UNIVERSITY^{OF} BIRMINGHAM University of Birmingham Research at Birmingham

Mathematical principles and models of plant growth mechanics

Smithers, Euan; Luo, Jingxi; Dyson, Rosemary

DOI: 10.1093/jxb/erz253

License: Other (please specify with Rights Statement)

Document Version Peer reviewed version

Citation for published version (Harvard):

Smithers, E, Luo, J & Dyson, R 2019, Mathematical principles and models of plant growth mechanics: from cell wall dynamics to tissue morphogenesis', *Journal of Experimental Botany*, vol. 70, no. 14, pp. 3587–3600. https://doi.org/10.1093/jxb/erz253

Link to publication on Research at Birmingham portal

Publisher Rights Statement:

This is a pre-copyedited, author-produced version of an article accepted for publication in Journal of Experimental Botany following peer review. The version of record Euan T Smithers, Jingxi Luo, Rosemary J Dyson, 'Mathematical principles and models of plant growth mechanics: from cell wall dynamics to tissue morphogenesis', Journal of Experimental Botany, Volume 70, Issue 14, 1 July 2019, Pages 3587–3600, is available online at: https://doi.org/10.1093/jxb/erz253 and https://academic.oup.com/jxb/article/70/14/3587/5498733

General rights

Unless a licence is specified above, all rights (including copyright and moral rights) in this document are retained by the authors and/or the copyright holders. The express permission of the copyright holder must be obtained for any use of this material other than for purposes permitted by law.

•Users may freely distribute the URL that is used to identify this publication.

•Users may download and/or print one copy of the publication from the University of Birmingham research portal for the purpose of private study or non-commercial research.

•User may use extracts from the document in line with the concept of 'fair dealing' under the Copyright, Designs and Patents Act 1988 (?) •Users may not further distribute the material nor use it for the purposes of commercial gain.

Where a licence is displayed above, please note the terms and conditions of the licence govern your use of this document.

When citing, please reference the published version.

Take down policy

While the University of Birmingham exercises care and attention in making items available there are rare occasions when an item has been uploaded in error or has been deemed to be commercially or otherwise sensitive.

If you believe that this is the case for this document, please contact UBIRA@lists.bham.ac.uk providing details and we will remove access to the work immediately and investigate.

1	Mathematical principles and models of plant growth
2	mechanics: from cell wall dynamics to tissue
3	morphogenesis
4	E.T. Smithers, J. Luo, R.J. Dyson
5	
6	16 th May 2019
7	
8	E.T.Smithers:ets796@bham.ac.uk
9	J. Luo: J.Luo.5@bham.ac.uk
10	R.J.Dyson: R.J.Dyson@bham.ac.uk
11	
12	Institution: The University of Birmingham
13	Address: Watson Building
14	University of Birmingham
15	Edgbaston
16	Birmingham B15 2TT
17	United Kingdom
18	Corresponding author telephone: 0121 4143415
19	
20	6 Figures
21	Word count: 8163
22	
23	Highlight (fewer than 30 words)
24	We explain the principles behind mathematical models of plant growth
25	mechanics and the biological insights they provide. We suggest open

questions for mathematicians and biologists to tackle in the future.

27 Abstract (max 200 words)

Plant growth research produces a catalogue of complex open questions. We argue that plant 28 growth is a highly mechanical process, and that mathematics gives an underlying framework 29 with which to probe its fundamental unrevealed mechanisms. This review serves to illustrate 30 31 the biological insights afforded by mathematical modelling and demonstrate the breadth of mathematically-rich problems available within plant sciences, thereby promoting a mutual 32 appreciation across the disciplines. On the one hand, we explain the general mathematical 33 principles behind mechanical growth models; on the other, we describe how modelling 34 35 addresses specific problems in microscale cell wall mechanics, tip growth, morphogenesis and stress feedback. We conclude by identifying possible future directions for both biologists 36 37 and mathematicians, including as-yet unanswered questions within various topics, stressing that interdisciplinary collaboration is vital for tackling the challenge of understanding plant 38 growth mechanics. 39

- 40
- 41

42 Keywords (6-8 words)

43 Mechanics, modelling, growth, morphogenesis, pollen tubes, shoot apical
44 meristem, microtubules

45

46 1 Introduction

47

48 Plant growth is a highly mechanical process, incorporating both reversible (elastic) and irreversible (plastic/viscoelastic) deformations. The cell wall withstands great tension, 49 equivalent to 100-1000 atmospheres of tensile stress (Cosgrove 2005), and consists of three 50 main components: cellulose, hemicellulose (e.g arabinoxylan or xyloglucan) and pectin 51 (Scheller and Ulvskov 2010, Höfte et al. 2012, Park and Cosgrove 2015, Jarvis 2009, 52 Cosgrove 2014). A cell wall inflated under the action of turgor pressure (causing wall stress) 53 will be stretched to mechanical equilibrium, exhibiting a constant elastic strain or 54 55 deformation. For growth to occur, there must be an irreversible deformation, which begins when the mechanical load exceeds some critical value (yield). Growth is carefully mediated 56

via active control of the wall's mechanical properties (e.g. by enzymatic action or new
material deposition), altering either the yield or the post-yield behaviour

59

Growth is inherently a multiscale process, from rearrangement of the cell wall microstructure 60 to the behaviour of a whole tissue (figure 1). On the microscale, bond breakage and polymer 61 network rearrangement (wall loosening) results in a relaxation of wall stress, allowing for 62 viscous flow of the cell wall, whilst thinning of the wall can be balanced by deposition of 63 wall material. Drawing water into the cell during extension allows for permanent volume 64 65 increase (plastic growth) and maintains a high level of turgor. Wall loosening can be mediated by the action of proteins or enzymes, such as expansins, xyloglucan 66 endotransglucosylase/hydrolase (XTH), pectin-modifying enzymes (PME) and/or regulated 67 by the action of hormones, such as auxin, gibberellins, abscisic acid, and so on (Cosgrove 68 2005, 2016). Turgor acts in all directions simultaneously as an isotropic force. To achieve 69 directional growth, cell walls can be mechanically anisotropic; this anisotropy is often 70 71 induced by the alignment of cellulose, thus cell walls highly regulate the direction of growth (Baskin and Jensen 2013, Kierzkowski and Routier-Kierzkowska 2019). On the macroscale, 72 plant cells are rigidly connected to one another through their cell walls (unlike animal cells); 73 74 no slippage can occur. As a result, macroscale morphogenesis and growth must be a collaborative process across the whole tissue (Hamant and Haswell 2017). 75

