel É, Perré P. 1999. Détermination des propriétés élastiques d'éléments individuels du plan ligneux du chêne par des essais de traction sur micro-éprouvettes. *Annals of Forest Science* **56,** 467–478.

Bader TK, Hofstetter K, Hellmich C, Eberhardsteiner J. 2011. The poroelastic role of water in cell walls of the hierarchical composite "softwood". *Acta Mechanica* **217,** 75–100.

Bargel H, Neinhuis C. 2005. Tomato (*Lycopersion esculentum* Mill.) fruit growth and ripening as related to the biomechanical properties of fruit skin and isolated cuticle. *Journal of Experimental Botany* **56,** 1049–1060.

Bargel H, Spatz HC, Speck T, Neinhuis C. 2004. Two-dimensional tension tests in plant biomechanics sweet cherry fruit skin as a model system. *Plant Biology* **6,** 432–439.

Baskin TI. 2005. Anisotropic expansion of the plant cell wall. *Annual Review of Cell and Developmental Biology* **21,** 203–222.

Besombes S, Mazeau K. 2005. The cellulose/lignin assembly assessed by molecular modeling. Part 1: adsorption of a *threo* guaiacyl β-*O*-4 dimer onto a Iβ cellulose whisker. *Plant Physiology and Biochemistry* **43,** 299–308.

Bjurhager I, Berglund LA, Bardage SL, Sundberg B. 2008. Mechanical characterization of juvenile European aspen (*Populus tremula*) and hybrid aspen (*Populus tremula*×*Populus tremuloides*) using full-field strain measurements. *Journal of Wood Science* **54,** 349–355.

Bjurhager I, Olsson AM, Zhang B, Gerber L, Kumar M, Berglund LA, Burgert I, Sundberg B, Salmén L. 2010. Ultrastructure and mechanical properties of *Populus* wood with reduced lignin content caused by transgenic down-regulation of cinnamate 4-hydroxylase. *Biomacromolecules* **11,** 2359–2365.

Bodner J, Grüll G, Schlag MG. 1996. In-situ fracturing of wood in the scanning electron microscope. *Holzforschung* **50,** 487–490.

Bolduc JF, Lewis LJ, Aubin CE, Geitmann A. 2006. Finite-element analysis of geometrical factors in micro-indentation of pollen tubes. *Biomechanics and Modeling in Mechanobiology* **5,** 227–236.

Bos HL, Van den Oever MJA, Peters O. 2002. Tensile and compressive properties of flax fibres for natural fibre reinforced composites. *Journal of Materials Science* **37,** 1683–1692.

Boyer JS. 2001. Growth-induced water potentials originate from wall yieling during growth. *Journal of Experimental Botany* **52,** 1483–1488.

Brändström J. 2001. Micro- and ultrastructural aspects of norway spruce tracheids: a review. *IAWA Journal* **22,** 333–353.

Burgert I. 2006. Exploring the micromechanical design of plant cell walls. *American Journal of Botany* **93,** 1391–1401.

Burgert I, Dunlop JWC. 2011. Micromechanics of cell walls. In: Wojtaszek P, ed. *Mechanical integration of plant cells and plants*. Heidelberg: Spinger Verlag, 27–52.

Burgert I, Eder M, Frühmann K, Keckes J, Fratzl P, Stanzl-Tschegg SE. 2005. Properties of chemically and mechanically isolated fibres of spruce (*Picea abies* [L.] Karst.). Part 1: mechanical characterisation. *Holzforschung* **59,** 354–357.

Burgert I, Eder M, Gierlinger N, Fratzl P. 2007. Tensile and compressive stresses in tracheids are induced by swelling based on geometrical constraints of the wood cell. *Planta* **226,** 981–987.

Burgert I, Fratzl P. 2009. Actuation systems in plants as prototypes for bioinspired devices. *Philosophical Transactions of the Royal Society A—Mathematical Physical and Engineering Sciences* **367,** 1541–1557.

Burgert I, Fruhmann K, Keckes J, Fratzl P, Stanzl-Tschegg SE. 2003. Microtensile testing of wood fibers combined with video extensometry for efficient strain detection. *Holzforschung* **57,** 661–664.

Butt HJ, Cappella B, Kappl M. 2005. Force measurements with the atomic force microscope: technique, interpretation and applications. *Surface Science Reports* **59,** 1–152.

