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REVIEW PAPER

On the role of stress anisotropy in the growth of stems

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This paper is dedicated to Professor Zygmunt Hejnowicz (University of Silesia, Poland) in recognition of his outstanding contributions to the understanding of plant growth.

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Abstract

We review the role of anisotropic stress in controlling the growth anisotropy of stems. Instead of stress, growth anisotropy is usually considered in terms of compliance. Anisotropic compliance is typical of cell walls, because they contain aligned cellulose microfibrils, and it appears to be sufficient to explain the growth anisotropy of an isolated cell. Nevertheless, a role for anisotropic stress in the growth of stems is indicated by certain growth responses that appear too rapid to be accounted for by changes in cell-wall compliance and because the outer epidermal wall of most growing stems has microfibrils aligned axially, an arrangement that would favour radial expansion based on cell-wall compliance alone. Efforts to quantify stress anisotropy in the stem have found that it is predominantly axial, and large enough in principle to explain the elongation of the epidermis, despite its axial microfibrils. That the epidermis experiences a stress deriving from the inner tissue, the so-called 'tissue stress', has been widely recognized; however, the origin of the dominant axial direction remains obscure. Based on geometry, an isolated cylindrical cell should have an intramural stress anisotropy favouring the transverse direction. Explanations for tissue stress have invoked differential elastic moduli, differential plastic deformation (so-called differential growth), and a phenomenon analogous to the maturation stress generated by secondary cell walls. None of these explanations has been validated. We suggest that understanding the role of stress anisotropy in plant growth requires a deeper understanding of the nature of stress in hierarchical, organic structures.

Key words: Cell wall, cellulose microfibril, elongation, growth anisotropy, maturation stress, multiscale model, radial expansion, residual stress, tissue stress, tissue tension.

Introduction

The plant stem is a thin cylinder, a shape that is mechanically efficient for the stem's function of positioning and supporting leaves, flowers, and fruits. But despite its simple shape, the growth of a plant stem is surprisingly complex. Originating as a minuscule region of a few hundred cells within a meristem, the stem attains macroscopic size by virtue of a prolonged period of highly anisotropic expansion. The form of the stem is achieved, with rarely a bulge or tear, by the coordinated expansion of hundreds of thousands of cells, different in size, shape, and composition. This anatomical complexity presents profound problems for understanding how anisotropic expansion within the stem is controlled.

The mechanical rigidity of a (non-woody) plant organ arises from a balance between an osmotic force drawing water into cells and an opposing mechanical force within cell walls. In a growing organ, these two forces are present but coupled to processes that allow water entry and irreversible cell-wall deformation. The osmotic force is isotropic (that is, equal in all directions), whereas the growth of a stem is anisotropic. Thus, the anisotropy of expansion depends on the cell wall. Expansion can be anisotropic when one direction experiences a greater stress than another or has a greater compliance. Efforts to understand expansion anisotropy have focused almost exclusively on compliance, whereas, for the most part, stress has been ignored.

Cell walls are indeed mechanically anisotropic, an attribute that arises at least in part from cellulose microfibrils. Objects of scrutiny for more than a century, cellulose microfibrils are long, stiff rods, typically occupying about a third of the cell wall's dry mass, and are usually arranged with great regularity. This asymmetric construction endows the wall with an anisotropic compliance that is undoubtedly relevant for understanding stem growth (Baskin, 2005). By contrast, anisotropy of stress is poorly characterized. Although for a single, isolated cell, a simple geometric derivation of intramural stress anisotropy is well known, this formulation does not apply to a multicellular tissue. Characterizing stress is difficult in a material like a stem that has a heterogeneous, multiscale structure; for example, the stress experienced by a single microfibril can have different characteristics from the cell wall in which it is embedded, or the tissue in which the cell wall sits. Attempts to model or measure stress anisotropy in a stem have been few and have produced somewhat conflicting results.

Here, we consider the role of stress anisotropy for the anisotropic expansion of the stem. As will emerge below, certain observations relating to expansion anisotropy in stems are difficult to account for based on compliance only. Furthermore, a sizable literature exists on cellular responses in the stem to stress, emphasizing the cytoskeleton (Williamson, 1990; Hejnowicz *et al*., 2000; Moulia and Fournier, 2009), yet these experiments seem difficult to interpret without reliable characterization of the stresses themselves. Here, we review attempts to quantify and model stress anisotropy in stems. We are particularly interested in the possibility that living cells of the stem are able to generate force in the cell wall actively, an ability long attributed to cells making secondary cell walls and widely alleged to be crucial for setting the mechanical properties of the mature plant body.

Mechanical framework and inevitable simplifications

We consider single cells as well as stems, and our assumed geometry is illustrated in Fig. 1. To avoid ambiguity and to help readers understand engineering of the non-genetic kind, we define key terms and basic mechanics in Box 1. For treating the mechanics, unless noted otherwise, we adopt the simplification that material is conserved. For the growing plant cell or stem, we recognize that water enters the system and material is continuously added to the cell wall. We suspect that water flow and cell-wall synthesis both need to be included before plant growth, anisotropic or otherwise, can be understood fully, and notable steps have been taken in this direction recently (Boyer, 2009; Rojas *et al*., 2011). Along these lines, we emphasize that our goal here is to introduce the reader to the issue of anisotropic stresses in stem growth and to review attempts to demonstrate their magnitudes. In no way are we attempting to treat stress anisotropy in a growing stem comprehensively.