76

77 Existing reviews which examine the principles of plant growth mechanics include Geitmann 78 and Dyson (2013), Geitmann and Ortega (2009), Prusinkiewicz (2004) and Bruce (2003). Chebli and Geitmann (2007) review specifically the mechanics of pollen tube growth. 79 80 Kierzkowski and Routier-Kierzkowska (2019) highlight the role of geometry in plant growth, citing studies which incorporate imaging approaches. Hamant and Haswell (2017) summarise 81 82 the role of mechanical cues. Ali et al. (2014) and Chickarmane et al. (2010) both examine morphogenesis, the latter specifically looking at the use of computational modelling. 83 Experimental procedures for quantifying mechanical behaviour have also been reviewed by 84 Bidhendi and Geitmann (2019), including discussion of how mathematics can aid in this 85 quantification. 86

87

There can be a lack of mutual appreciation between biologists and mathematicians about their respective disciplines. Papers in biological journals receive 28% fewer citations for each additional equation per page in the main article (Fawcett and Higginson 2012). This hinders

91 communication between researchers of different backgrounds. In this review we hope to tackle this issue by highlighting the crucial biological insights which are generated through 92 93 mathematical models, in a way that readers who are unfamiliar with the underlying theory 94 can appreciate. We lay out an argument that mechanics is fundamental to plant growth and 95 morphogenesis, and that mathematical frameworks are required to describe the mechanistic processes. Such frameworks allow access to details that experiments cannot determine 96 97 (Chickarmane et al. 2010), can bypass the practical challenges of experimentation on living tissue (Dupuy et al. 2007), and provide a means of testing whether proposed mechanisms are 98 sufficient to explain observed behaviour. We begin our review by explaining in section 2 the 99 mathematical frameworks that underlie various plant growth models. In section 3, we dissect 100 mathematical models concerning a number of plant systems, ending each subsection with an 101 102 overview of the main ideas and future outlook. We finish the review by describing in sections 4 and 5 some prospective future directions for the field in general, including both biological 103 and mathematical questions. 104



- 107 Figure 1: Various aspects of growth mechanics (bold) connected by mathematical modelling
- 108 concepts (in italics), inspired by Dumais et al. (2006)

110 2 Mathematical Principles

111

Here, we introduce the mathematical principles which underlie the models described in section 3. We give brief overviews of different classes of methodology, aiming to explain the basic concepts only; references are given for more details on each technique. Key words/concepts are indicated in italics, whilst interactions between concepts are summarised in Figure 1.

117

Any mechanical model of a material (e.g. solid or fluid) is a set of mathematical equations 118 which relate the material's intrinsic variables (including but not limited to deformations, flow 119 rates, potential energies and heat fluxes) to internal and external forces. Those equations 120 contain any number of *parameters*, which describe properties of the material. A parameter 121 122 can be *geometric*, meaning it involves dimensions of length only, such as an area or volume; 123 kinematic, involving dimensions of length and time, such as diffusivity; dynamic, involving 124 length, time and mass, such as Young's modulus; or *thermodynamic*, involving length, time, mass and temperature, such as heat capacity. The algebraic procedure of non-125 126 dimensionalisation creates dimensionless parameters from suitable combinations of the aforementioned dimensional ones, and uses scaling factors to remove dimensions (i.e. units) 127 128 from variables. Examples of dimensionless parameters include aspect ratios, efficiencies, and Reynold's number which is vital in many models of fluid flow. Expressing a mechanical 129 130 model in terms of dimensionless variables and parameters usually affords valuable insights into the physical system, as the absence of "sizes" in the model implies that its outputs will 131 132 hold irrespective of sizes in the system. An exemplary explanation of non-dimensionalisation can be found in Edelstein-Keshet (2005). 133

134

A primary example of a mechanical model of plant growth is the Lockhart equation(Lockhart 1965):

137

138

$$\frac{1}{V}\frac{dV}{dt} = \Phi_0(P - Y) \text{ if } P \ge Y \text{ and}$$
$$\frac{1}{V}\frac{dV}{dt} = 0 \text{ if } P < Y , \qquad (1)$$

139 where V is the volume of a growing cell, t is time, P is turgor pressure within the cell, the 140 parameter Y is a turgor yield threshold below which no growth occurs, and Φ_0 is an extensibility parameter. Despite its shortcomings, variations on the Lockhart equation havebecome a standard paradigm for plant cell and tissue growth, as we discuss in section 3.

143

144 2.1 Continuum mechanics

Mechanical modelling of a material cannot be achieved at the scale of individual particles 145 such as electrons and atoms, for two reasons. Firstly, the vast number of particles involved 146 would make calculations practically impossible; secondly and more fundamentally, 147 behaviours of a material at the macroscale are emergent phenomena which, despite being 148 caused by collective interactions between particles, cannot be predicted from those 149 interactions (Anderson 1972). A well-developed theoretical framework, which bypasses 150 particle interactions and models a material as an infinitely divisible medium, is that of 151 152 continuum mechanics.