Cavalier DM, Lerouxel O, Neumetzler L, *et al.* 2008. Disrupting two *Arabidopsis thaliana* xylosyltransferase genes results in plants deficient in xyloglucan, a major primary cell wall component. *Plant Cell* **20,** 1519–1537.

Cave ID. 1968. The anisotropic elasticity of the plant cell wall. *Wood Science and Technology* **2,** 268–278.

Chanliaud E, Burrows KM, Jeronimidis G, Gidley MJ. 2002. Mechanical properties of primary plant cell wall analogues. *Planta* **215,** 989–996.

Clair B, Almeras T, Pilate G, Jullien D, Sugiyama J, Riekel C. 2011. Maturation stress generation in poplar tension wood studied by synchrotron radiation microdiffraction. *Plant Physiology* **155,** 562–570.

Clair B, Arinero R, Leveque G, Ramonda M, Thibaut B. 2003. Imaging the mechanical properties of wood cell wall layers by atomic force modulation microscopy. *IAWA Journal* **24,** 223–230.

Cleland R, Cosgrove DJ, Tepfer M. 1987. Long-term acid-induced wall extension in an in-vitro system. *Planta* **170,** 379–385.

Cleland RE. 1971. The mechanical behavior of isolated *Avena* coleoptile walls subjected to constant stress. *Plant Physiology* **47,** 805–811.

Cosgrove DJ. 1988. Mechanism of rapid suppression of cell expansion in cucumber hypocotyls after blue-light radiation. *Planta* **176,** 109–116.

Cosgrove DJ. 1989. Characterization of long-term extension of isolated cell walls from growing cucumber hypocotyls. *Planta* **177,** 121–130.

Cosgrove DJ. 1993. Wall extensibility—its nature, measurement and relationship to plant-cell growth. *New Phytologist* **124,** 1–23.

Cosgrove DJ. 1999. Enzymes and other agents that enhance cell wall extensibility. *Annual Review of Plant Physiology and Plant Molecular Biology* **50,** 391–417.

Cosgrove DJ. 2000. Loosening of plant cell walls by expansins. *Nature* **407,** 321–326.

Cosgrove DJ. 2001. Wall structure and wall loosening: a look backwards and forwards. *Plant Physiology* **125,** 131–134.

Cosgrove DJ. 2005. Growth of the plant cell wall. *Nature Reviews Molecular Cell Biology* **6,** 850–861.

Cosgrove DJ. 2011. Measuring in vitro extensibility of growing plant cell walls. In: Popper ZA, ed. *Plant cell wall: methods and protocols*. USA: Humana Press, 291–303.

Cosgrove DJ, Jarvis MC. 2012. Comparative structure and biomechanics of plant primary and secondary cell walls. *Frontiers in Plant Science* **3,** 204.

de Borst K, Bader TK, Wikete C. 2012. Microstructure–stiffness relationships of ten European and tropical hardwood species. *Journal of Structural Biology* **177,** 532–542.

Donaldson L. 2008. Microfibril angle: measurement, variation and relationships—a review. *Iawa Journal* **29,** 345–386.

Donaldson LA. 2001. Lignification and lignin topochemistry—an ultrastructural view. *Phytochemistry* **57,** 859–873.

Doyle J, Walker JCF. 1985. Indentation hardenss of wood. *Wood and Fiber Science* **17,** 369–376.

Dunlop JWC, Fratzl P. 2010. Biological composites. *Annual Review of Materials Research* **40,** 1–24.

Ebenstein DM, Pruitt LA. 2006. Nanoindentation of biological materials. *Nano Today* **1,** 26–33.

Eder M, Arnould O, Dunlop JWC, Hornatowska J, Salmén L. 2013. Experimental micromechanical characterisation of wood cell walls. *Wood Science and Technology* **47,** 163–182.

Eder M, Burgert I 2010. Natural fibres—function in nature. In: Müssig J, ed. *Industrial applications of natural fibres—structure, properties and technical applications*. Weinheim: Wiley-VCH.

Eder M, Rueggeberg M, Burgert I. 2009. A close-up view of the mechanical design of arborescent plants at different levels of hierarchy—requirements and structural solutions. *New Zealand Journal of Forestry Science* **39,** 115–124.

