

REVIEW PAPER

Shrinking the hammer: micromechanical approaches to morphogenesis

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Abstract

Morphogenesis, the remarkable process by which a developing organism achieves its shape, relies on the coordinated growth of cells, tissues, and organs. While the molecular and genetic basis of morphogenesis is starting to be unravelled, understanding shape changes is lagging behind. Actually, shape is imposed by the structural elements of the organism, and the translation of cellular activity into morphogenesis must go through these elements. Therefore, many methods have been developed recently to quantify, at cellular resolution, the properties of the main structural element in plants, the cell wall. As plant cell growth is restrained by the cell wall and powered by turgor pressure, such methods also address the quantification of turgor. These different micromechanical approaches are reviewed here, with a critical assessment of their strengths and weaknesses, and a discussion of how they can help us understand the regulation of growth and morphogenesis.

Key words: Atomic force microscopy, biomechanics, cell wall, growth control, indentation, morphogenesis, pressure probe, turgor pressure.

Introduction

Morphogenesis is a beautiful and complex process requiring a precise translation of the genetic code, the activity of gene products, signalling events, chemical changes, and changes in the physical properties of the growing cell or tissue, which, finally, prescribe growth patterns. This review is focused on the last step of this cascade: the mechanical/physical aspects of morphogenesis in plants; specifically, how we can measure the micromechanics of growth and shape formation. Indeed, a number of approaches have been developed recently to address the biomechanics of growing plants at cellular and subcellular resolutions.

Plant cells are unique in two mechanical aspects that are relevant here. First, they include an enveloping cell wall, which connects cells to each other and prohibits cell migration. Secondly, the high pressure built up inside the plant cell holds

cells turgid. These two aspects are entangled: cell pressure, or turgor pressure, provides the push for growth, while the cell wall allows the build-up of a high pressure and restrains it, enabling control of growth. This principle has been developed in single cells, such as internode cells from Charales (Green, 1968; Proseus *et al.*, 1999), and in cylindrical organs such as the hypocotyl, stem, or coleoptile of various species (Cleland *et al.*, 1987; Cosgrove, 1989; McQueen-Mason *et al.*, 1992; see e.g. Baskin and Jensen, 2013, for a review). These systems continue to provide vital information today on the principle of the growth process and its control, and it is believed that this principle extends to more complex geometries or tissues.

The spatial and temporal regulation of the force of turgor and the resistance provided by the cell wall results in

morphogenesis. As such, it is imperative to measure both turgor pressure and cell-wall mechanical properties at least at cellular resolution. Methods for studying tissue-level mechanics have been available for a long time. As an example, the coleoptile bending studies and growth analyses performed by Cleland and colleagues (1987) are the basis of several principles now taught in classrooms: acid growth, auxin-induced elongation, and turgor-powered growth. The development of the modern 'extensometer' allowed more accurate quantifications of the forces involved in whole-organ deformation studies (Cosgrove, 1989; Durachko and Cosgrove, 2009); the principle of the extensometer is to apply an axial force to a cylindrical organ and monitor organ elongation under various conditions; it has been used for the hypocotyl, yielding the theory of expansin-induced growth and thereby increasing our understanding of cell-wall structure and its reaction to expansin activity. For a long time, pressure has been estimated via the plasmolytic technique developed by de Vries (de Vries, 1884; Beck, 1929); the principle is to increase the osmolality of the external medium and monitor the threshold at which the protoplast starts separating from the cell wall. While this method is indirect, it is still used today and can provide valuable information; however, it is only viable in large cells, with adequate imaging resolution, and with access to the tissues being analysed both by plasmolytic solutions and microscopic observation. The plasmolytic technique enables inferring internal osmolality of the protoplast at the abovementioned threshold, but this osmolality might differ from the osmolality of the unperturbed protoplast because of osmoregulation, i.e. the active regulation of internal osmolality in reaction to the osmotic stress induced by the external medium.

Recent years have seen the development of a series of micro-mechanical tools for studying both pressure and wall mechanics at single-cell levels and within more complex tissues. There are several excellent reviews on the methods of plant micro-mechanics available (Geitmann, 2006; Routier-Kierzkowska and Smith, 2013), in addition to introductions to the concepts of mechanics in the context of plant development (Geitmann and Ortega, 2009; Boudaoud, 2010; Mirabet *et al.*, 2011). This review will focus on indentation-based methods for determining pressure and cell-wall mechanical properties at cellular or subcellular resolution. Further discussion regarding the interpretation of these experiments will be presented, with particular attention paid to the necessity of computational modelling when complex mechanical properties are analysed. Lastly, an example case study will be presented detailing recent work on the mechanics of organ formation at the shoot apex; an in-depth treatment of this topic may be found in this special issue (Robinson *et al.*, 2013). Overall, a wealth of new methods and useful information is now available, and these methods are improving and will allow progress in plant development, plant biomechanics, phytopathology, and certainly beyond.

Measuring turgor pressure

The major role of turgor pressure in plant growth and development has been recognized since the early 20th century.

Nevertheless, it took more than half a century to see the development of the first method for direct examination of turgor pressure in an individual cell. This method, now known as the pressure probe, has been described by Paul Green and consists of the insertion of an oil-filled microcapillary directly into the vacuole of the studied cell (Green, 1968). The pressure of the cell contents compresses the air bubble trapped in the capillary and the compression is used to calculate the initial pressure of the cell. The first improvement was to replace the bubble with oil in contact with a reservoir, the pressure of which is monitored by a pressure sensor: turgor pressure would be the measured pressure when the meniscus between oil and cell sap is kept at the original position of the plasma membrane. This method was applied to giant-celled algae such as *Nitella*, *Chara*, and *Acetabularia* (Zimmermann and Steudle, 1978; Tomos, 1988). Since then, this technique has continuously evolved, permitting measurements in smaller cells such as stomatal guard cells (Hüsken *et al.*, 1978; Franks, 2003), as well as in growing cells (Triboulot *et al.*, 1995). In addition, this method enables the measurement of volumetric elastic moduli, which characterize the ratio of change in volume of a cell (as measured using the position of the meniscus) to a change in pressure applied with the capillary. This volumetric modulus depends on cell geometry, on the elasticity (see Box for the mechanics terminology) of its wall, and more weakly on neighbouring cells. Although the pressure probe is still today the only method allowing a direct measurement of turgor pressure over long periods of time, it presents some disadvantages. First, it is invasive and might elicit wound responses. Secondly, its use is rather tricky and slow, which restricts one to a dozen correct measurements per day. Lastly, the meniscus must be large enough to be positioned optically, and the capillary must not clog, preventing the use of very thin capillaries and making it impossible to measure turgor in cells from shoot apical or root apical meristems, which are model tissues for the study of plant morphogenesis. It seems that the technique has reached maturity, but unforeseen improvements are still possible.

In order to overcome some of these disadvantages, Lintilhac and Wei developed a non-invasive but non-direct method, ball tonometry, for the determination of turgor pressure in individual plant cells (Lintilhac *et al.*, 2000; Wei *et al.*, 2001). The turgor pressure was determined by observing and measuring the contact area formed during the application of a spherical probe to the cell surface, using an imposed load. If the elasticity of the cell wall is neglected, the resisting force from the cell is obtained by multiplying turgor pressure (a force per unit area) by the area of contact; at equilibrium, this resisting force balances the known load, and hence turgor is deduced from the ratio between the load and the contact area. This approach used 50–500 μm diameter spheres (see Figs 1A and 2) to indent onion epidermis cells and bean hypocotyl cells. The values of turgor pressure found agreed well with direct measurements using the pressure probe in onion epidermal peels, justifying the approximation to neglect wall elasticity in this case. Overall, ball tonometry has two strengths: it is much more rapid than the pressure probe and it allows a non-destructive measurement. Its main limitations stem from the requirement to quantify the contact area optically,

which is not possible with opaque samples or with very small cells. Yet, it is still possible to miniaturize the original set-up, thanks to current microfabrication techniques, but it remains to be shown that wall properties would not influence such a measurement.