153

154 Every material must obey the 'four fundamental axioms of mechanics', each of which is a *balance equation*, relating the rate of change of a variable – specifically mass, momentum 155 156 (mass \times velocity), angular momentum (mass \times orbital radius squared \times angular velocity), or energy – to the internal and external influences that could cause such a change (Eringen 157 1980). Reaction-diffusion equations are an important type of balance equation, which 158 determine how the concentrations of quantities vary in time and space. In the biological 159 context, many cell growth models involve a reaction-diffusion component which governs the 160 dynamics of certain chemicals (e.g. pollen tube growth, section 3.2). If a continuum model is 161 162 thermodynamic, i.e. involving some flux of heat, then an additional, fifth axiom of mechanics must be obeyed; this is the balance of entropy (Sandler 1999). An entropy balance equation 163 relates the rate of change of disorder in a physical system to heat transferred and/or 164 mechanical work done by the system. In some models of cell wall mechanics, 165 thermodynamic principles are used to determined energetically favourable wall structures 166 (e.g. micromechanics of cell wall construction, section 3.1). 167

168

As well as the universal balance equations, a mechanical model must include *dynamical equations* which are specific to the material. A typical dynamical equation is the *constitutive law*, relating a material's *stress* (internal forces) to its *deformation* (extension) (Astarita and Marrucci 1974, Paolucci 2016). The simplest constitutive law is Hooke's law: force is proportional to extension, with the constant of proportionality denoted by Young's modulus, 174 also known as the spring constant (see figure 2(a)). Some models of tissue-wide growth 175 phenomena involve variations of Hooke's Law (e.g. the shoot apical meristem, section 3.3). 176 In general, a constitutive law describes the mechanical behaviour of the given material (is the 177 material solid/fluid, hard/soft, does it display any directional dependence, etc.). Most balance 178 and dynamical equations are mathematical entities known as *partial differential equations* 179 (PDEs). For a good introduction to PDEs, and examples rooted in real-world problems, we 180 refer the reader to Mattheij et al. (2005).

181

182 Broadly speaking, the two types of material with which we are concerned are solids and *fluids* (more specifically, liquid fluids, as gaseous fluids are beyond the scope of this review). 183 The difference between them is clearly reflected in the constitutive law which, for a solid, 184 relates stress to strain, i.e. the amount of deformation, via a material property known as 185 stiffness; in contrast, the constitutive law for a fluid relates stress to strain-rate, i.e. the 186 velocity at which deformation occurs, via a property of the fluid called viscosity. For an 187 illustration of the various concepts we have introduced, see figure 2. However, the 188 categorisation of materials is far from binary. For instance, viscoelastic materials are 189 190 considered intermediate between solids and fluids, with constitutive laws relating stress to a 191 combination of strain and strain-rate (Dill 2007). Mathematical models of plant growth require a choice of constitutive law appropriate to capture the key behaviour for a given 192 193 system on the time and length scales of interest (for example treating the cell wall as a viscous fluid on a long timescale (see section 3.1). 194

195

196 Stress, strain and strain-rate in a material are represented mathematically by quantities known 197 as *tensors*. A tensor is written as an array of numbers and/or functions, with each entry known as a tensor component. A rank-1 tensor is commonly known as a vector, whilst a 198 199 rank-2 tensor can be represented by a matrix. In 3-dimensional space, a vector has 3 components, and a rank-2 tensor - the most common type in continuum mechanics - has 200 3^2 =9 components. In a stress tensor, each of the 9 components can be interpreted as stress in a 201 particular direction, such as normal stress (due to forces perpendicular to material cross-202 sections) or shear stress (due to forces parallel to material cross-sections). Of particular note 203 204 in plant biomechanics are those stress tensors that display anisotropy, i.e. directional variations. This type of stress is related to the geometry of cells (figure 1). For instance, 205 206 within spherical shapes the stress in the cell wall created by turgor tends to be isotropic (same in every directions) but in elongated cells the stress is anisotropic, which is why cells need to 207

have circumferential cellulose reinforcement to resist the stress. Moreover, stress distribution
depends on morphology, in the sense that areas of reduced stress correspond to elongation
(Kierzkowski and Routier-Kierzkowska 2019, Kierzkowski et al. 2012). A practical, physicsoriented description of generic tensors is contained in Arfken and Weber (1995), while the
technically-minded reader may enjoy the rigorous treatment of tensor algebra in Renteln
(2014) from the perspective of differential geometry. Specificities of stress, strain, strain-rate
and viscosity tensors are excellently elucidated in Spencer (2004).

215

We can roughly sub-categorise solids into elastic (whose deformations are entirely reversible) 216 and plastic (which exhibits irreversible deformation), and fluids into Newtonian ("normal" 217 fluids such as water) and non-Newtonian ("weird" fluids such as custard). For a Newtonian 218 fluid (which is assumed to be incompressible, i.e. with constant density), there is a simple 219 linear relation between stress and strain-rate, with the constant of proportionality being the 220 fluid viscosity, a measure of how much the fluid resists flow (see figure 2(b)). When a non-221 Newtonian fluid is considered, stress and strain-rate may be related by a viscosity tensor, 222 giving a non-linear relationship (Brujan 2011). For a model to describe growth, irreversible 223 deformation must be possible; when a plant cell wall is modelled as a fluid, it is typically 224 225 non-Newtonian (see section 3.1); For solids, there exist numerous types of elasticity and plasticity, each requiring its own model, which Spencer (2004) outlines succinctly. Each 226 227 model involves a strain energy function, which is differentiated to give the stress tensor. When deformations are small, so that a linear relationship between stress and strain is found, 228 229 the solid is said to be linear elastic; for large deformations, the solid is hyperelastic. For examples of hyperelastic models with sophisticated constitutive laws, such as the neo-230 231 Hookean, Mooney-Rivlin, and Ogden models - the latter of which is particularly applicable to biological tissue - the reader is referred to the comprehensive text by Ogden (2013). There is 232 233 a broad literature on the subjects of solids and fluids: Goodier and Hodge (1958) includes a rich catalogue of solid mechanics problems; Parker (2003) is a clear, elementary account of 234 Newtonian fluids; and Brujan (2011) concisely explains the basic concept of non-Newtonian 235 fluids, giving examples of constitutive laws from various well-known models. For an in-236 depth exposition of the vast number of non-Newtonian fluid models that exist, see Bird et al. 237 (1987). 238