Eder M, Stanzl-Tschegg S, Burgert I. 2008. The fracture behaviour of single wood fibres is governed by geometrical constraints: *in situ* ESEM studies on three fibre types. *Wood Science and Technology* **42,** 679–689.

Eichhorn SJ, Baillie CA, Zafeiropoulos N, *et al.* 2001*a*. Current international research into cellulosic fibres and composites. *Journal of Materials Science* **36,** 2107–2131.

Eichhorn SJ, Hughes M, Snell R, Mott L. 2000. Strain induced shifts in the raman spectra of natural cellulose fibers. *Journal of Materials Science Letters* **19,** 721–723.

Eichhorn SJ, Sirichaisit J, Young RJ. 2001*b*. Deformation mechanisms in cellulose fibres, paper and wood. *Journal of Materials Science* **36,** 3129–3135.

Fahlen J, Salmén L. 2003. Cross-sectional structure of the secondary wall of wood fibers as affected by processing. *Journal of Materials Science* **38,** 119–126.

Fahlen J, Salmén L. 2005. Pore and matrix distribution in the fiber wall revealed by atomic force microscopy and image analysis. *Biomacromolecules* **6,** 433–438.

Farber J, Lichtenegger HC, Reiterer A, Stanzl-Tschegg S, Fratzl P. 2001. Cellulose microfibril angles in a spruce branch and mechanical implications. *Journal of Materials Science* **36,** 5087–5092.

Fischer-Cripps AC. 2011. *Nanoindentation*. New York: Springer.

Forterre Y, Skotheim JM, Dumais J, Mahadevan L. 2005. How the venus flytrap snaps. *Nature* **433,** 421–425.

Fratzl P, Burgert I, Gupta HS. 2004*a*. On the role of interface polymers for the mechanics of natural polymeric composites. *Physical Chemistry Chemical Physics* **6,** 5575–5579.

Fratzl P, Burgert I, Keckes J. 2004*b*. Mechanical model for the deformation of the wood cell wall. *Zeitschrift für Metallkunde* **95,** 579–584.

Fratzl P, Weinkamer R. 2007. Nature's hierarchical materials. *Progress in Materials Science* **52,** 1263–1334.

Fruhmann K, Burgert I, Stanzl-Tschegg SE. 2003. Detection of the fracture path under tensile loads through *in situ* tests in an ESEM chamber. *Holzforschung* **57,** 326–332.

Fry SC, Smith RC, Renwick KF, Martin DJ, Hodge SK, Matthews KJ. 1992. Xyloglucan endotransglycosylase, a new wall-loosening enzyme activity from plants. *Biochemical Journal* **282,** 821–828.

Geitmann A. 2006. Experimental approaches used to quantify physical parameters at cellular and subcellular levels. *American Journal of Botany* **93,** 1380–90.

Gibson LJ. 2012. The hierarchical structure and mechanics of plant materials. *Journal of the Royal Society Interface* **9,** 2749–2766.

Gierlinger N, Schwanninger M, Reinecke A, Burgert I. 2006. Molecular changes during tensile deformation of single wood fibers followed by Raman microscopy. *Biomacromolecules* **7,** 2077–2081.

Gindl W, Gupta HS, Schoberl T, Lichtenegger HC, Fratzl P. 2004. Mechanical properties of spruce wood cell walls by nanoindentation. *Applied Physics A—Materials Science & Processing* **79,** 2069–2073.

Gindl W, Schoberl T. 2004. The significance of the elastic modulus of wood cell walls obtained from nanoindentation measurements. *Composites Part A—Applied Science and Manufacturing* **35,** 1345–1349.

Goswami L, Dunlop JWC, Jungnikl K, Eder M, Gierlinger N, Coutand C, Jeronimidis G, Fratzl P, Burgert I. 2008. Stress generation in the tension wood of poplar is based on the lateral swelling power of the G-layer. *The Plant Journal* **56,** 531–538.

Green NH, Allen S, Davies MC, Roberts CJ, Tendler SJB, Williams PM. 2002. Force sensing and mapping by atomic force microscopy. *Trends in Analytical Chemistry* **21,** 64–73.

Groom L, Mott L, Shaler S. 2002*a*. Mechanical properties of individual southern pine fibers. Part I. determination and variability of stress-strain curves with respect to tree height and juvenility. *Wood and Fiber Science* **34,** 14–27.