Measuring cell-wall mechanics

The cell wall of a growing plant has to satisfy two conflicting requirements: on the one hand, the wall must be strong enough to withstand turgor pressure, and on the other hand, it needs to be soft enough to allow expansion of the cell. The wall accommodates these requirements through its composite and dynamic structure, presenting stiff structural elements (such as the cellulose microfibrils) embedded in a more plastic or viscous matrix of polysaccharides and structural proteins. The main components of the cell wall are well identified (Cosgrove, 2005), and it is now essential to understand their role in the maintenance and/or remodelling of the cell-wall structure and their influences on its mechanical properties. Thus, measuring the physical properties of growing cell walls is a necessary step towards understanding the mechanical control of morphogenesis. Classical methods in material engineering use micro-indentation methods to measure the mechanical properties of thin layers such as the cell wall. However, it is challenging to extend these methods *in vivo*. Indeed, the cell-wall thickness ranges from 0.1 to a few micrometers, so that indentation at depths greater than a micrometer will probably be more sensitive to turgor pressure, as in the case of ball tonometry. Therefore, it is necessary to use precise nano-indentation systems, such as nano-indenters or atomic force microscopy (AFM) (Figs 1B, C and 2).

For almost two decades now, AFM has been used to image the surface of living animal cells, under quasi-physiological conditions and in a non-destructive manner. In plants, very

few studies have used this technology. Among these, observations have been done on extracted or isolated cell walls from various tissues and species in order to examine cell-wall structure and texture (van der Wel *et al.*, 1996; Davies and Harris, 2003; reviewed by Yarbrough *et al.*, 2009; Ding and Liu, 2012). AFM has also allowed visualization and characterization of the cellulose microfibril network in the presence of expansin, a cell-wall loosening agent, demonstrating that it acts selectively on the cross-linking polymers between parallel microfibrils, rather than more generally on the wall matrix (Marga *et al.*, 2005). However, AFM is more than a topographic imaging tool, as it can be used to measure physical properties when applied as a material indenter (Figs 1B and 2).

AFM-based studies of mechanical properties are performed by collecting force-indentation curves at various cell-surface points. Each curve comprises the approach and retraction of the AFM cantilever, considered as a small elastic beam, from the surface, while recording its deformation. The cantilever has a calibrated stiffness constant so that the attractive and/or repulsive forces between the tip and the sample surface can be measured and mechanical properties such as adhesion and elasticity can be quantified. This technique has been used to study the mechanical properties of animal and bacterial cells. In plants, AFM was initially used to study the nano-mechanical properties of fibre walls in normal and tension wood (Clair *et al.*, 2003). Because wood fibres contain only dead cells, this study was performed on horizontal wood sections and allowed the authors to describe the elastic properties of the different cell-wall layers forming the wood fibre. This approach was further extended to cell cultures of *Vitis vinifera* by Lesniewska *et al.* (2004). Cells were deposited on a polylysine-coated substrate, and AFM was used to quantify the topography and stiffness of cell walls in living cells. However, exploring morphogenesis requires that measurements are performed on growing tissues of organisms during development.

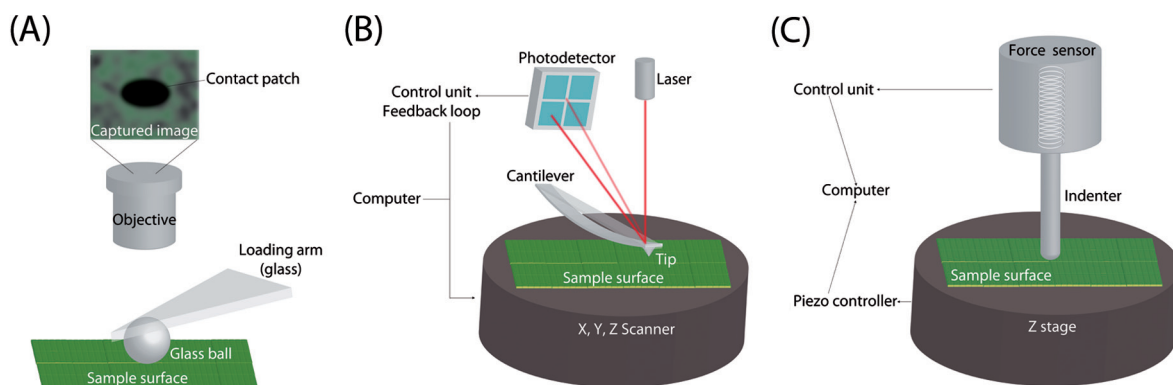


Fig. 1. Schematics of the main indentation set-ups. (A) Ball tonometry. The sample is indented by a glass ball. A microscope objective allows visualization of the contact patch. The load-dependent area of this contact patch is used to determine turgor pressure. (B) Atomic force microscopy. A sharp tip is attached on a flexible cantilever and is used to indent the sample surface. The deformation of the cantilever when a known force is applied on the sample is monitored via a laser beam reflecting from the top surface of the cantilever into a photodetector. This provides force–displacement curves from which are extracted the mechanical properties of the sample. (C) Micro-indentation. A hard micrometric indenter (equipped with a force sensor) penetrates the sample. During indentation, the applied force and depth of penetration are measured. The corresponding force–displacement curves can be used to extract the mechanical properties of the sample. Note that AFM and micro-indenters come in different configurations but with the same working principle.