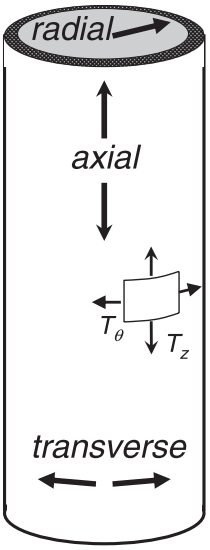

Fig. 1. A schematic of a cell or stem, indicating axial, transverse (i.e. circumferential), and radial directions. The in-plane tensions acting on a small element are T_z in the axial direction and T_θ in the transverse direction. The structure is viewed as a two-component system, with the outer (black) component in tension and the inner (grey) component in compression. For a cell, the components are cell wall and protoplasm; for a stem, they are epidermis and inner tissue.

A challenging observation

Isn't the usual explanation based on mechanical anisotropy and cell-wall compliance demonstrably sufficient? We think the answer is no, in part because of a remarkable and little-known pair of papers (Perley *et al*., 1975; Taiz and Métraux, 1979). The former used lupine (*Lupinus angustifolius*) hypocotyls and measured growth with position transducers; the latter used pea (*Pisum sativum*) epicotyls and measured growth with laser reflection.

In the experiments with pea, growth in length and radius was quantified for stems treated with acid, the growth hormone auxin, or the fungal toxin fusicoccin (Fig. 2). Each of these compounds stimulates elongation rate, as expected, but they each affect transverse expansion distinctly. For acid, as elongation rate increases, transverse expansion rate becomes negative (Fig. 2A), but on auxin, the transverse rate is essentially zero, despite the stimulated elongation (Fig. 2B). For the fungal toxin, after about an hour, transverse expansion rate is stimulated, so much that expansion becomes essentially isotropic (Fig. 2C). For acid and auxin, Perley *et al*. (1975) reported all but identical results (they did not use fusicoccin).

These data deserve to be better known. Besides offering evidence against the notion that auxin stimulates elongation by acidifying the cell wall, the observed growth responses are difficult to account for solely by compliance. The effect of fusicoccin is consistent with compliance, provided that an hour be sufficient time to weaken the usual resistance of cell walls to transverse deformation. But for acid or auxin treatments, the growth responses appear to be

Box 1. Stress, strain, and anisotropy

Stress is a force per unit area, acting on an oriented surface. This surface may be a physical interface (e.g. between neighbouring cells) or a virtual slice through a block of tissue. A normal stress is one that acts perpendicular to the surface. A shear stress acts tangential to the surface. Pressure is therefore a normal stress; by convention, a positive pressure is compressive. Tension is a force (in a fibre) or a force per length (in a sheet) but is also used generically to refer to a negative or expansive stress. In general, a surface will be subject to both normal and shear stresses. For a small cube of material (e.g. within a cell wall), normal and shear stresses act on each of the cube's six faces. The stress exerted on the cube by a uniform external pressure is isotropic (the pressure has the same magnitude on each face). In general, however, the shear and normal stresses acting on the different faces of the cube will have different magnitudes, making the local stress distribution anisotropic. However, if the cube is at rest (or at least not accelerating rapidly), then the net forces acting on the surfaces of the cube must balance; these conditions place restrictions on the differences in normal and shear stresses acting across the cube.

Deformation of a small cube of material is defined using strain, a measure of the degree to which the cube's length changes in a particular direction relative to its original length, and strain rate, which measures the rate at which strain changes with respect to time. The physical properties of the cube are embodied in a constitutive relation, an equation (strictly, a set of equations) that relates the normal and shear stresses acting on the cube's faces to the components of strain and strain rate that characterize the cube's deformation. For an elastic material, the constitutive relation relates stress to strain, and it embodies any material anisotropy of the material in the cube. An elastic material under load deforms reversibly and instantaneously, meaning that when the load is removed, the material returns immediately to its original configuration. The constitutive relation also incorporates material properties through sets of parameters that characterize resistance to elongation, represented by one or more Young's moduli; resistance to shear deformation, represented by one or more shear moduli; and the degree to which an extension in one direction induces strains in other directions, represented by one or more Poisson's ratios. For small elastic deformations, the relation between the components of stress and the components of strain is embodied by a set of linear equations, leading to straight-line graphs of stress versus strain. For large deformations, stress is a non-linear function of strain and additional parameters are needed to characterize the material properties. An isotropic elastic material has a single Poisson's ratio, ν; for an incompressible (volume-preserving) material, ν=1/2. An orthotropic material (one having three orthogonal symmetry planes) is characterized by nine independent parameters: three Young's moduli, three shear moduli, and three Poisson's ratios.

Irreversible or non-instantaneous deformations (i.e. not elastic) are described by a family of constitutive relations that incorporate viscous effects. Conceptual models for viscoelastic materials include a spring and dashpot in series (representing a fluid-like material that will continually elongate under sustained load) or a spring and dashpot in parallel (a solid-like material that undergoes a reversible but delayed deformation under sustained load). Both responses are examples of creep, and both models can be described using linear relations between stress, strain, and their rates of change. Plastic materials demonstrate a non-linear response to an imposed stress: if the stress is below a threshold, the deformation is elastic (and reversible); however, if the stress exceeds the threshold, the material yields, deforming irreversibly.