Groom L, Shaler S, Mott L. 2002*b*. Mechanical properties of individual southern pine fibers. Part III. Global relationships between fiber properties and fiber location within an individual tree. *Wood and Fiber Science* **34,** 238–250.

Hanus J, Mazeau K. 2006. The xyloglucan–cellulose assembly at the atomic scale. *Biopolymers* **82,** 59–73.

Hinterdorfer P, Baumgartner W, Gruber HJ, Schilcher K, Schindler H. 1996. Detection and localization of individual antibody–antigen recognition events by atomic force microscopy. *Proceedings of the National Academy of Sciences, USA* **93,** 3477–3481.

Hoenicka H, Lautner S, Klingberg A, *et al.* 2012. Influence of over-expression of the *FLOWERING PROMOTING FACTOR 1* gene (*FPF1*) from *Arabidopsis* on wood formation in hybrid poplar (*Populus tremula* L.×*P. tremuloides* Michx.). *Planta* **235,** 359–373.

Hofstetter K, Hellmich C, Eberhardsteiner J. 2005. Development and experimental validation of a continuum micromechanics model for the elasticity of wood. *European Journal of Mechanics A—Solids* **24,** 1030–1053.

Hutter JL, Bechhoefer J. 1993. Calibration of atomic force microscoy tips. *Review of Scientific Instruments* **64,** 1868–1873.

Jager A, Bader T, Hofstetter K, Eberhardsteiner J. 2011*a*. The relation between indentation modulus, microfibril angle, and elastic properties of wood cell walls. *Composites Part A—Applied Science and Manufacturing* **42,** 677–685.

Jager A, Hofstetter K, Buksnowitz C, Gindl-Altmutter W, Konnerth J. 2011*b*. Identification of stiffness tensor components of wood cell walls by means of nanoindentation. *Composites Part A—Applied Science and Manufacturing* **42,** 2101–2109.

Jarvis MC. 2009. Plant cell walls: supramolecular assembly, signalling and stress. *Structural Chemistry* **20,** 245–253.

Jayne BA. 1959. Mechanical properties of wood fibers. *Tappi Journal* **42,** 461–467.

Kamiyama T, Suzuki H, Sugiyama J. 2005. Studies of the structural change during deformation in *Cryptomeria japonica* by time-resolved synchrotron small-angle X-ray scattering. *Journal of Structural Biology* **151,** 1–11.

Kasas S, Longo G, Dietler G. 2013. Mechanical properties of biological specimens explored by atomic force microscopy. *Journal of Physics D—Applied Physics* **46,** 133001.

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. *Nature Materials* **2,** 810–814.

Kempson GE, Freeman MAR, Swanson SAV. 1971*a*. Determination of a creep modulus for articular cartilage from indentation tests on human femoral head. *Journal of Biomechanics* **4,** 239–250.

Kempson GE, Swanson SAV, Spivey CJ, Freeman MAR. 1971*b*. Patterns of cartilage stiffness on normal and degenerate human femoral heads. *Journal of Biomechanics* **4,** 597–609.

Kersevage PC. 1973. Moisture content effect on tensile properties of individual Douglas-fir latewood tracheids. *Wood Fiber Science* **5,** 105–117.

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

Kirby AR, Gunning AP, Waldron KW, Morris VJ, Ng A. 1996. Visualization of plant cell walls by atomic force microscopy. *Biophysical Journal* **70,** 1138–1143.

Köhler L, Spatz HC. 2002. Micromechanics of plant tissues beyond the linear-elastic range. *Planta* **215,** 33–40.

Kölln K, Grotkopp I, Burghammer M, Roth SV, Funari SS, Dommach M, Müller M. 2005. Mechanical properties of cellulose fibres and wood. Orientational aspects *in situ* investigated with synchrotron radiation. *Journal of Synchrotron Radiation* **12,** 739–744.

Konnerth J, Eiser M, Jager A, Bader TK, Hofstetter K, Follrich J, Ters T, Hansmann C, Wimmer R. 2010. Macro- and micro-mechanical properties of red oak wood (*Quercus rubra* L.) treated with hemicellulases. *Holzforschung* **64,** 447–453.

Konnerth J, Gierlinger N, Keckes J, Gindl W. 2009. Actual versus apparent within cell wall variability of nanoindentation results from wood cell walls related to cellulose microfibril angle. *Journal of Materials Science* **44,** 4399–4406.