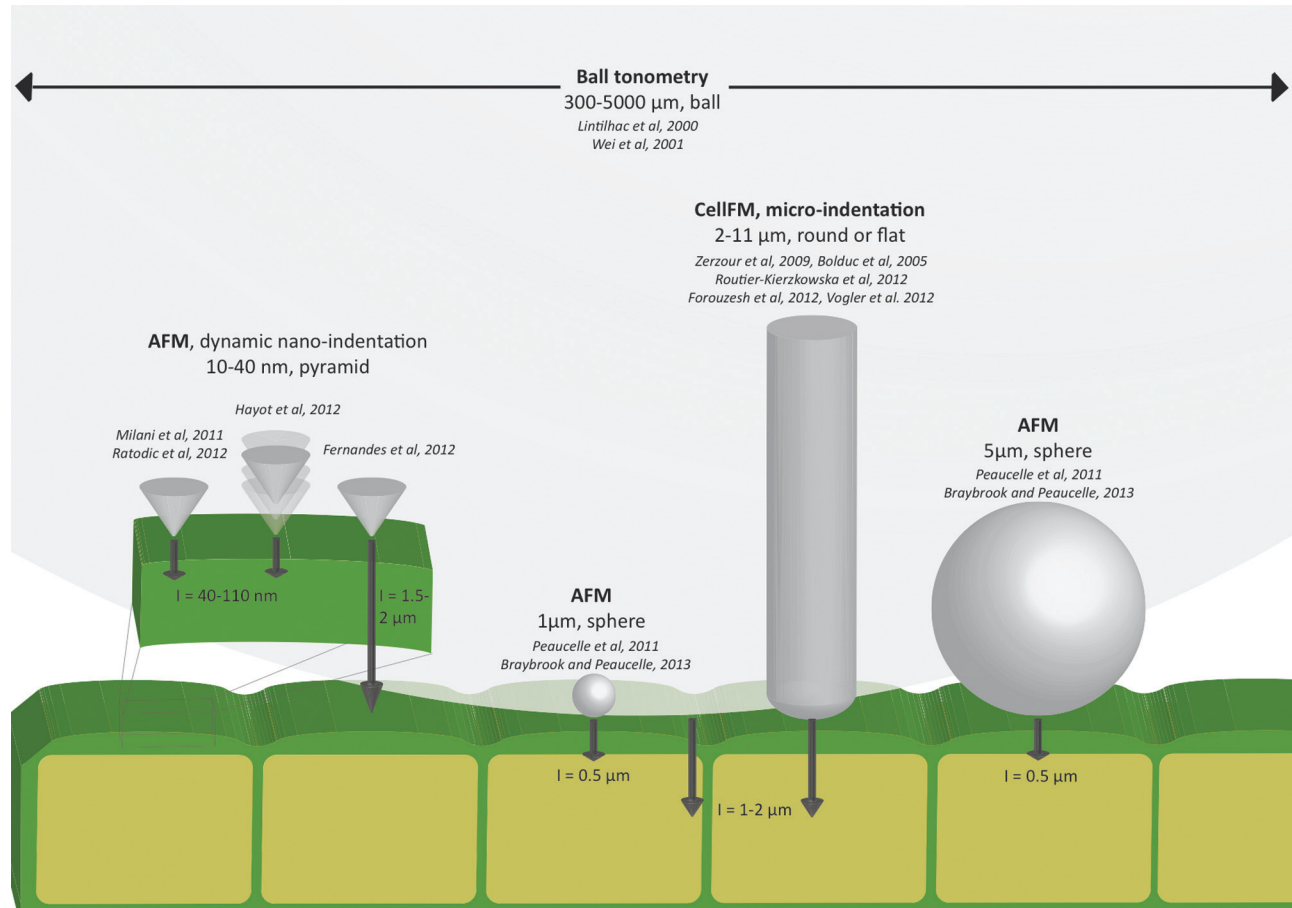


Fig. 2. The panorama of indentation methods applied to plant cells. Schematics of the probes are shown, together with arrows representing typical indentation depths and the reference in which they were used. The cones are proxy for pyramids and the superimposition of cones stands for the dynamic mechanical analysis (DMA) in which the probe is oscillated sinusoidally on top of the vertical translation to the maximal depth. The probe used in ball tonometry is shown for comparison, although it was only used on much bigger cells.

Recent studies have begun addressing nano-mechanical properties of primary cell walls in living, growing tissues. Milani *et al.* (2011) used AFM for the shoot apical meristem (SAM) of *Arabidopsis thaliana*. Shallow indentations (40–100 nm deep) were carried out using low force (order of nN) with a sharp and pyramidal shaped tip (10–40 nm tip radius; see Fig. 2) to measure the mechanical properties of the SAM epidermis. Such measurements are potentially sensitive to many parameters: elasticity, viscosity, and plasticity (see Box) of the cell wall or of the cuticle; adhesion between the probe and the surface; turgor pressure; and flow of water in the symplast, in the apoplast or between the two. In order to minimize the impact of these parameters, several experimental procedures were employed: data was extracted from indentation depths greater than 40 nm to exclude contributions of the cuticle, adhesion was found to be small when the sample is submerged in water, the approach and retraction curves were close suggesting a minor contribution for dissipative effects such as viscosity, and it was shown that these measurements are insensitive to turgor pressure as they were unchanged by plasmolysis (probably owing to indentation depths significantly smaller than wall thickness). The study revealed spatial patterns in the

mechanical properties of epidermal cell walls, at both cellular and subcellular levels. The cell walls at the SAM tip presented a higher elastic modulus (i.e. were stiffer) than at the flanks of the SAM, which grow at a higher rate than at the tip (Kwiatkowska, 2008), suggesting that, at least in the apex area, the growth rate may depend on the local mechanical properties of the cell wall. However, it is unclear which properties of the cell wall are measured, because the force is roughly perpendicular to cellulose microfibrils. In addition, the pattern of stiffness was identified based on morphological criteria (tip/flanks of the apex), raising questions about how well stiffness patterns are correlated with the central and peripheral zones as might be defined by gene expression.

Using a similar method (Fig. 2), Fernandes *et al.* (2012) performed force-indentation curves along the root, extending shootwards from the tip. In this case, the authors focused on the material plasticity of the cell walls revealed by the difference in the approach and retraction curves. In particular, they extracted from these experiments a plasticity index quantifying the irreversibility of the imposed deformation. Using plasmolysis, they found this index to be largely independent of turgor, suggesting that irreversibility originates in the cell

wall. This study did not reveal any spatial difference in plastic or viscoelastic behaviour along the root length. Future work should address whether the absolute values of stiffness vary.

Peaucelle *et al.* (2011) performed moderately deep AFM-based indentations with larger probe sizes (spheres 1 or 5 μm in diameter; see Fig. 2); in this case, preliminary measurements were highly sensitive to turgor and, as such, all experiments were performed on plasmolysed tissue. The authors characterized the SAM in terms of cell-wall mechanical properties, both elastic and viscoelastic. First, viscoelasticity was examined by cyclically applying a 5 μm ball mounted on a cantilever at different positions of the SAM. There did not appear to be spatial differences in the viscoelastic relaxation time (ratio of viscous modulus to elastic modulus) within the central zone, periphery, or emerged organs, suggesting that the viscous behaviour originates in the cell wall. It is important to note that the analysis model used for viscoelasticity was a linear one, and a more sophisticated analysis model may reveal subtler differences. More dynamic information on viscoelastic behaviours may be obtained by dynamic nano-mechanical analysis (nanoDMA; discussed further below), which employs oscillations to quantify in detail the viscosity (or the loss modulus) of a system. In the experiments of Peaucelle *et al.* (2011), the relaxation time was of the order of 1 s, which is much smaller than the characteristic time for viscous growth (1 d), questioning the relevance of the measured viscoelastic behaviour to growth.

In a second set of experiments by Peaucelle *et al.* (2011), rapid indentation, down to a depth of 500 nm by a 1 or a 5 μm ball, provided maps of the apparent elastic modulus of the SAM, interpreted as maps of the epidermis and of the two first internal layers, respectively. While this interpretation was consistent with biochemical and histological data, it remains to be confirmed by mechanical modelling. This study concluded that the cell-wall mechanical properties were different within the meristem; cell walls in the central zone were less stiff (had a higher elastic modulus) than those in emerged primordia, and the cell walls of subepidermal tissues had a higher elasticity at initia. In this study, the authors correlated observed changes in elasticity with changes in pectin matrix chemistry, specifically demethylesterification. Braybrook and Peaucelle (2013) further demonstrated that the phytohormone auxin triggers increases in cell-wall elasticity at the shoot apex prior to organ emergence and that this increase in elasticity requires the demethylesterification of pectins. The strength of these two studies was to couple cell-wall biochemistry with its biomechanics, but theoretical work is still needed to validate interpretations.