Growth of an elastic material can be described in terms of a prescribed distribution of strain (or strain rate), which may vary with position through a material. The strain field must satisfy certain compatibility constraints (mathematical conditions imposed on its spatial derivatives) for there to be a single-valued displacement field that is consistent with the proposed strain. If a compatibility constraint is violated, or the strain field is not consistent with external boundary conditions, then an internal residual stress will be generated in the material (Skalak *et al*., 1996). Residual stress is also known as self-stress (Howell *et al*., 2009) or auto-stress (Moulia and Fournier, 2009).

established within minutes: while one can readily imagine a pattern of microfibril arrangement at time zero consistent with the response to either acid or auxin, it is difficult to imagine a pattern consistent with both. Additionally, Taiz and Métraux made a further observation. The buffers used for treatments contained on the order of 10 mM osmoticum (salt or organic compound): when this was removed from the acid treatment, expansion rate in length was scarcely affected but radial shrinkage ceased instantly (Fig. 2A). It seems unlikely that removal of a modest supply of osmoticum could change the mechanical anisotropy of the cell wall with such rapidity.

To be sure, both studies had small sample sizes, and until the experiments are repeated and extended, firm conclusions from them are premature. Nevertheless, these growth patterns are not the only reason prompting us to examine stress anisotropy.

Giant steps from giant cells

The paradigmatic object for studies of expansion anisotropy is the internode of *Nitella axillaris* (and the related *N. flexilis* and *N. opaca*). In the thallus of this alga, nodes alternate with internodes, with the latter being just one cell but one that enlarges to reach many centimetres in length and only a millimetre or two in width. Because of their size and accessibility, these cells were used in a series of pioneering

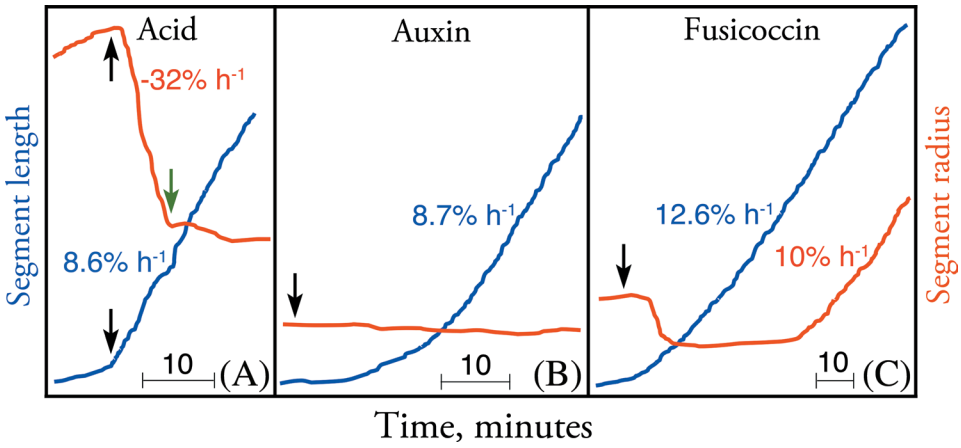

Fig. 2. Stem growth kinetics. Stem segments (~1 cm) were isolated from the epicotyl of etiolated pea (*Pisum sativum*) seedlings and placed in an apparatus for high-resolution measurement of length (blue lines) and radius (red lines). Treatment began, as indicated by black arrows, and comprised 1 mM MES (pH 4.0) (A), 10 μM indole acetic acid (B), or 10 μM fusicoccin (C). All treatment solutions contained ~10 mM osmoticum (sucrose or 10 mM polyethylene glycol 600). In (A), the osmoticum was removed at the time indicated by the green arrow. A time interval of 10 min is shown for each panel. Strain rates shown were estimated for the linear portion of each curve as $(100/t)\ \ln(D_f/D_i)$, where D_f is the final dimension (length or radius) and D_i is the initial dimension, and t is the time in hours between D_i and D_f. Data are redrawn from Taiz and Métraux (1979).

experiments half a century ago. The anisotropy of expansion rate of these cells is constant—with elongation rate being about four- to fivefold faster than transverse expansion rate. The ratio is constant even while the absolute rates change during development. Likewise, compliance is anisotropic to roughly a similar extent, where compliance is assessed as the Young's modulus of cell-wall strips cut from the cell at different azimuths (Probine and Preston, 1962; Métraux and Taiz, 1978; Wei *et al*. 2006). This anisotropy of cell-wall mechanical properties correlates with structure. In growing cells, the orientation of cellulose microfibrils is mainly transverse, parallel to the direction of lowest compliance (Green, 1958; Probine and Preston, 1961). It is intuitively reasonable that aligned microfibrils are more readily separated perpendicular to their orientation compared with parallel, and it became widely accepted that depositing aligned cellulose microfibrils perpendicular to the cell's long axis endows a cell wall with a mechanical anisotropy sufficient to account for the observed expansion anisotropy.

But what is the intramural stress anisotropy of a single cylindrical cell, such as the *N. axillaris* internode? This question is readily answered for a right circular cylinder with a wall whose thickness is much less than the cylinder's radius. In a cell, stress in the cell wall arises because of hydrostatic pressure of the cell contents; therefore, we consider a pressurized cylinder, and neglect forces imposed by gravity. Because pressure is isotropic, one might suppose that the stress in the wall generated by pressure would likewise be isotropic. But, in fact, the intramural stress is anisotropic because the shape of the cylinder is asymmetric. For the cylinder, the ratio of intramural stresses can be solved (Box 2). The transverse stress is twice the axial stress, a fact that explains why, when water pipes freeze, the crack runs axially.

Returning to the cell, the next question is: given the anisotropic loading based on geometry, what is the response of the cell wall? Because growth involves irreversible deformation, one might plausibly answer with an analysis that treats the growing cell wall as a viscous, rather than a purely elastic, material (Box 2). Unfortunately, calculations from such a treatment are difficult to test against observations because there are too many experimental uncertainties (e.g. cell-wall viscosity). Therefore, we will treat the cell wall as an elastic material, which in fact is the approach taken in most if not all of the foundational work on the growth of plant cells.