Konnerth J, Harper D, Lee SH, Rials TG, Gindl W. 2008. Adhesive penetration of wood cell walls investigated by scanning thermal microscopy (SThM). *Holzforschung* **62,** 91–98.

Krotil HU, Stifter T, Waschipky H, Weishaupt K, Hild S, Marti O. 1999. Pulsed force mode: a new method for the investigation of surface properties. *Surface and Interface Analysis* **27,** 336–340.

Kutschera U, Kohler K. 1994. Cell elongation, turgor and osmotic-pressure in developing sunflower hypocotyls. *Journal of Experimental Botany* **45,** 591–595.

Kutschera U, Schopfer P. 1986*a*. Effect of auxin and abscisic acid on cell wall extensibility in maize coleoptiles. *Planta* **167,** 527–535.

Kutschera U, Schopfer P. 1986*b*. *In-vivo* measurement of cell-wall extensibility in maize coloeptiles: effects of auxin and abscisic acid. *Planta* **169,** 437–442.

Landahl S, Herppich WB, Herold B, Geyer M, De Baerdemaeker J. 2004. A comprehensive evaluation of the interactions between produce elasticity and water status. *European Journal of Horticultural Science* **69,** 250–257.

Lichtenegger H, Reiterer A, Stanzl-Tschegg SE, Fratzl P. 1999. Variation of cellulose microfibril angles in softwoods and hardwoods—a possible strategy of mechanical optimization. *Journal Of Structural Biology* **128,** 257–269.

Marga F, Grandbois M, Cosgrove DJ, Baskin TI. 2005. Cell wall extension results in the coordinate seperation of parallel microfibrils: evidence from scanning electron microscopy and atomic force microscopy. *The Plant Journal* **43,** 181–190.

Mark RE, Gillis PP. 1970. New models in cell-wall mechanics. *Wood Fiber* **2,** 79–95.

Mark RE, Gillis PP. 1973. The relationship between fiber modulus and S2 angle. *Tappi Journal* **56,** 164–167.

Martone PT, Boller M, Burgert I, Dumais J, Edwards J, Mach K, Rowe N, Rueggeberg M, Seidel R, Speck T. 2010. Mechanics without muscle: biomechanical inspiration from the plant world. *Integrative and Comparative Biology* **50,** 888–907.

Matas AJ, Cobb ED, Bartsch JA, Paolillo DJ, Niklas KJ. 2004. Biomechanics and anatomy of *Lycopersicon esculentum* fruit peels and enzyme-treated samples. *American Journal of Botany* **91,** 352–360.

McCann MC, Roberts K. 1991. Architecture of the primary cell wall. In: Lloyd CW, ed. *The cytosceletal basis of plant growth and form*. New York: Academic Press, 109–129.

Meng Y, Wang S, Cai Z, Young TM, Du G, Li Y. 2013. A novel sample preparation method to avoid influence of embedding medium during nano-indentation. *Applied Physics A—Materials Science & Processing* **110,** 361–369.

Milani P, Braybrook SA, Boudaoud A. 2013. Shrinking the hammer: micromechanical approaches to morphogenesis. *Journal of Experimental Botany* **64**, 4651–4662.

Moran N. 2007. Osmoregulation of leaf motor cells. *FEBS Letters* **581,** 2337–2347.

Morris S, Hanna S, Miles MJ. 2004. The self-assembly of plant cell wall components by single-molecule force spectroscopy and Monte Carlo modelling. *Nanotechnology* **15,** 1296–1301.

Mott L, Shaler S, Groom L, Liang BH. 1995. The tensile testing of individual wood fibers using environmental scanning electron microscopy and video imaging analysis. *Tappi Journal* **78,** 143–148.

Nakahori K, Katou K, Okamoto H. 1991. Auxin changes oth the extensibility and the yield threshod of the cell wall of *Vigna* hypocotyls. *Plant Cell Physiology* **32,** 121–129.

Navi P, Rastogi PK, Gresse V, Tolou A. 1995. Micromechanics of wood subjected to axial tension. *Wood Science Technology* **29,** 411–429.

Nazarian A, Stauber M, Muller R. 2005. Design and implementation of a novel mechanical testing system for cellular solids. *Journal of Biomedical Materials Research Part B—Applied Biomaterials* **73B,** 400–411.