Interpretation of indentation experiments in terms of cell-wall mechanics

While recent work in the field has begun to unravel some of the chemical mechanisms underlying changes in cell-wall mechanics, the details of interpreting indentation experiments remain challenging. As mentioned previously, changes in the pectin matrix appear to be strongly correlated with organ emergence in the SAM and also to be strictly required, downstream of auxin, for this process; however, many technical questions

remain. Are different indentation methods more sensitive to different components of the composite cell wall? How does the indentation direction relate to growth direction? And lastly, how does the sample geometry affect the data collected? While answers to these questions are still being investigated (they should be considered when undertaking these types of experiments), progress has been achieved in many directions.

The cell wall is often considered mechanically as a homogeneous material, when in fact it is a composite material similar to a fibre-reinforced gel. As such, varying indenter size, shape, and depth of indentation may reveal different properties of the composite. Small, shallow indentations may be more likely to reveal properties of the gel matrix, whereas larger deformations would involve the bending of cellulose fibres and hemi-cellulose linkages. While this seems intuitive enough, the hypothesis remains to be tested. Radotić *et al.* (2012) applied AFM-based indentation methods to study the material properties of the cell wall as a composite. In this work, the authors extracted mechanical properties at different, small indentation depths, using so-called stiffness tomography (Fig. 2). While their measurements are likely to be independent of turgor thanks to the small depths used, it would have been useful to test it directly in their study system, *Arabidopsis* culture cells. They found that the cell wall became more heterogeneous across its thickness during growth, which might be connected with the multinet theory of wall texture (see e.g. Lloyd, 2011). This method may be adapted to examine the properties of wall components, as shown in animal cells and in artificial gels by Roduit *et al.* (2009). It should be noted that, although this approach demonstrated its ability to distinguish structures of known, higher stiffness buried into the bulk of the sample, Lee *et al.* (2009) show from numerical investigation that it is difficult to distinguish different layers from indentation experiments. Nevertheless, the approach remains attractive for future development and use.

Often, indentation tests occur perpendicular to the direction of growth, and as such it is interesting to consider what type of information could be relevant for morphogenesis. For example, indentations that probe gel matrix properties may provide easily transferable information; gels tend to behave as isotropic materials and the direction of indentation is not so important. On the other hand, indentations that stretch the cellulose fibres and their connections are more likely to relay anisotropic information. However, this information does not necessarily reflect properties in the directions tangent to the wall because the probe is displaced in the direction normal to the cell wall (see Milani *et al.*, 2011). In addition, because the anisotropic properties of cell walls are so important in controlling the direction of growth, methods that reveal these differences are of importance. There is a strong possibility that further refinement of AFM-based methods will allow a hybrid measurement between surface topography (Yarborough *et al.*, 2009) and cellulose fibre stiffness.

Lastly, the effect of geometry on the data, in terms of probe shape, sample shape, wall thickness, and cell shape must be carefully considered. The geometry of the sample, cell, or organ can have a profound effect on the data obtained. For instance, theoretical interpretations assume the direction of

indentation to be normal to the sample surface, which is difficult to achieve in experiments. Two methods were used to evaluate this issue. [Routier-Kierzkowska *et al.* \(2012\)](#) computed a correction for the bias due to non-normal indentation assuming that the probe slides on the surface of the sample, so that regions with a slope appear softer than regions normal to the indenter, which was consistent with experiments on onion where the thick cuticle probably lubricates the contact between the indenter and the sample. [Braybrook and Peaucelle \(2013\)](#) employed a mock sample, which they produced by taking a replica of a shoot apex and then moulded a silicon elastomer into this replica; thus, they obtained a sample with the same geometry as the biological sample of interest but with uniform mechanical properties; they used this mock sample to assess the geometrical bias with AFM and found regions with a slope to be slightly stiffer than regions normal to the AFM probe; this apparent discrepancy with the results of [Routier-Kierzkowska *et al.* \(2012\)](#) is probably due to absence of sliding between the small AFM probes and contacting surfaces. Hence, friction might also affect measurements when indentation is not normal. Overall, the extent of geometrical bias appears to be dependent on the sample and the methodology, and as such must always be evaluated.

Cell-wall thickness also plays a large part in data interpretation. The Hertzian contact model, which is used to extrapolate Young's moduli, is designed for the indentation of homogeneous, elastic, infinite-depth surfaces. While these idealized conditions are far from the reality of a cell wall, they are the simplest currently available as they allow the extraction of a single number that is qualitatively related to the elastic modulus of the wall. In the study of [Milani *et al.* \(2011\)](#), indentation depths and contact width were significantly smaller than the wall thickness, which makes the assumption of infinite thickness acceptable, allowing the extraction of an average modulus (in the direction normal to wall) of the external part of the wall. For deeper indentations, the cell wall will deform according to a bending mode where the whole top wall of the cell bends down. This possibility has been considered for unpressurized shells by [Ogbonna and Needleman \(2011\)](#), who concluded that, when the ratio of shell thickness to object radius was below 0.4, with sufficiently shallow indentations, the material behaved like an infinite half space. A similar conclusion was reached by [Forouzesh *et al.* \(2013\)](#) in a study discussed below. This issue can be examined experimentally by looking at patterns of measured stiffness within a cell. If bending occurs, the cell would be expected to be softer in its middle than close to its periphery (where anticlinal walls give it more support), as can be experienced when pressing on the cover of box, and as measured by [Peaucelle *et al.* \(2011\)](#) and [Routier-Kierzkowska *et al.* \(2012\)](#). [Milani *et al.* \(2011\)](#) found no such pattern and concluded that bending did not occur.

Disentangling turgor pressure and cell-wall mechanics

In the previous sections, we have described methods allowing the exclusive measurement of turgor pressure or cell-wall

mechanical properties in a growing organism. In recent years, several other indentation-based techniques have been developed, and it appears that they are sensitive to both turgor and wall elasticity, giving access to new information. These studies have highlighted the need for interpretative mechanical models. For instance, tomato culture cells were compressed between a micromanipulation probe (200 μm in diameter) and a glass chamber ([Blewett *et al.*, 2000](#)). The probe was mounted on a force transducer attached to a micromanipulator. The experiments were designed to measure the force required to puncture the cell, and analysis of the force-deflection curves provided information on the whole-cell mechanical behaviour. Later, application of a theoretical model on the obtained force-indentation curves allowed an extrapolation of the elastic modulus of the wall ([Wang *et al.*, 2004](#)). In another study, the compression system was used for the exclusive quantification of turgor pressure by determining the ratio between the applied force and the area of the compression zone ([Wang *et al.*, 2006](#)). Here, measurements were performed simultaneously with a pressure probe, validating the capability of the micromanipulation method to quantify turgor accurately. As this last study showed that micromanipulation was mostly sensitive to turgor, it is unlikely that the elastic modulus of the wall was determined robustly by [Wang *et al.* \(2004\)](#). These types of whole-cell mechanical experiments examine the cell in its turgid (pressurized) state and allow analysis of the whole wall (as opposed to shallow indentations); however, they call for careful mechanical modelling for a robust interpretation.

Analytical mechanical models used to extract quantitative data work best with restrictive assumptions and for simple cell and indenter geometries. For example, by considering cells as spherical or ellipsoidal, elastic, and pressured shells, the analytical studies performed by Vella and co-workers have shown that it is possible to extract cells' mechanical properties and turgor from indentation approaches ([Vella *et al.*, 2012a,b](#)), as long as it is possible to obtain indentation force-displacement curves for turgid and flaccid cells. These analyses were supported by indentation experiments on silicone elastic shells with different aspect ratios ([Lazarus *et al.*, 2012](#)) and were used to characterize osmoregulation in yeast ([Vella *et al.*, 2012a](#)). However, it might be difficult to extend the results of these studies to more realistic geometries.