For our pressurized cylinder, modelled as a (linearly) elastic sheet, bi-axially loaded in a 2:1 ratio favouring width, with an isotropic cell wall, transverse expansion rate would exceed elongation rate by at least a factor of two. The actual factor depends on the Poisson's ratio of the cell-wall material, and has been given as: $(2 - \nu)/(1 - 2\ \nu)$, where ν is the Poisson's ratio (derived from equation 2.2.15 in Howell *et al*., 2009). This expression equals five when the Poisson's ratio equals 0.3, which is the value measured for non-growing internodes (Tazawa and Kamiya, 1965). In fact, five is about the strain rate anisotropy (favouring transverse expansion) observed when the synthesis of aligned microfibrils is inhibited chemically (Green *et al*., 1970). Therefore, to overcome the prevailing stress favouring swelling sufficiently to elongate four or five times faster than expanding transversely, the cell needs perhaps a tenfold difference in cell-wall compliance. Again, this is about the difference that was observed when isolated cell-wall cylinders were pressurized with mercury (Richmond *et al*., 1980). Interestingly, this compliance difference was in plastic deformation; by contrast, the comparable ratio for elastic compliance was only about two. Thus, with the caveat that the elastic analysis might be inappropriate, the accepted roles for intramural stress and compliance appear plausible for the anisotropic growth of a single cell.

Box 2. Stress in an isolated cylindrical cell

Consider an isolated, rigid, closed-ended, circular cylinder of radius R, at uniform internal pressure P (Fig. 1). At equilibrium, for an element on the curved surface of the cylinder, the pressure acts on the flat ends of the cylinder to induce an axial tension T_z and acts on the sides of the cylinder to induce a transverse tension T_θ (each a force per unit length). Balancing the force on the end plate due to pressure, $\pi R^2 P$, with the tension acting around the perimeter of the end plate, $2\pi R T_z$, gives $T_z = RP/2$. A radial force balance on an element of the curved surface, accounting for the fact that the transverse tensions acting on either edge of an element of curved surface pull in slightly different directions, gives $T_\theta = PR$ (a result known as the law of Laplace). Thus, $T_\theta = 2T_z$. If the curved wall of the cylinder has uniform thickness h, then the stresses in the wall are T_z/h and T_θ/h, again differing by a factor of 2. It should be emphasized that this result is specific to the particular shape of a circular cylinder and will not be exact close to the ends.

While the 2:1 ratio of stresses is independent of the material properties of the cylinder, the response of the cylinder to a small increase in P is strongly dependent on the material properties of the wall. For a linearly elastic, isotropic material, the Poisson's ratio plays a defining role and one that is amplified by any material anisotropy in the cell wall. Suppose the cylinder is an elastic material that is substantially stiffer in the axial direction compared with the transverse direction: then the cylinder can be expected to shrink in length and increase in radius upon inflation. In contrast, a wall that is stiffer in the transverse direction will elongate on inflation. Conditions on the relevant Poisson's ratios and Young's moduli that govern the consequent strain anisotropy can be derived from equation (B5) in Hejnowicz and Sievers (1995*a*).

To model growth of a cylindrical cell, it is more appropriate to treat the wall as a viscous material. Similarly to an incompressible isotropic elastic sheet (with Poisson's ratio 1/2), an incompressible, isotropic viscous sheet wrapped into a cylinder will not elongate on inflation. However, if the wall is reinforced with inextensible fibres, then radial expansion can be inhibited (or even reversed), allowing pronounced elongation (Dyson and Jensen, 2010). The fibres embedded in the wall control the anisotropy of expansion; the evolving fibre orientation, together with the viscosity of the matrix in which the fibres are embedded, determine the rate of elongation under a given load.

Before leaving the pliant *N. axillaris* cells, we point out that growth is tied intimately to metabolism (Ray, 1992; Boyer, 2009). Far from conservation of material, an apt conservation law could be conservation of cell-wall thickness, an invariance that requires cell-wall synthesis to be regulated specifically to balance thinning from deformation. A consequence of this linkage is that the removal of metabolism (i.e. cell death) can, and probably does, alter the compliance of the cell wall. In other words, the relevant compliance is that generated instantaneously by metabolism acting on the cell wall. This means that data obtained for isolated walls, whether pulled on in one direction or pushed on in all directions, are at best approximate and at worse systematically wrong.

Embracing the stem

Understanding a stem requires handling multicellularity (Box 3). In engineering terms, the stem is similar to a thin-walled pressurized foam. For such a material, the distribution of stress cannot usually be solved analytically, even when the walls of the foam are uniform. Adding to the difficulty, although all of the cell walls in the stem are 'thin' according to the engineering criterion, they differ from each other in thickness, composition, and mechanical properties. Given this complexity, progress requires making reasonable simplifications.

Among tissues of a stem, the epidermis has cell walls that are demonstrably thicker than other tissues (Fig. 3). Although this is arguably true for any stem, this difference between epidermis and other tissues is salient for growing stems, because the vasculature and supporting fibre cells have yet to undergo much secondary cell-wall thickening. Therefore, growing stems are plausibly simplified as a two-component system: epidermis and inner tissue. The inner tissue comprises most of the stem and has thin, relatively compliant cell walls, whereas the epidermis comprises the outer-cell layers, which have thick, relatively inextensible cell walls (Fig. 1). Although the epidermis typically amounts to one or two cell layers, in the hypocotyl of *Arabidopsis thaliana*, it appears that the stiff component constitutes only the outer epidermal cell wall (Crowell *et al.*, 2011). While this two-component reduction is undoubtedly a simplification (Fig. 3), it has been widely adopted and, as described partly below, appears to help explain several observable features of stem behaviour.