Figure 2. Stress, strain, and strain-rate. (a) The stretching and compressing deformation of a spring under an applied force is considered a one-dimensional system. Under the force F, the spring is in equilibrium (held at constant length), having extended from its natural length of L to its deformed length of $L+\Delta x$. To say the spring obeys Hooke's law means that there is some constant k, called the spring's stiffness (or spring constant), such that $F = k \Delta x$. An equilibrium state with twice the force will exhibit twice the extension, halving the force halves the extension, and so on. (b) The strain-rate (velocity) of an (infinitesimal volume of) Newtonian fluid, side-view. If the fluid flow is uniform in the z-direction (out of page), then the system is two-dimensional in (x,y). The bottom plate flows at velocity v while the top place, separated from the bottom by a distance Δy , flows at velocity $v + \Delta v$. The shear stress τ , defined as the shearing force F per unit area of the top plate, is related to Δy and Δv via a constant parameter called the fluid's viscosity μ : τ $=\mu \Delta v/\Delta y$. (c) The strain (deformation) of an elastic solid under applied forces. The force on each surface is normal (perpendicular) to that surface, causing a strain according to a generalised Hooke's law: the vector (F_x, F_y, F_z) is related to the vector $(\Delta x, \Delta y, \Delta z)$ via some stiffness matrix with constant coefficients. If the force on any surface is not normal to that surface, then it will cause a shearing deformation. For example, if the force on the rightmost surface can be resolved into F_x along the x-axis and F_{xy} along the y-axis, then F_{xy} will cause a shear in the x-y plane. Components of the stress tensor are related to the (per-unit-area) forces, F_{x} , F_{y} , F_{z} , F_{xy} , F_{yz} , F_{zx} (at equilibrium, F_{xy} , = F_{yx} etc.). For further mathematical details concerning the stress tensor, as well as the analogous strain tensor and strain-rate tensor, we refer the reader to Spencer (2004).

240 2.2 Asymptotics

PDEs in continuum mechanics models rarely admit exact solutions; in most situations, 241 242 approximate solutions are sought. One of the most commonly used techniques for finding approximate solutions to a PDE is that of *asymptotics*. This method relies on the existence in 243 the system of a dimensionless parameter, say ϵ , whose value is 'small'. For example, ϵ might 244 represent the ratio of cell wall thickness to cell length, or the ratio of some cross-sectional 245 area to surface area. One may then assume that any variable, say T, can be written as a 246 regular asymptotic expansion in the form of $T = T_0 + \epsilon T_1 + \epsilon^2 T_2 + ...$, where T_0 is known as 247 the 0th-order solution and $T_{j>0}$ is known as the jth-order correction. By substituting the 248 asymptotic expansion into the system, one may determine $T_{i\geq 0}$ in succession; including 249 higher-order corrections generally makes the solution more accurate. Steinrück (2010) 250 formalises the general principles of asymptotics that we have described, and provides 251 advanced examples. Further examples, which require the advanced method of matched 252 asymptotics, are presented in Kevorkian (2000). For an interesting historical note on the 253 development of asymptotic methods, we refer the reader to O'Malley (2014). 254

- 255
- 256

257 2.3 Finite elements

While *analytical* methods such as asymptotics are valuable in sufficiently simple systems, 258 more complicated systems may require a *numerical* approach. The method of finite elements 259 is a popular one for solving continuum mechanics models over a finite domain. The basis of 260 the method is to partition the domain, such as the cell wall of a pollen tube, into a number of 261 appropriately defined, usually small, regions called finite elements. One then looks for an 262 263 approximate global solution which is represented within each element by a simple function. When the domain is a plant tissue, cells can be represented as vertices interconnected by 264 edges representing cell walls; these edges are typically modelled as springs with some 265 prescribed mechanical behaviour. This type of finite-element modelling approach is known as 266 267 the vertex element method and will be explained further in section 3.3.

268

In Evans et al. (2000), the reader will find a detailed and practical introduction to finiteelement methods, with worked examples that demonstrate solving some well-known physical problems. Elman et al. (2005) describes fast finite-element algorithms which are suited specifically to equations of fluid mechanics. A recent review by Bidhendia and Geitmann (2018) offers a critical analysis of the use of FE methods in mechanical plant cell modelling,
and advocates the use of FE for various plant growth problems, provided that good modelling
practice is followed.

276

277 3 Models of Growth Mechanics

278

In this section, we summarise the insights into some plant growth scenarios provided by 279 mathematical modelling techniques. Where appropriate, we explain how the models have 280 281 been derived and solved. Beginning with the microscale and cellular aspects of growth in section 3.1, we review how cell wall components hold stress and how they are arranged. In 282 283 section 3.2 we give an overview of models of tip-growing cells, specifically pollen tubes. 284 This is followed by section 3.3, where we look at models on a larger scale, which deal with the mechanical process in morphogenesis, specifically in pavement cells and shoot apical 285 meristem (SAM), and tissue signalling (thus following the flow of figure 1 by starting at the 286 287 top and reading down).

288

We assume in this review that cellulose deposition angle is highly influenced by microtubules; even though it has been shown that this is not necessarily the case, and there is ongoing debate about the regulatory effect of microtubules on cellulose alignment and anisotropy (Baskin 2005, Cosgrove 2014, Baskin 2001). We will expand on this issue in Section 4. We will also be focusing only on the primary cell wall.

294

295 3.1 Cell wall properties and construction

Models of cellular and microscale dynamics within the cell wall may help to resolve the longstanding apparent paradox that the wall is weak enough to yield under turgor, yet strong enough for the cell to remain intact and resist bursting. The models described below incorporate elements of the cell wall microstructure to determine the emergent growth behaviour (see figure 1) and/or macroscale mechanical characteristics

301

It has been a matter of debate as to how cellulose fibres are connected within the cell wall.
This is an important question, as the links between cellulose microfibrils by matrix
polysaccharides determine most of the physical properties of the cell wall (Cosgrove 2005).

305 One early theory was the tethered/sticky network model, which assumed cellulose molecules were joined continuously along their lengths, and peel off as they get increasingly stretched 306 during cell growth (Cosgrove 1993). There has been growing experimental evidence against 307 this theory; for instance the observation that xyloglucan-digesting enzymes (xyloglucan is a 308 309 hemicellulose that is said to crosslink the microfibrils) do not have a significant impact on the strength of the cell wall (Cosgrove 2014). According to a finite-element model featuring a 310 network of cellulose molecules tethered together by hemicellulose via hydrogen bonds, a 311 deformed network is not strong enough to withstand the strain (Yi and Puri 2012). This is 312 313 evidence that the tethered network model is not a feasible explanation as to how the cell wall retains integrity. A plausible alternative theory of cell wall connectivity is the biomechanical 314 hotspot hypothesis, which suggests that wall extensibility is controlled by a limited number of 315 cellulose-cellulose contacts, potentially coordinated by xyloglucan (Cosgrove 2014). One 316 hotspot model considers a network of cellulose connected by hotspots represented as linear 317 springs (Nili et al. 2015). The model implies that a group of short xyloglucan strands is stiffer 318 than a single long strand, and it can produce the requisite wall stiffness to oppose turgor. The 319 hotspot hypothesis also claims that a small amount of degradation of the hotspots could lead 320 321 to the load being carried by pectin, which then enables viscous flow of the cell wall, 322 providing a possible mechanism for growth.