Niklas KJ, Spatz HC. 2012. *Plant Physics*. London: University of Chicago Press.

Niklas KJ. 2009. Functional adaptation and phenotypic plasticity at the cellular and whole plant level. *Journal of Biosciences* **34,** 613–620.

Oliver WC, Pharr GM. 1992. An improved technique for determining hardness and elastic modulus using load and displacement sensing indentation experiments. *Journal of Materials Research* **7,** 1564–1583.

Oyen ML. 2005. Spherical indentation creep following ramp loading. *Journal of Materials Research* **20,** 2094–2100.

Oyen ML. 2011. *Handbook of nanoindentation with biological applications*. Singapore: Pan Stanford Publishing.

Page DH, El-Hosseiny F. 1983. The mechanical properties of single wood pulp fibres. Part IV. Fibril angle and the shape of the stress-strain curve. *Journal of Pulp and Paper Science* **9,** 1–2.

Page DH, El-Hosseiny F, Winkler K. 1971. Behaviour of single wood fibres under axial tensile strain. *Nature* **229,** 252–253.

Paris O, Burgert I, Fratzl P. 2010. Biomimetics and biotemplating of natural materials. *MRS Bulletin* **35,** 219–225.

Park YB, Cosgrove DJ. 2012*a*. Changes in cell wall biomechanical properties in the xyloglucan-deficient *xxt1/xxt2* mutant of *Arabidopsis*. *Plant Physiology* **158,** 465–475.

Park YB, Cosgrove DJ. 2012*b*. A revised architecture of primary cell walls based on biomechanical changes induced by substrate-specific endoglucanases. *Plant Physiology* **158,** 1933–1943.

Pauly M, Albersheim P, Darvill AG, York WS. 1999. Molecular domains of the cellulose/xyloglucan network in the cell walls of higher plants. *The Plant Journal* **20,** 629–639.

Peaucelle A, Braybrook SA, Le Guillou L, Bron E, Kuhlemeier C, Hoefte H. 2011. Pectin-induced changes in cell wall mechanics underline organ initiation in *Arabidopsis*. *Current Biology* **21,** 1720–1726.

Peaucelle A, Louvet R, Johansen JN, Hoefte H, Laufs P, Pelloux J, Mouille G. 2008. *Arabidopsis* phyllotaxis is controlled by the methyl-esterification status of cell-wall pectins. *Current Biology* **18,** 1943–1948.

Peetla P, Schenzel KC, Diepenbrock W. 2006. Determination of mechanical strength properties of hemp fibers using near-infrared fourier transform raman microspectroscopy. *Applied Spectroscopy* **60,** 682–691.

Pena MJ, Ryden P, Madson M, Smith AC, Carpita NC. 2004. The galactose residues of xyloglucan are essential to maintain mechancial strength of the primary cell walls in arabidopsis during growth. *Plant Physiology* **134,** 443–451.

Perez S, Kouwijzer M, Mazeau K, Engelsen SB. 1996. Modeling polysaccharides: present status and challenges. *Journal of Molecular Graphics & Modelling* **14,** 307

Preston R. 1974. *The physical biology of plant cell walls*. London: Chapmann & Hall.

Preston RD. 1982. The case for multinet growth in growing walls of plant cells. *Planta* **155,** 356–363.

Proseus TE, Boyer JS. 2006. Calcium pectate chemistry controls growth rate of *Chara corallina*. *Journal of Experimental Botany* **57,** 3989–4002.

Proseus TE, Ortega JKE, Boyer JS. 1999. Separating growth from elastic deformation during cell enlargement. *Plant Physiology* **119,** 775–784.

Radotic K, Roduit C, Simonovic J, Hornitschek P, Fankhauser C, Mutavdzic D, Steinbach G, Dietler G, Kasas S. 2012. Atomic force microscopy stiffness tomography on living *Arabidopsis thaliana* cells reveals the mechanical properties of surface and deep cell-wall layers during growth. *Biophysical Journal* **103,** 386–394.

Refregier G, Pelletier S, Jaillard D, Hofte H. 2004. Interaction between wall deposition and cell elongation in dark-grown hypocotyl cells in *Arabidopsis*. *Plant Physiology* **135,** 959–968.

Reiterer A, Lichtenegger H, Tschegg S, Fratzl P. 1999. Experimental evidence for a mechanical function of the cellulose microfibril angle in wood cell walls. *Philosophical Magazine A* **79,** 2173–2184.