Extraction of data from indentation experiments on cells with more complex geometries and in a tissue environment has been achieved through the use of different interpretation models, mostly based on the numerical solution of a mechanical model that is defined according to the geometry of the experiment. [Hayot *et al.* \(2012\)](#) used a dynamic nano-mechanical analysis (nanoDMA) technique, which consists of superimposing a nanometric oscillation on the vertical translation of the indenter probe, allowing them to quantify mechanical properties such as storage and loss moduli (see Box) of the cell wall of leaf epidermis in *Arabidopsis thaliana* ([Fig. 2](#)). Elastic moduli were extracted using a mechanical model, with a spherical probe indenting a circular bulging wall, solved numerically using the finite element method (FEM). Despite a relatively low indentation depth (110 nm), simulations

suggest that the wall thickness and turgor pressure are important factors that influence estimation of the elastic modulus. This apparent contradiction with the results of [Milani *et al.* \(2011\)](#) discussed above might be ascribed to the difference in tissues, although it probably originates from a major difference between the geometry of the probes: [Milani *et al.* \(2011\)](#) used sharp pyramidal tips with an opening angle of about 20°, while [Hayot *et al.* \(2012\)](#) used Berkovitch tips (a flat three-sided pyramid) with an angle of about 70° so that the area of contact with the sample was ~60 times larger in the latter study, making it more sensitive to pressure. More recently, DMA was used for the determination of turgor pressure ([Forouzesh *et al.*, 2013](#)) by applying deeper indentations (1.8 µm) with a spherical probe ([Fig. 2](#)). In this experiment, the pressure was inferred, using the same mechanical model, in leaves under different osmotic conditions and at various developmental stages. This approach provided values of pressure that varied as expected from the water status and the leaf age; the absolute values of pressure were a bit higher than those measured with a psychrometer by [Hayot *et al.* \(2012\)](#). The strength of these two studies resides in the use of DMA, which provides much more information than simple, linear indentation. Improvements coupling a precise positioning within the cell probed and a determination of the cell geometry with mechanical measurements will be useful, so as to use more realistic interpretation models (e.g. with geometries closer to the actual puzzle-piece shape of leaf epidermal cells).

Such a coupling between the quantification of cell topography and indentation was developed by [Routier-Kierzkowska *et al.* \(2012\)](#), also allowing the visualization of the indentation by a standard inverted light microscope in transparent tissues. This technology, named cellular force microscopy (CFM), combines high-resolution indentation with the measurement of the topography of the sample, expanding the range of the AFM to higher forces. The authors used CFM to quantify the stiffness of onion epidermal cells, under various osmotic conditions. They used a realistic mechanical model (solved with FEM), having a geometry close to that of the tissue, to interpret their measurements. For indentation depths sufficiently smaller than the wall thickness and for turgid onion cells, the measurements revealed differences in the outer wall, as in the AFM study of [Milani *et al.* \(2011\)](#). The main findings were that, for deeper indentation, the measurements were mostly sensitive to turgor, and that the sample slope could induce a bias. Future work should enable the extraction of the values of pressure from such measurements.

Despite the difficulty in interpreting data from indentation experiments, use of nano- and micro-indentation approaches has proved crucial for the characterization of cellular mechanical properties *in vivo* on tissues such as SAM and therefore in a great advance in further understanding the mechanisms governing growth and morphogenesis processes. Micro-indentation methods have also been useful for the analysis of polar growth process in the study of pollen tubes. Indeed, these tip-growing cells provide an ideal system for the study of mechanical properties at the single-cell level. Initial studies showed that the pollen tube apex is apparently softer than the more distal region ([Geitmann and Parre, 2004](#); [Zerzour *et al.*, 2009](#)). The authors concluded that such data were in

agreement with expansion taking place exclusively at the tip of the growing pollen tube. A more recent study used a combination of CFM and FEM to measure the apparent stiffness of the pollen tube and deduce turgor pressure and elastic modulus of the wall. In good agreement with previous studies, this work concluded that the apex of the pollen tube seemed softer ([Vogler *et al.*, 2013](#)). The interpretation, however, was quite different; their modelling approach indicates that the measured differences could be explained by the geometry of the pollen tube alone. More precisely, when micro-indentation measurements are made in the vertical direction, the tilt angle between the indenter and the sample surface is increased towards the apex, thus underestimating force measurements, a possibility that was already considered in the model by [Bolduc *et al.* \(2006\)](#), which did not account for turgor pressure. Note that these interpretations would depend on the friction, or lack thereof, at the probe–pollen tube interface. Interestingly, there are well-documented biochemical and structural gradients in the cell wall between the tip and the more distal region, which should have mechanical relevance (see e.g. [Fayant *et al.*, 2010](#)). Here again, it is likely that geometry and turgor pressure mask differences in wall elasticity measurement in these regions. A solution to reveal patterns in wall elasticity would be to perform indentations normal to the cell surface, smaller indentations, and indentations on cells with reduced pressure. AFM would certainly enable such experiments, while interpretation would be eased: walls are probably isotropic at the tube tip because cellulose microfibrils are absent there.

A quick guide to indentation experiments and their interpretation

We will now sketch some general trends that emerge from all of the studies discussed thus far. Varying the tip size and indentation depth enables probing the tissue at different scales, while realistic mechanical models facilitate the interpretation of experiments; however, the relationship between the properties of the wall that are extracted and its biochemical state remains to be fully explored ([Braybrook and Peaucelle, 2013](#)).

- (i) Indentation depths and contact areas sufficiently smaller than wall thickness (equivalently, small depths and sharp tips) give access to the mechanical properties of the external part of the cell wall ([Milani *et al.*, 2011](#); [Radotić *et al.*, 2012](#)). The depths need to be large enough so as to discard the cuticle. Mechanical models ([Milani *et al.*, 2011](#)) suggest that the measurements are mostly sensitive to the elastic modulus in the direction normal to the wall and that the Hertz contact model (which is strictly valid for infinitely deep isotropic solids) enables the extraction of the normal elastic modulus. Refined analyses might also give information on the properties of cell wall according to depth ([Radotić *et al.*, 2012](#)).
- (ii) For deeper indentation or a larger contact area, the measurements are sensitive to both wall mechanics and turgor pressure ([Bolduc *et al.*, 2006](#); [Wang *et al.*, 2006](#); [Zerzour *et al.*, 2009](#); [Forouzesh *et al.*, 2012](#); [Hayot *et al.*, 2012](#);

Routier-Kierzkowska *et al.*, 2012; Vogler *et al.*, 2013). The combination of indentation experiments with different values of osmolality of the external medium and of realistic modelling are necessary to extract the values of turgor and of elastic moduli.

(iii) Plasmolysing the tissue removes turgor and enables measuring only cell-wall mechanics (although this alters the normal contact between the wall and membrane and changes the tensional state of the wall). Apparent moduli can then be extracted from indentation experiments using the Hertz model (Peaucelle *et al.*, 2011; Braybrook and Peaucelle, 2013). In addition, increasing the size of the tip seems to allow probing the mechanics of internal layers. As model assumptions (homogeneous solid) are not verified, such apparent moduli are a necessary oversimplification of a complex tissue with a mechanical behaviour that encompasses a fluid-filled cellular structure. Further realistic mechanical modelling is necessary to solidify the above interpretations. Nevertheless, the clear advantage of plasmolysis is that intrinsic properties of the cell wall are revealed by indentation.