323

324 The micromechanics of cell wall construction allows for controlled creep and determines the ability of the cell wall to withstand turgor pressure, but the exact roles of the different wall 325 326 elements in strength and in wall loosening are still unknown (Park and Cosgrove 2015, Cosgrove 2014, Braybrook et al. 2012). The fibres may play different roles at different states 327 328 of the cell wall. It has been suggested that pre-yield (low strain) dynamics of the cell wall are dominated by hemicellulose fibres stretching and breaking, while post-yield behaviour (high 329 330 strain) is dominated by pectins (Dyson et al. 2012). This was investigated using a fluid mechanical model of the cell wall, considering growth as a fluid flow which drives the 331 stretching/straining of a network of hemicellulose fibres, each represented by a spring with 332 stiffness κ , rest-length L_0 , and evolving length L which is a function of time. These fibres 333 334 connect cellulose molecules, with a breakage rate depending on the current strain. The stress resultant, Σ (essentially the axial tension), of the cell wall is found by summing (integrating) 335 the effect of all bonds across the wall thickness, giving 336

337
$$\Sigma = \int_0^n n\kappa (L - L_0) \,\mathrm{d}y + \frac{\alpha}{\phi_M},\tag{2}$$

where h is the thickness of the cell wall, n the density of hemicellulose bonds, y the 338 coordinate across the wall thickness, α the strain (growth) rate, and α/ϕ_M represents the 339 contribution of pectin. The concentration of fibres might also affect wall strength. By re-340 deriving the Lockhart equation from thermodynamic principles, it can be shown that the cell 341 wall yield is primarily determined by the concentration of xyloglucans and cellulose, and not 342 the bonds between them (Veytsman and Cosgrove 1998). Even though this model considers a 343 small time-scale on which material deposition is negligible, wall yield depends on xyloglucan 344 concentration which in turn is determined by wall deposition. This implies an influential role 345 of deposition in wall loosening and consequently in growth. The flexibility of the fibres also 346 347 influences cell wall stress because hemicellulose could be trapped within the cellulose fibres. However, interactions with pectin are not incorporated within this framework. 348

349

The CMF is said to have a highly regulatory effect on extensibility and maintaining cell 350 351 shape, but the detailed consequences of reorientation, distribution and crosslinking during growth are missing (Anderson et al 2010). Using linear elasticity under imposed turgor to 352 353 examine the impact of CMF orientation, it has been found that it affects the radial elastic deformation at the ends of the cell, but that the presence of CMF on the cell end plates makes 354 little difference to the cell's axial expansion (Ptashnyk and Seguin 2016). The model predicts 355 356 that shifting the positions of the cells out of alignment in the tissue (i.e. lined up like bricks in a wall) allows for larger strains and increases the effect of varying microfibril configurations 357 on axial expansion. A variety of orientations throughout the wall also reduces axial expansion 358 and slightly increases radial growth. Another model has also found that the cell radius is 359 maintained via the CMF (Dyson and Jensen 2010). Representing the cell wall as a fibre-360 reinforced thin sheet of viscous fluid, this model includes fibres (representing the CMF) 361 which are convected by growth, and is analysed and solved using asymptotics. The model 362 also finds that a variety of fundamental geometric and mechanical parameters related to the 363 composite cell wall properties govern the cell wall extensibility. 364

365

Efforts have been made to understand pectins' regulatory effects on growth. Pectins are known to form hydrated gels that can force microfibrils apart, allowing for wall extensibility to increase and the microfibrils to slip (Cosgrove 2005). Deposition of pectin is also said to play a role, although its significance is still not completely understood. Using thermodynamic constitutive laws which involve turgor, temperature, volume, free and bound pectate, and the synthesis of pectates, a growth rate that principally depend on pectate crosslink synthesis can

be derived (Barbacci et al. 2013) (see figure 3). The comparison of this model's predictions 372 with data concerning Chara corallina is "quite good". Meanwhile, by balancing cell wall 373 growth rate and pectin insertion rate, it has been argued that turgor-driven deposition leads to 374 cell wall polymerisation (see figure 3), which is a primary growth control mechanism (Ali 375 and Traas 2016). The insertion rate in the model is derived from the thermodynamics 376 principle of balancing the free energy difference between bound and unbound pectin states. 377 Although the results match data qualitatively to *Chara corallina*, the authors acknowledge 378 that other mechanisms are at play. Both of the pectin studies consider Chara corallina which 379 380 contain a high amount of pectin, so these results may not be generalisable. It seems that hemicellulose connections do influence the yield threshold of growth, and these studies 381 382 emphasise the role of pectins insertion in the viscous flow of the cell wall.

383

There are still remaining mysteries in cellular and microscale growth. For instance, despite 384 growing evidence for the biomechanical hotspot hypothesis as we have described, there is no 385 universal consensus on how the cell wall polymers are connected. Moreover, most studies 386 heavily rely on xyloglucan being the load-bearing component in the cell wall, but it has been 387 noted that xyloglucan possibly covers only a small portion of cellulose surface in the onion 388 389 wall (Zheng et al. 2018), and that Arabidopsis mutants containing small amounts of xyloglucan have only minor changes in growth phenotype (Cosgrove 2016). The exact role of 390 391 pectin in the cell wall structure has also not been carefully investigated. All of these are prerequisites for understanding how the cell wall expands. There has also not been substantial 392 393 work on the action of enzymes. Some models have shown that in order to match experimental data, expansin must affect extensibility (Pietruszka 2011). Apart from this, not much work 394 395 has been done to model how enzymes manipulate the cell wall to cause wall relaxation, strengthening, etc., and we will expand on this point in section 4. 396