Richmond PA, Métraux JP, Taiz L. 1980. Cell expansion patterns and directionality of wall mechanical properties in *Nitella*. *Plant Physiology* **65,** 211–217.

Roelfsema MRG, Hedrich R. 2005. In the light of stomatal opening: new insights into 'the Watergate'. *New Phytologist* **167,** 665–691.

Routier-Kierzkowska AL, Weber A, Kochova P, Felekis D, Nelson BJ, Kuhlemeier C, Smith RS. 2012. Cellular force microscopy for in vivo measurements of plant tissue mechanics. *Plant Physiology* **158,** 1514–1522.

Ruggeberg M, Speck T, Paris O, Lapierre C, Pollet B, Koch G, Burgert I. 2008. Stiffness gradients in vascular bundles of the palm *Washingtonia robusta*. *Proceedings of the Royal Society B—Biological Sciences* **275,** 2221–2229.

Ryden P, Sugimoto-Shirasu K, Smith AC, Findlay K, Reiter WD, McCann MC. 2003. Tensile properties of *Arabidopsis* cell walls depend on both a xyloglucan cross-linked microfibrillar network and rhamnogalacturonan II–borate complexes. *Plant Physiology* **132,** 1033–1040.

Salmén L, Bergstrom E. 2009. Cellulose structural arrangement in relation to spectral changes in tensile loading FTIR. *Cellulose* **16,** 975–982.

Salmén L, Burgert I. 2009. Cell wall features with regard to mechanical performance. A review COST Action E35 2004–2008: wood machining—micromechanics and fracture. *Holzforschung* **63,** 121–129.

Salmén L, de Ruvo A. 1985. A model for the prediction of fiber elasticity. *Wood and Fiber Science* **17,** 336–350.

Salmén L, Olsson AM. 1998. Interaction between hemicelluloses, lignin and cellulose: structure-property relationships. *Journal of Pulp and Paper Science* **24,** 99–103.

Saren MP, Serimaa R, Andersson S, Paakkari T, Saranpaa P, Pesonen E. 2001. Structural variation of tracheids in Norway spruce (*Picea abies* [L.] Karst.). *Journal of Structural Biology* **136,** 101–109.

Schopfer P. 2001. Hydroxyl radical-induced cell wall loosening *in vitro* and *in vivo*: implications for the control of elongation growth. *The Plant Journal* **28,** 679–688.

Sedighi-Gilani M, Sunderland H, Navi P. 2005. Microfibril angle non-uniformities within normal and compression wood tracheids. *Wood Science and Technology* **39,** 419–430.

Shao ZP, Fang CH, Huang SX, Tian GL. 2010. Tensile properties of Moso bamboo (*Phyllostachys pubescens*) and its components with respect to its fiber-reinforced composite structure. *Wood Science and Technology* **44,** 655–666.

Sidorenko A, Krupenkin T, Taylor A, Fratzl P, Aizenberg J. 2007. Reversible switching of hydrogel-actuated nanostructures into complex micropatterns. *Science* **315,** 487–490.

Somerville C, Bauer S, Brininstool G, *et al.* 2004. Toward a systems approach to understanding plant cell walls. *Science* **306,** 2206–2211.

Spatz HC, Köhler L, Niklas KJ. 1999. Mechanical behaviour of plant tissues: composite materials or structures? *Journal of Experimental Biology* **202,** 3269–3272.

Speck T, Burgert I. 2011. Plant stems: functional design and mechanics. *Annual Review of Materials Research* **41,** 169–193.

Sturcova A, Eichhorn SJ, Jarvis MC. 2006. Vibrational spectroscopy of biopolymers under mechanical stress: processing cellulose spectra using bandshift difference integrals. *Biomacromolecules* **7,** 2688–2691.

Suslov D, Verbelen JP. 2006. Cellulose orientation determines mechanical anisotropy in onion epidermis cell walls. *Journal of Experimental Botany* **57,** 2183–2192.

Takeichi Y, Yoshida M, Kitano K, Terashima N, Yamamoto H. 2013. *In situ* measurement of tensile elastic moduli of individual component polymers with a 3D assembly mode in wood cell walls. *Journal of Wood Science* **59,** 104–111.