In all three cases, ensuring that the indentation is approximately normal to the wall eases interpretation by minimizing friction and geometrical bias. Otherwise, the development of comprehensive analytical and experimental corrections and controls is essential for accurate interpretation of data.

As a final word of caution, forces in the range of 100 μ N are generated by pathogens, e.g. by fungi, and elicit a range of

cellular responses (Hardham *et al.*, 2008). While such forces are higher than those achieved in the indentation experiments discussed here, it should be checked that a given measurement is not influenced by the existence of a previous one (through a putative active cell reaction to indentation).

A case study: organ formation at the shoot apex

The formation of new organs at the shoot apex is a fascinating biological event. This process has a rich experimental history involving genetics, chemistry, and mechanics. As mentioned earlier, several recent studies have uncovered new cell-wall mechanical properties associated with organ formation at the apex. Milani *et al.* (2011) described a striking spatial difference in *pin1* mutant meristems, which do not form organs due to a genetic defect; cell walls in the peripheral zone (where organs could initiate) were on average three times less stiff than those in the meristem tip (Fig. 3). These data suggest that there is a change in cell-wall mechanics that can predict the zone of organ formation. Consistent with this study, Peaucelle *et al.* (2011) used slightly larger tips and deeper indentations to reveal differences in wall mechanics within the peripheral zone that predicted the sites of new organ emergence (Fig. 3); interestingly, the authors hypothesized that the decreased stiffness seen at organ initia occurred in subepidermal walls. In the same study, the authors saw decreased stiffness in all emerged organs. As auxin is a known initiator of organ

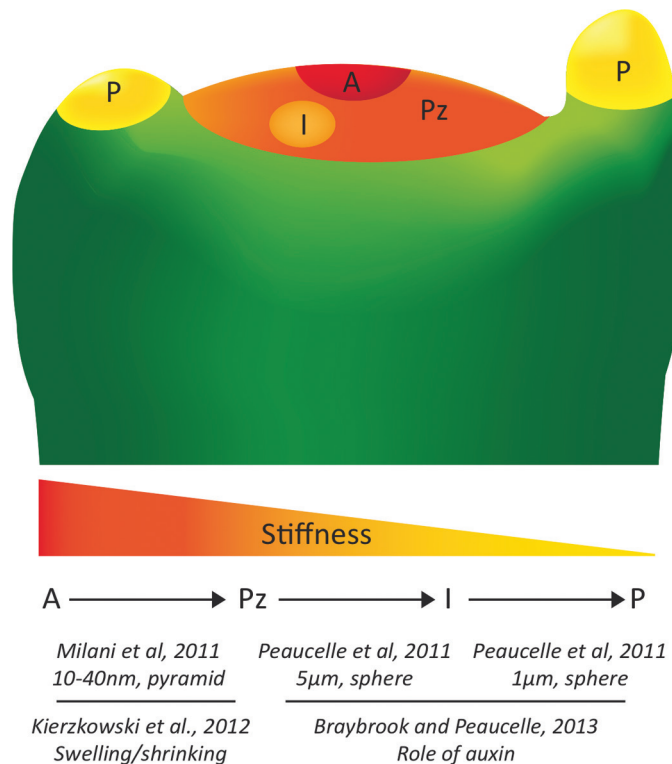


Fig. 3. Schematic of a shoot tip, with the apex (A) surrounded by the peripheral zone (Pz) within which initia (I) are the sites from which organ primordia (P) grow. Overall, micromechanical measurements and turgor manipulation indicate a ranking in stiffness according to the identity of the regions (A>Pz>I>P). Relevant references are noted with brief experimental detail.

outgrowth at the apex, [Braybrook and Peaucelle \(2013\)](#) investigated the connection between auxin and cell-wall elasticity. In this study, they concluded that auxin triggered changes in wall elasticity at the apex, and identified changes in pectin chemistry required for both the reduction in stiffness and also organ formation.

The assessment of indentation-based data is also imperative, preferably via alternative methodologies. For the case of organ emergence, an elegant approach was introduced by [Kierzkowski *et al.* \(2012\)](#), generalizing the principle of the measurement of cell volumetric moduli (discussed above, together with the pressure probe). [Kierzkowski *et al.* \(2012\)](#) manipulated turgor pressure, by changing the osmolality of external medium, within a tomato vegetative apical meristem and examined the resulting shape changes in epidermal cell surface. These shape changes can be ascribed to the elastic stretching or shortening of cell walls, which results in the inflation or deflation of cells, respectively. The meristem tip and the peripheral zone displayed differential cell mechanics, which correlated with measured growth rates. More precisely, upon an increase in turgor, highly growing cells expanded more than slowly growing cells. This very interesting result shows that growth (irreversible deformations) is correlated with elastic deformations. In addition, the authors found an asymmetry between inflation and deflation, which they interpreted as a strain-stiffening behaviour (the cell wall becomes stiffer when stretched). As this asymmetry might also be ascribed to an asymmetry in osmoregulation between hyper-osmotic and hypo-osmotic stress, it would be very useful to combine the approach of [Kierzkowski *et al.* \(2012\)](#) with micro-indentation.

Despite their limitations, the studies considered here ([Milani *et al.*, 2011](#); [Peaucelle *et al.* 2011](#); [Kierzkowski *et al.*, 2012](#); [Braybrook and Peaucelle, 2013](#)) lead to similar general conclusions, so that it is now safe to state that cell mechanics correlates with growth and morphogenetic events at the shoot apex ([Fig. 2](#)). Future work will certainly deepen these

conclusions and make links with the genetic regulation of morphogenesis.

Conclusions

The field of plant biomechanics has a long history, but has been renewed over the past few years. The renaissance of a physical view of morphogenesis in plants has provided us with many new tools but also a wealth of exciting questions and research avenues. These micromechanical tools require a careful assessment of their output, and a critical view on the assumptions in interpretative mechanical models. Methodologies will certainly improve in the coming years, yielding more and more useful information. At least three directions can be foreseen. First, most of the studies presented considered elastic or viscoelastic short-term properties of the cell wall, which are not necessarily relevant to growth and morphogenesis. Two types of data are nevertheless encouraging: viscous moduli are found to correlate with elastic moduli ([Peaucelle *et al.* 2011](#); [Hayot *et al.*, 2012](#)); growth is correlated with the expansion of cells upon turgor manipulation ([Kierzkowski *et al.*, 2012](#)). Future work will deepen these links between cell-wall elasticity and growth. Secondly, all indentation methods are just 'scratching the surface' and give little information about deep cell layers. Destructive experiments involving dissection enable the comparison of the mechanical state and properties of different cell layers, as performed for the epidermis of hypocotyl (see [Kutschera and Niklas, 2007](#)). Similarly, pressure-probe measurements revealed differences in turgor between cell layers in roots and hypocotyls (e.g. [Meshcheryakov *et al.*, 1992](#); [Zimmermann *et al.*, 1992](#)). Non-destructive methods might stem from improving the resolution of biomedical elastography, which derives three-dimensional maps of elastic modulus from the velocity of sound in tissues ([Greenleaf *et al.*, 2003](#)). Thirdly, biomechanical measurements will be coupled to other techniques that give information on the cell-wall biochemistry

Mechanics terminology

Strain: relative deformation (ratio of displacement to initial length, unitless). Strain rate refers to the time rate of strain.