397

398 3.2 Tip-growing cells

There are a number of cells such as pollen tubes and root hairs which extend via tip growth, where growth is highly localised to one area of the cell wall. Growth rates are typically very high, despite turgor pressures similar to other cell types (Beauzamy et al. 2014). Tight control of both mechanical properties and new material deposition is therefore required, and modelling can help understand these coupled processes (Geitmann and Emons 2000). Here we review tip growth in pollen tubes. 405

Pollen tubes are of particular interest due to their high growth rate (1cm/h) (Bove et al. 2008) 406 407 and their oscillatory growth patterns (Kroeger and Geitmann 2012), but their growth is as yet not fully understood. To model pollen tube growth, one needs to couple cell mechanics to 408 409 biochemical processes (Cameron and Geitmann 2018). Measurements of turgor do not show significant oscillations, implying that it is likely not a driving mechanism behind growth 410 oscillations (Beauzamy et al. 2014). One possible explanation for oscillatory growth is a 411 vesicle recycling mechanism dependant on calcium concentration, where the fusion of 412 413 vesicles at the apex is stimulated by calcium ions (Kroeger et al. 2008). In this framework, the pollen tube invading the external media is modelled as one viscous fluid being injected 414 into another, surrounded by a viscoelastic membrane representing the wall. The overall 415 416 growth velocity is calculated via Darcy's law of pressure-driven fluid flow

417

$$\mathbf{u} = \frac{\kappa}{\mu} \nabla p , \qquad (3)$$

418 where \boldsymbol{u} is the tip apex velocity, K denotes the permeability of the external medium, μ the viscosity of the cell, p is the pressure, and ∇p gives the magnitude and direction of the 419 420 pressure gradient. The idea behind this model is that the rate of flow/growth is proportional to 421 the difference in pressure between two different regions (there is higher pressure in the cell 422 due to turgor which drives the flow outwards), similar to diffusion. An effective elastic 423 constant is incorporated into the fluid flow/growth rate, which in turn is dependent on calcium concentration at the cell walls (see figure 3). The calcium exocytosis rate on the cell 424 425 wall is determined from a reaction-diffusion equation. These results agree qualitatively with experimental observations, with some discrepancies due to calcium absorption by the cell 426 427 wall being neglected; this suggests that the assumption of calcium dynamics driving vesicle

428 recycling could be robust. The pollen tube growth phenomenon of pearled morphology 429 (oscillations in diameter to form a wavy boundary) is also not understood. A possible 430 explanation is that it occurs as a result of the extension and deposition rates being out of phase (Rojas et al. 2011). Employing a principle that deposition causes crosslink turnover, 431 this model considers a computational lattice network of nodes which are connected if there is 432 a crosslink (modelled as linear springs), incorporating both esterified and de-esterified pectin 433 (see figure 3). The model predicts both steady and oscillatory growth, agreeing with 434 experimental data. Alternative morphologies have been observed in pollen tubes consisting of 435 436 swelling or tapering of the pollen tube head; unlike the causes above which both relate to deposition, other mechanisms could be at play here. One model claims that the swelling 437

arises due to an insufficiently steep decrease in Young's modulus along the growth direction
(Fayant et al. 2010). This claim is validated by the distribution of de-esterified (stiffer) pectin.
The model also suggests that cellulose are important in resisting radial expansion, despite the
randomness of their orientations. In conclusion, the model posits that the features affecting
growth are spatially-varying components of the cell wall.



Figure 3: Pollen tube and pectin driven growth model principles. Kroeger et al. (2008) predict that the pressure gradient and calcium ion concentration both effect growth, **u** (equation 3) with the former determining the direction and latter affecting the elasticity constant, λ which in turn affects the extension rate (labelled [1]). Rojas et al. (2011) assume the deposition of pectin with rate D causes crosslink turnover, leading to extension in length from $L^{(1)}$ to $L^{(2)}$ with rate R (labelled [2]). Note diagram depicts only demethylesterified pectin. This pectin deposition driven growth framework also demonstrates the modelling ideas behind Ali and Traas (2016) and Barbacci et al. (2013) in *Chara corallina* with the former model using turgor driven extension of the wall (denoted as *f*), driving further polymerisation (labelled [3]).

There remain unexplored territories within pollen tube modelling. Most models assume axisymmetric growth with a straight centreline (in order to reduce complexity and computational time), and therefore are unsuited for investigating complex mechanisms such as steering, which to our knowledge has not been explicitly modelled. Regarding oscillatory growth, there is evidence that calcium-ion oscillations occur in non-growing pollen tubes, showing it could be independent of growth (Cameron and Geitmann 2018), motivating

- 452 further work in this area.
- 453

454 3.3 Models of tissue growth

We turn our attention to larger-scale models which are evaluated across tissues, where 455 mechanics influences both shape and growth (see figure 1). Plant cells are tightly fixed to 456 457 each other, therefore growth and morphogenesis arise due to collaborative expansion, cell division and communication between cells (Cosgrove 2005, Hamant and Haswell 2017, 458 Mirabet et al. 2011). For example, careful control of organ growth is observed in leaves with 459 a reduced cell number, which still reach normal size by increasing the rate or duration of their 460 cell expansion, demonstrating shape-sensing mechanisms (Hervieux et al. 2016). In this 461 section, we review mathematical insights into problems posed by pavement cells, shoot apical 462 463 meristems (SAM) and stress signalling.

464



Figure 4: The concept of a shell model approximating a 3D tissue as a thin shell of epidermis inflated by pressure from the inner tissue. This significantly reduces computational time. The square objects on the shell represent a simple example mesh and do not correspond to cell walls.

- 465 We first explain the concept of a shell model. This is a 2D representation, a simplification of
- the 3D tissue whereby only the outer epidermal layer is explicitly modelled, and tension from
- the inner layers is imposed (see figure 4). The epidermis bears higher resistance to the tension, as demonstrated experimentally. For example, a peeled, isolated epidermis will contract, showing that it is under high stress (Savaldi-Goldstein et al. 2007, Hamant and

470 Haswell 2017, Beauzamy et al. 2015). Since the outer epidermis heavily restricts plant471 growth, the 2D shell can be a realistic assumption.