A manifest difference between epidermis and inner tissue was discussed prominently in the 19th century by von Sachs, and ever since the implications of this difference for growth have received attention (Peters and Tomos, 1996; Kutschera and Niklas, 2007). The key observation is that the epidermis of a growing stem contracts axially when it is peeled off from the inner tissue or when the stem is partially bisected. This implies that, in the intact state, the epidermis is in tension while the inner tissue is under axial compression (Fig. 4A). Where do these stresses originate? Surprisingly the stress on the epidermis is too large to be generated by the osmotic pressure of the epidermal cells themselves; instead, the force originates from the inner tissue. That the inner tissue is restrained from elongation by the epidermis is evidenced by the peeled inner tissue elongating instantaneously when immersed in water (Peters and Tomos, 2000). This sharing of loading and resistance between inner tissue and epidermis is referred to as *tissue stress*. Although *tissue tension* is a synonym, this term is potentially misleading

Box 3. Mechanics of multiscale materials

Plant tissues have a heterogeneous structure across a hierarchy of length scales, from hydrogen bonds up to the whole plant. In characterizing the mechanical properties of a stem, tissue layer, or cell wall, it is necessary to consider the stresses acting on, and deformations of, a small representative cube of material (Fig. 4B). This cube should be substantially smaller than the object of interest (a stem, say) but larger than the constituent components (individual cells, or molecules, again depending on the scale). It is necessary to average over the fine structure of the components to assign effective material properties to the cube. This concept is the basis of the continuum hypothesis that underpins mechanics. Of course, if there is an insufficient gap in scale (between, for example, cell and stem diameter), then a continuum description will break down, making it necessary to model the behaviour of individual components.

Tissue stress involves spatial averaging over individual cells; it represents the stress acting on a cube of homogenized material that in reality contains multiple individual cells (Fig. 4). Tissue stress is an example of residual stress (Box 1): a stress field that remains hidden until the material is cut, at which point the material spontaneously deforms. The spatial averaging process that underpins any continuum model hides information about fine-scale stress distributions. For example, imagine taking a slice through a few cells, intersecting cell walls, cytoplasm, and vacuoles (Fig. 4A). Within each protoplast (combination of cytoplasm and vacuole), the stress is predominantly a compressive, isotropic pressure. Within a cell wall, the stress is extensional and anisotropic, dominated by axial and transverse tensions acting in the plane of the cell wall. Averaging over a few cells (i.e. integrating the fluctuating stress field), the large differences between compressive (protoplast) and tensile (intramural) stress components cancel out to give a net tissue stress. A positive tissue stress denotes that, after averaging spatially over the cells in a representative tissue cube, the tensile stress resisting elongation in cell walls exceeds the local pressure that promotes tissue elongation, so that the tissue cube, if isolated, would contract. The effective material properties assigned to an element of tissue (such as a set of Poisson's ratios) thus reflect the integrated effect of structural information at the level of constituent cells and at the level of the fibrous microstructure within the cell walls.

Theoretical and computational models are only beginning to capture the macroscopic effects of this multiscale structure (Merks *et al*., 2011; Uyttewaal *et al*., 2012; Yi and Puri, 2012). By having its periphery under tension and its interior under compression, the distribution of axial tissue stress across the stem mirrors the distribution of axial stress across individual cells (Fig. 4), a hierarchical symmetry that provides mechanical resilience at different scales.

because, strictly speaking, the tissues share stresses, not tensions (Box 1).

In engineering terms, tissue stress is an example of a residual stress (Boxes 1 and 3). As such, putting the epidermis in tension has been shown to play an important role in enhancing the resistance of the stem to bending (Niklas and Paolillo, 1997; Vandiver and Goriely, 2008). Tissue stress is a stress integrated over (and acting upon) multiple cells, arising from large but fine-scale fluctuations of stress that occur at the subcellular level, and involving compression of protoplasts and tension in neighbouring cell walls (Fig. 4A). At the level of a cell, tissue stress represents the net stress acting on that cell due to its neighbours; it disrupts the balance between cell-wall tension and protoplast pressure that would occur were the cell to be isolated.

The consequences of tissue stress have been considered almost exclusively for elongation. For example, evidence has been collected to support the hypothesis that auxin stimulates elongation in stems specifically acting on the epidermis to make it more extensible (Kutschera and Niklas, 2007). However, the relationship between tissue stress and anisotropy of expansion has been little explored.

Stressing tissues in two dimensions

Expansion anisotropy in stem growth might have been generally ignored because the single-cell framework has been implicitly imposed on the stem. That is, one imagines that all cell walls in the stem have transverse microfibrils, thereby making all walls more deformable in the axial direction. In this view, the basic construction of stem cell walls would specify limited, if any, radial expansion, and the key variable would be the magnitude of elongation rate.

Besides this happy view being confounded by data such as those in Fig. 2, it founders on the fact that epidermal cell walls of growing stems rarely have cellulose microfibrils that are transverse (Baskin, 2005). In a variety of species, microfibril order in the cell walls of the great majority of growing epidermal cells is longitudinal, whether assessed cumulatively with polarized light microscopy (Paolilo, 2000) or directly at the innermost layer with transmission electron microscopy (Takeda and Shibaoka, 1981). In addition, imaging of tagged cellulose synthase proteins in the hypocotyl of *A. thaliana* reveals no preference for transverse alignment at the outer epidermal cell wall (Chan *et al*., 2010; Crowell *et al*., 2011). For a growing stem, given that the compliance of the epidermis favours transverse expansion but growth is axial, we conclude that the tissue stress acting on the epidermis must itself be anisotropic, with axial tissue stress exceeding both radial and transverse components considerably.