Telewski FW. 2006. A unified hypothesis of mechanoperception in plants. *American Journal of Botany* **933,** 1466–1476.

Tetard L, Passian A, Farahi RH, Kalluri UC, Davison BH, Thundat T. 2010*a*. Spectroscopy and atomic force microscopy of biomass. *Ultramicroscopy* **110,** 701–707.

Tetard L, Passian A, Thundat T. 2010*b*. New modes for subsurface atomic force microscopy through nanomechanical coupling. *Nature Nanotechnology* **5,** 105–109.

Thygesen LG, Eder M, Burgert I. 2007. Dislocations in single hemp fibres-investigations into the relationship of structural distortions and tensile properties at the cell wall level. *Journal of Materials Science* **42,** 558–564.

Tze WTY, Wang S, Rials TG, Pharr GM, Kelley SS. 2007. Nanoindentation of wood cell walls: continuous stiffness and hardness measurements. *Composites Part A—Applied Science and Manufacturing* **38,** 945–953.

VanLandingham MR. 2003. Review of instrumented indentation. *Journal of Research of the National Institute of Standards and Technology* **108,** 249–265.

Vlassak JJ, Ciavarella M, Barber JR, Wang X. 2003. The indentation modulus of elastically anisotropic materials for indenters of arbitrary shape. *Journal of the Mechanics and Physics of Solids* **51,** 1701–1721.

Wang CX, Wang L, Thomas CR. 2004. Modelling the mechanical properties of single suspension-cultured tomato cells. *Annals of Botany* **93,** 443–453.

Wang XQ, Ren HQ, Zhang B, Fei BH, Burgert I. 2012. Cell wall structure and formation of maturing fibres of moso bamboo (*Phyllostachys pubescens*) increase buckling resistance. *Journal of the Royal Society Interface* **9,** 988–996.

Wegst UGK, Ashby MF. 2004. The mechanical efficiency of natural materials. *Philosophical Magazine* **84,** 2167–2181.

Whitney SEC, Brigham JE, Darke AH, Reid JSG, Gidley MJ. 1995. In-vitro assembly of cellulose/xyloglucan networks—ultrastructural and molecular aspects. *The Plant Journal* **8,** 491–504.

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 Physiology* **121,** 657–663.

Williams SE, Bennett AB. 1982. Leaf closure in the Venus flytrap: an acid growth response. *Science* **218,** 1120–1122.

Wimmer R, Lucas BN, Tsui TY, Oliver WC. 1997. Longitudinal hardness and Young's modulus of spruce tracheid secondary walls using nanoindentation. *Wood Science and Technology* **31,** 131–141.

Wu Y, Wang S, Zhou D, Xing C, Zhang Y. 2009. Use of nanoindentation and Silviscan to determine the mechanical properties of 10 hardwood species. *Wood and Fiber Science* **41,** 64–73.

Yamamoto H, Kojima Y. 2002. Properties of cell wall constituents in relation to longitudinal elasticity of wood. Part 1. Formulation of the longitudinal elasticity of an isolated wood fiber. *Wood Science and Technology* **36,** 55–74.

Yamamoto H. 1998. Generation mechanism of growth stresses in wood cell walls: roles of lignin deposition and cellulose microfibril during cell wall maturation. *Wood Science and Technology* **32,** 171–182.

Yarbrough JM, Himmel ME, Ding SY. 2009. Plant cell wall characterization using scanning probe microscopy techniques. *Biotechnology for Biofuels* **2**.

Yu Y, Jiang ZH, Fei BH, Wang G, Wang HK. 2011. An improved microtensile technique for mechanical characterization of short plant fibers: a case study on bamboo fibers. *Journal of Materials Science* **46,** 739–746.

Zabler S, Paris O, Burgert I, Fratzl P. 2010. Moisture changes in the plant cell wall force cellulose crystallites to deform. *Journal of Structural Biology* **171,** 133–141.

Zamir EA, Taber LA. 2004. On the effects of residual stress in microindentation tests of soft tissue structures. *Journal of Biomechanical Engineering* **126,** 276–283.

Zauner M, Keunecke D, Mokso R, Stampanoni M, Niemz P. 2012. Synchrotron-based tomographic microscopy (SbTM) of wood: development of a testing device and observation of plastic deformation of uniaxially compressed Norway spruce samples. *Holzforschung* **66,** 973–979.