472

The diversity of shapes of epidermal cells in leaves (pavement cells) is truly spectacular. 473 474 Some form highly undulating anticlinal walls, as in Arabidopsis, which lead to jigsaw-like patterns of cells (Vőfély et al. 2018). Mechanical stress is said to play an important role in 475 determining these cells' geometry, but why and how the jigsaw patterns form has not been 476 elucidated (Sapala et al. 2019). Here we shall refer to the indents and protrusions of a 477 478 pavement cell as necks and lobes, respectively. Stress is found to be higher in the neck regions, by extracting 3D cell shapes using MorphoGraphX (de Reuille et al. 2015) and 479 meshing the resulting surface, then incorporating fibre directions in a hyperelastic model 480 (Sampathkumar et al. 2014). Moreover, by considering idealised ellipsoidal cells with or 481 without protrusions, stress is found to transfer from the centres of cells to the neck regions, 482 consequently reducing the overall stress (Sapala et al. 2018). Since the spongy mesophyll 483 layer, which lies underneath the epidermis, contains air holes, it might not be able to provide 484 strength to the tissue, therefore the epidermis must withstand most of the total stress. Thus, 485 the stress reduction provided by the jigsaw morphology could be advantageous, as it could 486 487 help reduce the resources needed to strengthen the tissue and/or reduce the chance of the tissue rupturing. As for how the jigsaw patterns come about, it has been shown that 488 489 microtubules align with the direction of maximal stress, which can then reinforce the necks through the deposition of cellulose (Sampathkumar et al. 2014). The cellulose can then 490 491 restrict expansion at the necks, which in turn increases the stress, creating a feedback loop where microtubules continue to align with the stress. This is a possible mechanism for how 492 493 the lobes become more prominent and enlarge, but it still does not answer how the lobes *initially* form. To that end, a positive relationship between isotropic growth and 'lobeyness' 494 495 has been proposed (Sapala et al. 2018). The model is validated by a 2D simulation of cells, where the walls are represented as nodes connected by linear springs (figure 6 - see 496 description later in this section), with additional intra-cell springs to represent stiffening 497 components such as cellulose. 498

499

It has also been suggested that lobe formation is a result of wall heterogeneities which cause buckling when the leaf epidermis is under tension from variations in growth rates across different cell layers (Majda et al. 2017). However, when this 2D modelling approach of only including the anticlinal walls (wall perpendicular to leaf surface) was recreated, the lobe 504 amplitude was not as significant; including the effect of the periclinal walls (wall parallel to leaf surface) the lobes are found to disappear (Bidhendi and Geitmann 2019). An alternative 505 model including the periclinal wall was developed using finite element methods assuming the 506 cell wall is neo-Hookean hyperelastic (Bidhendi et al. 2019). Upon analysis it was found that 507 varying periclinal wall stiffness between neighbouring cells can induce lobe formation; a 508 potential buckling mechanism is proposed due to cell geometry and internal pressure. 509 Reduction of stress is therefore most likely a factor in why pavement cells form these 510 interesting geometries but the origins of how they form could be connected with periclinal 511 512 wall mechanics and possible buckling.

513

The shoot apical meristem (SAM) has often been of interest to plant biologists, due to its 514 complex morphology of forming organ primordia, and because of its importance in leaf and 515 floral meristems and stem development (Soyars et al. 2016). The SAM must maintain a 516 source of undifferentiated cells, and differentiate cells in order to initiate organogenesis. This 517 requires strict co-ordination and structure, which are not yet understood (Truskina and 518 Vernoux 2018). The SAM's elastic and plastic properties which enable bulge initiation are 519 520 also a mystery. There surely must be varying properties in the region to allow for the 521 different growth rates in the shoot apex central zone and periphery. It has been proposed that the slow-growing area at the shoot tip is significantly strain-stiffened, and this may control 522 523 the expansion process of the tip (Kierzkowski et al. 2012) (see figure 5). This model concludes that the difference between tip and peripheral growth rates is not due to variations 524 525 in stress, but instead due to variations in other parameters such as yield threshold. In terms of 526 methodology, the model approximates the surface as a shell of Ogden hyperelastic material 527 (cf. section 2 and figure 4) with two regions of differing elastic properties: the shell tip where stiffness increases with strain, and the periphery where stiffness is constant. Validation of the 528 529 model is provided by reproducing material behaviour from osmotic treatment experiments. Differing regions of elastic properties are also echoed by another model, which tests different 530 mechanisms of organ emergence (Boudon et al. 2015). It finds that a bulge (similarly to the 531 above) could be produced by changing the stiffness of the outer cell layers near the bulge tip, 532 but not through variations in turgor or wall stiffness from interior layers. By creating a highly 533 rigid ring around the bulge of cells, to promote further growth, the model produces a more 534 distinct bump (see figure 5). This is a 3D model of tissue growth which includes gene 535 regulation, and a generalised Lockhart equation relating the plastic deformation tensor to the 536 elastic strain tensor. Both of the SAM models we have discussed illustrate the necessary 537

mechanical features to allow bulge initiation and maintain SAM morphogenesis, namely: in
order to adapt the stiffness properties, cell wall properties of the surrounding cells vary with
the distance from the tip of the initiation site.