What is the origin of stress anisotropy within the stem? The stem is cylindrical and comprises roughly cylindrical units. As described above, isolated cylindrical units are expected to generate intramural stress anisotropy favouring transverse expansion (Box 2). Gluing cylindrical cells

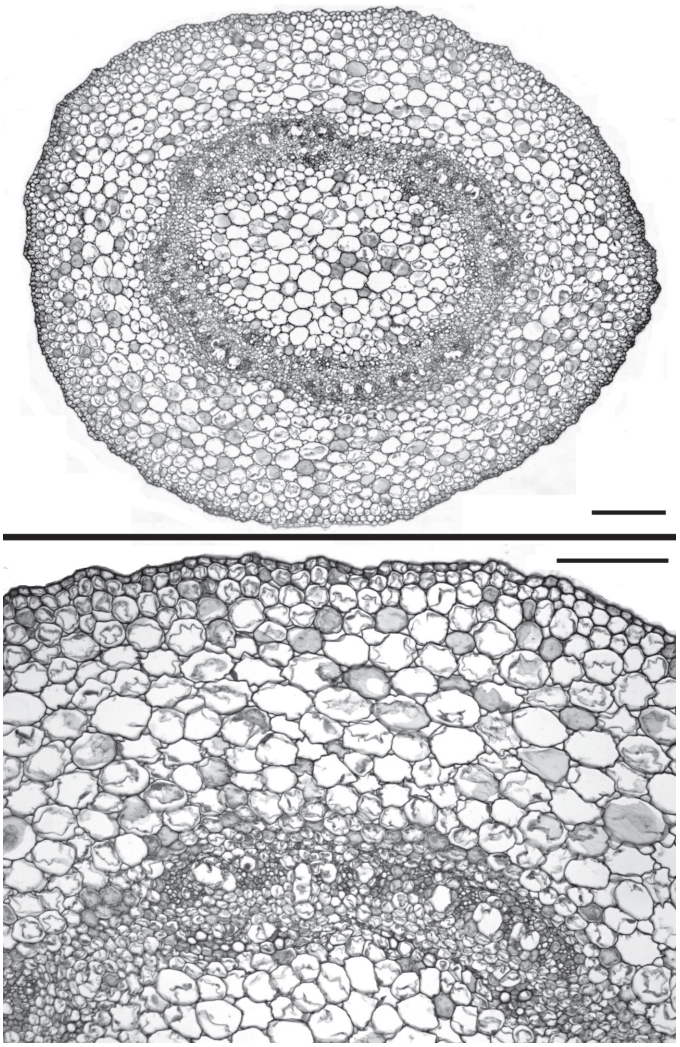

Fig. 3. Transverse section through the growing portion of a soybean (*Glycine max*) hypocotyl, stained with saffranin. Note that, although the vascular tissue anatomy is well established, few vascular cells have undergone appreciable cell-wall thickening. Bars, 200 μm (upper); 150 μm (lower).

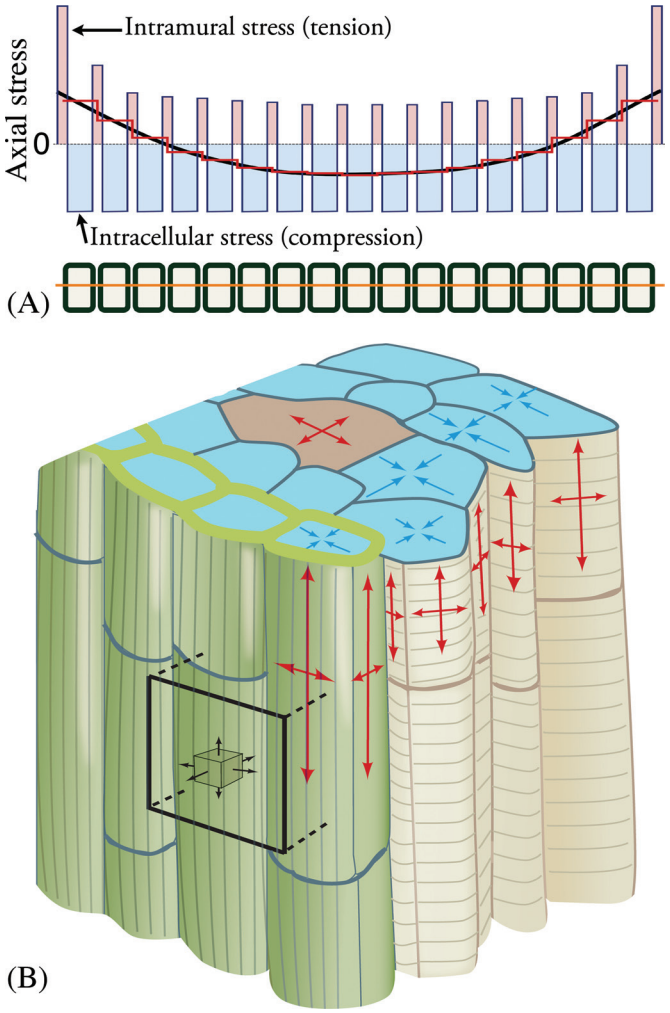

Fig. 4. (A) Schematic representation of the distribution of axial stress across a stem (top), evaluated along a transverse line of cells (orange line, bottom). At the cellular scale, positive intramural stress (tension) alternates with negative stress (compression) in the protoplast. For simplicity, the negative stress is assumed to be uniform across the stem. Cell walls towards the outer edge of the stem carry higher intramural stress than those nearer the centre. The red line shows the axial stress when averaged across individual cells. Each step illustrates the axial load borne by the relevant cell. At the tissue scale, this 'staircase' distribution is smoothed (black line), giving the continuous distribution referred to as 'tissue stress'. Stresses are measured relative to atmospheric pressure. (B) Visualization of part of a stem illustrating difficulties for understanding how stress is generated and propagated through this structure. Arrows indicate stress magnitudes and directions qualitatively (red for positive and blue for negative stresses). Lines in the walls indicate the predominant direction of cell-wall microfibrils. Two 'stress cubes' show different scales at which stress might be evaluated. Among the difficulties are the steeply varying mechanical properties and orientations of the cell walls and the presence of individual cells whose pressure can vary independently.