Stress: force per unit area (unit N m^{-2} =Pascal=Pa).

Elasticity: describes the instantaneous reversible deformation of a solid under load. Experimental assays are based on measuring the strain of the solid versus the stress applied on it. The degree of elasticity can be quantified using stiffness (ratio of force to displacement, unit N m^{-1}) for a spring or using the elastic modulus (also called Young's modulus, ratio of stress to strain, unit Pa) for linearly elastic solids.

Viscosity: describes the time-dependent dissipative deformation of a fluid under load. Experimental assays are based on measuring the strain rate of the fluid versus applied stress. The degree of viscosity can be quantified using the kinetic viscosity (ratio of stress to strain rate, unit Pa s^{-1}) for Newtonian (linear) fluids.

Viscoelasticity: describes the combination of viscosity and elasticity and applies to both fluids and solids. Common experimental assays are stress-relaxation (measuring stress under constant applied strain), creep (measuring strain rate under constant applied stress), and dynamic mechanical analysis (DMA, measuring stress under cyclical strain with varied frequency). For linearly viscoelastic materials, viscoelasticity can be quantified using a storage modulus (which reduces to the elastic modulus for pure elasticity) and loss modulus (which reduces to the product of viscosity and frequency for pure viscosity, and so has the same units as the storage modulus).

Plasticity: describes the irreversible deformation of a solid above a threshold in strain; the solid is elastic below this threshold. In general, above the threshold, the rate of irreversible deformation depends on applied stress, which corresponds to a viscoplastic behaviour. Plant growth is sometimes thought of as the combination of viscoplasticity and wall synthesis.

(Mouille *et al.*, 2003; Braybrook *et al.* 2012), on the cell differentiation state, or on the cell physiological state.

Finally, it also appears that dynamic mechanical models of morphogenesis are increasingly used to test hypotheses drawn from observations (see e.g. Chickarmane *et al.* 2010; Jönsson and Krupinski, 2010; Fayant *et al.* 2010; Kennaway *et al.* 2011; Prusinkiewicz and Runions, 2012), with the aim of predicting morphogenetic events from sets of elementary rules. However, these models cannot be fully predictive as long as they do not incorporate quantitative data about cell mechanics. The tools discussed in this review will also become essential in connection with such modelling approaches. Thus, we are led to a new, exciting frontier, integrating genetic and molecular regulation, cell mechanics, and morphogenesis.

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References

- Baskin TI, Jensen OE.** 2013. On the role of stress anisotropy in the growth of stems. *Journal of Experimental Botany* **64**, this issue.
- Beck WA.** 1929. Determining the osmotic value at incipient plasmolysis. *Transactions of the American Microscopic Society* **48**, 204.
- Blewett J, Burrows K, Thomas C.** 2000. A micromanipulation method to measure the mechanical properties of single tomato suspension cells. *Biotechnology Letters* **22**, 1877–1883.
- Bolduc JE, 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.
- Boudaoud A.** 2010. An introduction to the mechanics of morphogenesis for the plant biologist. *Trends in Plant Sciences* **15**, 353–360.
- Braybrook SA, Hofte H, Peaucelle A.** 2012. Probing the mechanical contributions of the pectin matrix: insights for cell growth. *Plant Signaling and Behaviour* **7**, 1037–1041.
- Braybrook SA, Peaucelle A.** 2013. Mechano-chemical aspects of organ formation in *Arabidopsis thaliana*: the relationship between auxin and pectin. *PLoS ONE* **8**, e57813.
- Chickarmane V, Roeder AHK, Tarr PT, Cunha A, Tobin C, Meyerowitz EM.** 2010. Computational morphodynamics: a modeling framework to understand plant growth. *Annual Review of Plant Biology* **61**, 65–87.
- Clair B, Arinero R, Lévêque 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 RE, Cosgrove D, Tepfer M.** 1987. Long-term acid-induced wall extension in an in-vitro system. *Planta* **170**, 379–385.
- Cosgrove DJ.** 1989. Characterization of long-term extension of isolated cell walls from growing cucumber hypocotyls. *Planta* **177**, 121–130.
- Cosgrove DJ.** 2005. Growth of the plant cell wall. *Nature Reviews Molecular Cell Biology* **6**, 850–861.
- Davies LM, Harris PJ.** 2003. Atomic force microscopy of microfibrils in primary cell walls. *Planta* **217**, 283–289.
- de Vries.** 1884. Eine Methode zur Analyse der Turgorkraft. *Jahrbücher für wissenschaftliche Botanik* **14**, 427.
- Ding SY, Liu YS.** 2012. Imaging cellulose using atomic force microscopy. *Methods in Molecular Biology* **908**, 23–30.
- Durachko DM, Cosgrove DJ.** 2009. Measuring plant cell wall extension (creep) induced by acidic pH and by α -expansin. *Journal of Visualized Experiments* **11**, 1263.
- Fayant P, Girlanda O, Chebli Y, Aubin CE, Villemure I, Geitmann A.** 2010. Finite element model of polar growth in pollen tubes. *Plant Cell* **22**, 2579–2593.
- Fernandes AN, Chen X, Scotchford CA, Walker J, Wells DM, Roberts CJ, Everitt NM.** 2012. Mechanical properties of epidermal cells of whole living roots of *Arabidopsis thaliana*: an atomic force microscopy study. *Physical Review E* **85**, 021916.
- Forouzesh E, Goel A, Mackenzie SA, Turner JA.** 2012. *In vivo* extraction of *Arabidopsis* cell turgor pressure using nanoindentation in conjunction with finite element modeling. *The Plant Journal* **73**, 509–520.
- Franks PJ.** 2003. Use of the pressure probe in studies of stomatal function. *Journal of Experimental Botany* **54**, 1495–1504.
- Geitmann A, Ortega JKE.** 2009. Mechanics and modeling of plant cell growth. *Trends in Plant Sciences* **14**, 467–478.
- Geitmann A, Parre E.** 2004. The local cytomechanical properties of growing pollen tubes correspond to the axial distribution of structural cellular elements. *Sexual Plant Reproduction* **17**, 9–16.
- Geitmann A.** 2006. Experimental approaches used to quantify physical parameters at cellular and subcellular levels. *American Journal of Botany* **10**, 1380–1390.
- Green PB.** 1968. Growth physics in *Nitella*: a method for continuous *in vivo* analysis of extensibility based on a micro-manometer technique for turgor pressure. *Plant Physiology* **43**, 1169–1184.
- Greenleaf JF, Fatemi M, Insana M.** 2003. Selected methods for imaging elastic properties of biological tissues. *Annual Review of Biomedical Engineering* **5**, 57–78.
- Hardham AR, Takemoto D, White RG.** 2008. Rapid and dynamic subcellular reorganization following mechanical stimulation of *Arabidopsis* epidermal cells mimics responses to fungal and oomycete attack. *BMC Plant Biology* **8**, 63.
- Hayot CM, Forouzesh E, Goel A, Avramova Z, Turner JA.** 2012. Viscoelastic properties of cell walls of single living plant cells determined by dynamic nanoindentation. *Journal of Experimental Botany* **63**, 2525–2540.
- Hüsken D, Steudle E, Zimmermann U.** 1978. Pressure probe technique for measuring water relations of cells in higher plants. *Plant Physiology* **61**, 158–63.