together into a tissue evidently disrupts the prevailing anisotropy of stress, but in what way? How do the stresses, orientations, and material properties of individual cell walls taken together determine stress anisotropy at the tissue level (Fig. 4B)? Before offering potential explanations, we first consider investigations of the actual stress anisotropy in the stem.

Anisotropic statics of stems

Arguably the most comprehensive attempt to quantify the anisotropy of tissue stress was made by Hejnowicz and Sievers for the growing stem of sunflower (*Helianthus annuus*). Their approach begins by accounting for the coupling between stresses in orthogonal directions: pull on an object in one direction and you will induce a strain in other directions. The coupling is represented by *Poisson's ratios* (Box 1). For a stem, the induced strain will be resisted by surrounding tissues, generating a stress orthogonal to the primary load. Hejnowicz and Sievers (1995*a*) observed that microfibrils in epidermal cells are indeed oriented

longitudinally overall and their measured Poisson's ratios for isolated epidermis are consistent with this orientation, provided one accepts their anisotropic linear elasticity model. Such a model is reasonable for describing short-term, small-amplitude deformations but, insofar as growth in stems occurs over days, a viscous or viscoelastic model might be more suitable.

With Poisson's ratios measured, they next work out the transverse tissue stress induced by an axial stress imposed on the outer tissue layer (Hejnowicz and Sievers, 1995*b*). They do so by modelling the outer tissue as an isolated flat sheet, wrapped around the inner tissue, and stuck to it so that the transverse tissue stress is transmitted to the inner tissue. Combined with measurements of the stress needed to extend an epidermal strip as well as that needed to prevent the inner tissue from expanding in water, the authors report that, in the inner tissue, the axial (compressive) tissue stress is about twice that in the transverse, whereas, in the epidermis, the axial (tensile) tissue stress exceeds the transverse by about sixfold.

While this analysis indeed recovers a dominant axial tissue stress, it is perhaps premature to place undue emphasis on the reported values. The wrapping and sticking arguably change the problem and would seem to require validation. Furthermore, transverse tissue stress in the outer layer comes from two independent sources: axial loading via the Poisson's ratio and radial loading from the inner tissue (equations B1 and B3, respectively, in Hejnowicz and Sievers, 1995*b*). These contributions were equated, whereas it seems more plausible to add them, with appropriate weighting, to satisfy appropriate compatibility conditions (Box 1). The way such stresses interact has been addressed systematically for stems, accounting for non-linear elasticity (specifically strain-stiffening of the epidermis) but not anisotropy (Vandiver and Goriely, 2008).

The stress distribution in stems was also investigated by Niklas and Paolillo (1998). They used the stems of dandelion (*Taraxacum officinale*) taking advantage of the stem's hollow structure, allowing it to be experimentally pressurized, a protocol that potentially gives insight into the underlying anisotropy of tissue stress. They compared both mature and immature stems and the results were similar for both; however, maturity was distinguished based on the state of the capitulum, and it is not clear whether the immature stems were actually growing when they were sampled. Be that as it may, the authors found microfibrils in the epidermis to be axial (in these stems, the effective outer layer comprises several cell layers) and axial stiffness to exceed transverse by about tenfold. They then elaborate a mechanical model based on two or more layers glued together and containing microfibrils that differ in orientation by 90°, and being effectively pressurized by increasing the temperature. Despite ignoring Poisson's ratio couplings, their model accounts for the observed behaviour, including the response to cuts in the stem, and predicts the dominant tissue stress in the epidermis to be strongly axial.

Both groups agree in finding dominant axial tissue stress in the epidermis. That the mechanical models invoked are distinct gives confidence that epidermal stress is indeed predominantly axial. This allows us to understand why, despite having axial microfibrils, epidermal tissue does not swell transversely. But the question posed at the end of the last section remains salient: how does a material comprising cylindrical units, which in isolation would have predominantly transverse intramural stress, generate predominantly axial tissue stress?

More than one way to stress a stem

One way to account for a large axial tissue stress in a stem is through *differential growth*. On first principles, this explanation seems untenable because plant organ growth is symplastic—the inner tissue can grow more than the outer only with bending or tearing. But, strictly speaking, differential growth refers to irreversible extension (i.e. plastic deformation). Symplastic growth constrains the sum of plastic and elastic deformations to be equal among tissues but does not forbid plastic deformation to be less in epidermis than inner tissue, provided that elastic deformation be correspondingly more. If this occurs, then the epidermis would have a comparatively large elastic strain and, it is conjectured, stress.