- Jönsson H, Krupinski P.** 2010. Modeling plant growth and pattern formation. *Current Opinion in Plant Biology* **13**, 5–11.
- Kennaway R, Coen E, Green A, Bangham A.** 2011. Generation of diverse biological forms through combinatorial interactions between tissue polarity and growth. *PLoS Computational Biology* **7**, e1002071.
- Kierzkowski D, Nakayama N, Routier-Kierzkowska AL, Weber A, Bayer E, Schorderet M, Reinhardt D, Kuhlemeier C, Smith RS.** 2012. Elastic domains regulate growth and organogenesis in the plant shoot apical meristem. *Science* **335**, 1096–1099.
- Kutschera U, Niklas KJ.** 2007. The epidermal-growth-control theory of stem elongation: an old and a new perspective. *Journal of Plant Physiology* **164**, 1395–1409.
- Kwiatkowska D.** 2008. Flowering and apical meristem growth dynamics. *Journal of Experimental Botany* **59**, 187–201.
- Lazarus A, Florijn HC, Reis PM.** 2012. Geometry-induced rigidity in nonspherical pressurized elastic shells. *Physical Review Letters* **109**, 144301.
- Lee D, Barber JR, Thouless MD.** 2009. Indentation of an elastic half space with material properties varying with depth. *International Journal of Engineering Science* **47**, 1274–1283.
- Lesniewska E, Adrian M, Klinguer A, Pugin A.** 2004. Cell wall modification in grapevine cells in response to UV stress investigated by atomic force microscopy. *Ultramicroscopy* **100**, 171–178.
- Lintilhac PM, Wei C, Tanguay JJ, Outwater JO.** 2000. Ball tonometry: a rapid, nondestructive method for measuring cell turgor pressure in thin-walled plant cells. *Journal of Plant Growth Regulation* **19**, 90–97.
- Lloyd C.** 2011. Dynamic microtubules and the texture of plant cell walls. *International Review of Cell & Molecular Biology* **287**, 287–329.
- Marga F, Grandbois M, Cosgrove DJ, Baskin TI.** 2005. Cell wall extension results in the coordinate separation of parallel microfibrils: evidence from scanning electron microscopy and atomic force microscopy. *The Plant Journal* **43**, 181–190.
- McQueen-Mason S, Durachko DM, Cosgrove DJ.** 1992. Two endogenous proteins that induce cell extension in plants. *Plant Cell* **4**, 1425–1433.
- Meshcheryakov A, Steudle E, Komor E.** 1992. Gradients of turgor, osmotic pressure, and water potential in the cortex of the hypocotyl of growing ricinus seedlings: effects of the supply of water from the xylem and of solutes from the Phloem. *Plant Physiology* **98**, 840–852.
- Milani P, Gholamirad M, Traas J, Arneodo A, Boudaoud A, Argoul F, Hamant O.** 2011. *In vivo* analysis of local wall stiffness at the shoot apical meristem in *Arabidopsis* using atomic force microscopy. *The Plant Journal* **67**, 1116–1123.
- Mirabet V, Das P, Boudaoud A, Hamant O.** 2011. The role of mechanical forces in plant morphogenesis. *Annual Review of Plant Biology* **62**, 365–385.
- Mouille G, Robin S, Lecomte M, Pagant S, Hofte H.** 2003. Classification and identification of *Arabidopsis* cell wall mutants using Fourier-transform infrared (FT-IR) microspectroscopy. *The Plant Journal* **35**, 393–404.
- Ogbonna N, Needleman A.** 2011. Conical indentation of thick elastic spherical shells. *Journal of Mechanics of Materials and Structures* **6**, 443–451.
- Peaucelle A, Braybrook SA, Le Guillou L, Bron E, Kuhlemeier C, Höfte H.** 2011. Pectin-induced changes in cell wall mechanics underlie organ initiation in *Arabidopsis*. *Current Biology* **21**, 1720–1726.
- Proseus TE, Ortega JK, Boyer JS.** 1999. Separating growth from elastic deformation during cell enlargement. *Plant Physiology* **119**, 775–784.
- Prusinkiewicz P, Runions A.** 2012. Computational models of plant development and form. *New Phytologist* **193**, 549–569.
- Radotić K, Roduit C, Simonović J, Hornitschek P, Fankhauser C, Mutavdžić 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.
- Robinson SJ, Burian A, Couturier E, Landrein B, Louveaux M, Neumann E, Peaucelle A, Weber A, Nakayama N.** 2013. Mechanical control of morphogenesis at the shoot apex. *Journal of Experimental Botany* **64**, this issue.
- Roduit C, Sekatski S, Dietler G, Catsicas S, Lafont F, Kasas S.** 2009. Stiffness tomography by atomic force microscopy. *Biophysical Journal* **97**, 674–677.
- Routier-Kierzkowska AL, Smith RS.** 2013. Measuring the mechanics of morphogenesis. *Current Opinion in Plant Biology* **16**, 25–32.
- 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.
- Tomos AD.** 1988. Cellular water relations of plants. In: Franks F, ed. *Water science review*. Cambridge: Cambridge University Press, 186–277.
- Triboulot MB, Pritchard J, Tomos D.** 1995. Stimulation and inhibition of pine root growth by osmotic stress. *New Phytologist* **130**, 169–175.
- van der Wel NN, Putman CAJ, van Noort SJJ, de Grooth BG, Emons AMC.** 1996. Atomic force microscopy of pollen grains, cellulose microfibrils, and protoplasts. *Protoplasma* **194**, 29–39.
- Vella D, Ajdari A, Vaziri A, Boudaoud A.** 2012a. The indentation of pressurized elastic shells: from polymeric capsules to yeast cells. *Journal of the Royal Society Interface* **9**, 448–455.
- Vella D, Ajdari A, Vaziri A, Boudaoud A.** 2012b. Indentation of ellipsoidal and cylindrical elastic shells. *Physical Review Letters* **109**, 144302.
- Vogler H, Draeger C, Weber A, et al.** 2013. The pollen tube: a soft shell with a hard core. *The Plant Journal* **73**, 617–627.
- Wang CX, Wang L, Thomas CR.** 2004. Modelling the mechanical properties of single suspension-cultured tomato cells. *Annals of Botany* **93**, 443–453.

Wang L, Hukin D, Pritchard J, Thomas C. 2006. Comparison of plant cell turgor pressure measurement by pressure probe and micromanipulation. *Biotechnology Letters* **28**, 1147–1150.

Wei C, Lintilhac PM, Tanguay JJ. 2001. An insight into cell elasticity and load-bearing ability. Measurement and theory. *Plant Physiology* **126**, 1129–1138.

Yarbrough JM, Himmel ME, Ding SY. 2009. Plant cell wall characterization using scanning probe microscopy techniques. *Biotechnology for Biofuels* **2**, 2–17.

Zerzour R, Kroeger J, Geitmann A. 2009. Polar growth in pollen tubes is associated with spatially confined dynamic changes in cell mechanical properties. *Developmental Biology* **334**, 437–446.

Zimmermann U, Rygol J, Balling A, Klöck G, Metzler A, Haase A. 1992. Radial turgor and osmotic pressure profiles in intact and excised roots of *Aster tripolium*: pressure probe measurements and nuclear magnetic resonance-imaging analysis. *Plant Physiology* **99**, 186–196.

Zimmermann U, Steudle E. 1978. Physical aspect of water relations of plant cells. *Advances in Botanical Research* **6**, 45–117.