Besides the uncertainty about the relationship between elastic strain and stress, a problem with accepting differential growth is that the process is implicitly time dependent. One might expect the tissue stresses to change during the many days over which a typical stem grows, as the 'growth' differentials became greater or less; likewise, when tissue stresses are reset, for example by cycles of plasmolysis, again one might expect the observed residual stresses to be changed. Although anisotropy of tissue stress has rarely been explored, longitudinal stresses themselves have frequently been observed and such time-dependent behaviour has been rarely if ever reported (e.g. Kutschera, 1992). Furthermore, if substantially different elastic strains were present between epidermis and inner tissue, one might expect a plasmolysed stem to buckle, bulge, or tear, but such distortions are, to our knowledge, absent.

As an alternative to differential growth, Hejnowicz and Sievers (1996) supported a view we term *differential moduli*. They modelled axial tissue stress in the epidermis based on the presence of internal pressure and distinct stiffness of epidermis and inner tissue, and tested their model with experiments. Their model roughly reproduces the magnitudes of the experimentally estimated axial stresses, as well as their dependence on the magnitude of internal pressure. However, as an important caveat, these authors did not consider transverse stresses so it is not known to what extent their model predicts the correct tissue stress anisotropy.

A further caveat is that both groups (Niklas and Paollilo, and Hejnowitcz and Sievers) considered only (short-term) elastic properties of the system, whereas growth is a slow, viscous process, during which the cell wall will age and remodel (Ray, 1992; Boyer, 2009). Stiffnesses and viscosities

will be influenced by microfibril rearrangements, crosslink dynamics, and the incorporation of cell-wall material. For a fundamentally viscous process like growth, it is dangerous to rely too much on elastic measurements and linear regimes. However, one can consider the viscous analogue of the differential moduli concept, for which material properties associated with viscous growth (yield and extensibility) vary across the stem, giving rise to non-uniform tissue stress. This idea was exploited in a stem-growth model accounting for non-uniform turgor pressure and a stiff epidermis (Passioura and Boyer, 2003), but again anisotropy was not treated.

A third explanation, and none of these is necessarily exclusive, is *growth stress* (sometimes called maturation stress), a phenomenon widely recognized to occur in the secondary cell walls of wood (Okuyama *et al*., 1994; Clair *et al*., 2011; Mellerowicz and Gorshkova, 2012). As the secondary cell wall is being synthesized, microfibrils are modified so as to be placed in tension, although the mechanisms used to do this are a matter of dispute.

That a phenomenon analogous to growth stress in trees plays a role in stem-growth anisotropy was posited by Hejnowicz and Borowska-Wykręt (2005). These authors plasmolysed epidermal peels of sunflower hypocotyls and observed fine buckles in the cell wall with a wavelength of about 0.5 µm. The buckles were strictly transverse and were present only in the inner portion (roughly half) of the cell wall. This is a difference between different regions of the epidermal cell wall, and not between the cell walls of different tissues.

The authors explain the buckling wavelength by positing a substantially greater axial intramural stress in the outer-cell-wall layers compared with the inner, and they point out that greater tension in the outer layers is reasonable because, as microfibrils age and move from inner to outer portions of the cell wall, strain continues and stress accumulates. In fact, such stress accumulation has been recently quantified by a theoretical model of the intramural distribution of crosslinks connecting microfibrils, tracking the crosslinks from their initial formation near the inner surface of the cell wall to their rupture after elongation nearer the outer surface (Dyson *et al*., 2012). In that study, stress accumulated among crosslinks rather than microfibrils, but, regardless, stress accumulation is a time-dependent process associated with elongation.

What about the transverse direction? In the inner tissue, how does a newly deposited, transverse microfibril become load-bearing? In that direction, there is little strain. Making an explicit analogy to the growth stresses of trees, Hejnowicz and Borowska-Wykręt (2005) suggest that, soon after deposition, microfibrils contract. This would be sufficient to place them in tension and become load-bearing. If such a contraction likewise occurred for epidermal microfibrils deposited longitudinally, it would be amplified by subsequent growth and might contribute to the dominance of axial stress in the epidermis.

Return to the beginning

We have seen how the account of growth anisotropy for the single cell based on compliance cannot be applied to the stem in any straightforward manner. Recognizably, the stem's behaviour is complicated by its manifold tissues with their distinct shapes and cell walls (Fig. 4B). We have seen that, although the compliance of the epidermis in a growing stem is usually greater transversely than axially, the stem is able to exert a large, axial tissue stress on the epidermis sufficient to drive highly anisotropic expansion. Nevertheless, this stress has an unknown origin: it might arise from differential growth, differential moduli, or growth stress, alone or in combination.

And then there is Fig. 2. The rapid, non-linear growth behaviour seen in this figure implies that something is missing. None of the above-named mechanisms readily explains how removing 10 mM osmoticum from an acid buffer rapidly and profoundly alters the rate of transverse expansion without changing elongation rate. We hypothesize that the missing component is hydraulics. Removal of an osmoticum might trigger the gating of aquaporins or some other channel leading to a rapid change of pressure, a response that could occur specifically in a given tissue. The attempts to model stress anisotropy reviewed above have considered the pressure of each cell to be constant; however, in growing stems, steep radial gradients of pressure typically occur as one moves away from the xylem, both towards the epidermis and towards the stem centre (Passioura and Boyer, 2003). These gradients are arguably not required for the existence of tissue stress, because, for example, they disappear when the stem is cut and floated in water whereas stresses persist, but hydraulic properties could condition expansion anisotropy. It would be revelatory to examine the consequences for stress anisotropy of changing internal pressure in selected layers.

The stem reminds us of how much there remains to be learned about plant growth. If a stem passes our understanding, how can we possibly understand a leaf, let alone an orchid petal that grows into the shape of a bee? But the simple shape of the stem also gives hope that, with a combination of experiments and modelling, this understanding can be accomplished.

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