541

542 Further on the topic of SAM, it has been noted that isotropic walls grow slower than anisotropic walls (Armezzani et al. 2018). This confused the understanding of emerging 543 primordia, as their microtubules are in an isotropic setup while still growing faster than the 544 surrounding meristem (Sassi et al. 2014). There also seem to be no change in cellulose 545 546 deposition to alter the strength of the wall. Therefore there is likely to be some kind of signalling to promote wall loosening (Kierzkowski and Routier-Kierzkowska 2019). Through 547 modelling and experimentation, evidence has been presented that microtubule re-organisation 548 to an isotropic distribution can activate wall loosening genes (and vice versa), allowing organ 549 550 emergence independently of auxin (Armezzani et al. 2018) (see figure 5). The model makes use of a 2D shell based on Hooke's law, which incorporates fibre orientation and plastic 551 552 spring growth (figure 4 and 6). The signalling to genes is an essential part, without which isotropic walls in an anisotropic environment are unable to increase growth rates in organ 553 outgrowth. These models all inform us that organ emergence requires not only differential 554 555 mechanical properties from the tip to the periphery, but also a coupling between genes and the degree of anisotropy. 556

557

The thick, relatively stiff cell walls of the epidermis have been postulated to regulate stem 558 559 growth via intricate interactions between cells and whole tissues (Baskin and Jensen 2013). It was found that the tissue structure is stabilised by the outer layer's strain stiffening 560 561 behaviour, without which the tissue could buckle (Vandiver and Goriely 2008). This model was developed by creating a cylindrical model of the stem, consisting of two material layers 562 with different properties. They defined a deformation gradient as the product of a growth 563 tensor, which governs the unrestricted growth of both layers, and an elastic deformation 564 tensor, which couples the layers together. To determine the growth and bending rate of the 565 composite Arabidopsis root, a model assigns a yield threshold, wall viscosity and thickness to 566 each individual cell wall segment, varying across cell files (Dyson et al. 2014). This was 567 integrated over the cross section to obtain a tissue-wide Lockhart equation (c.f. eq. (1)). 568 Parametrising with experimental data such as wall thickness and turgor values, they found 569 570 that the epidermis plays a dominant (6-fold influence) role in regulating extension and described the effectiveness of targeting this layer to cause root bending. Both papers therefore 571

572 demonstrate the absolute mechanical importance of the epidermis in regulating and 573 stabilising tissues.



574

Figure 5: The requisite shoot apical meristem features for growth and organ emergence. Kierzkowski et al. (2012) demonstrated that in the slow growing apex, region A, there is strain stiffening behaviour and the faster growing periphery, region B, displayed linear behaviour (see (a)). This is depicted in (b) where the dotted line shows the strain at typical levels of turgor. The blue/red line depicts behaviour from region A/B showing the stress increasing rapidly/linearly demonstrating strain hardening in the former. The conditions for organ emergence was modelled by Boudon et al. (2015) who showed reducing the rigidity of the cells in region D allowed for organ emergence site and the creation of a rigid ring of cells in region E around the site can create a distinct bulge emergence. They also note that increased pressure from the bottom layers (region F) does not aid in organ emergence. Armezzani et al. (2018) also predict the necessary isotropic setup of microtubules which signals appropriate wall loosening genes in order for bulge initiation (region D).

Stress patterning can be crucial in tissue-wide signalling, as there is increasing evidence that 575 576 cells can sense these mechanical forces, and that microtubules play a fundamental role in this phenomenon (Hamant and Haswell 2017). Evidence for this claim comes from observations 577 that microtubules align with the stress direction, which allows for the deposition of cellulose 578 to reinforce the cell in the principle direction of stress (Bozorg et al. 2014). Indeed this paper 579 also shows that the microfibril direction is aligned with maximal stress direction and 580 perpendicular to maximal strain direction in the SAM. Using mathematical modelling, one 581 582 can directly approximate the distribution of stress and then compare it with the organisation of the microtubules (Mirabet et al. 2011). Moreover, by examining stress-feedback and the 583 584 microtubule patterning at root hair initiation sites, it has been found that circumferential principal stresses around the loosened region lead to radial star configurations of 585

microtubules (Krupinski et al. 2016). More evidence is found in sepals where microtubules have been shown to align with maximal tension in sepals (Hervieux et al. 2016). At the sepal tip where growth slows down, microtubules are orientated in a setup that corresponds to fast anisotropic growth. The model includes a generalised Hooke's law, with a Young's modulus that increases from the base to the tip, and is solved by finite-element methods. Comparing this model with experiments leads to the hypothesis that microtubule stress feedback operates as a shape sensor at the tip and resists further radial expansion.

593 This microtubule function has also been found in the *Arabidopsis* shoot apex, whose shape is 594 theorised to depend on the microtubule cytoskeleton, which is regulated in turn by the 595 mechanical stress in a feedback loop (Hamant et al. 2008). The study combines experimental 596 work, including fluorescent marking of microtubules and tissue imaging, with a shell model 597 (see figure 4) that includes growing elastic walls elements, proliferation and anisotropy. The 598 model defines the potential energy in the shell as

599
$$U = \sum_{w \in walls} \frac{k_w}{2} \left(\frac{l_w - l_w^0}{l_w}\right)^2 - \sum_{c \in cells} P_c A_c - \sum_{c \in cells} P_{c,int} V_{c,int}, \qquad (4)$$

where Σ denotes summation of the wall elements, $w \in walls$ (e.g. segment AC in figure 6) or 600 cells, $c \in cells$ (e.g. the square ABCD in figure 6). The first term is the contribution of the 601 wall element elastically stretching, where anisotropy is included in the stiffness k_w of the w^{th} 602 wall element (this term increases when the w^{th} wall element aligns more closely with a 603 defined direction of microtubules), which also changes due to stress feedback (microtubule 604 directions are updated). Plastic spring growth is incorporated by increasing l_w^0 . The second 605 term in eq. (4) is the force from the internal pressure between cells in the simulated 2D shell 606 607 layer (see figure 4), and the third is pressure emerging from the inner tissues including dependence on both area, Ac and volume, V(c,int). Models are often written in terms of the 608 609 energy, because to stretch fibres (elastic deformation), break bonds and/or allow fibres to slip past each other (viscous/plastic deformation), we require energy. The energy comes from the 610 611 action of turgor pressure pushing against the walls in the normal direction. There must then 612 be a balance between turgor and wall stretching/yielding (figure 6). This is essentially what is



Figure 6: Principles of the vertex element method, plastic spring growth and mechanical energy balance/minimisation. A network of vertices (A,B,C,D) are attached via elements (AB,BD,CD,CA) which are represented as springs. In particular, A, B, C are outside elements with D on the inside. This simplistic system demonstrates the modelling of cell wall extension via plastic spring growth, with dynamically increasing resting lengths $l_0^{(1)}$ and $l_0^{(2)}$ (thus permanently extending the spring). Here the elements AC and BD have the same length *l* but AC has higher stress because its resting length $l_0^{(2)}$ is shorter than the BD resting length $l_0^{(1)}$ (so AC has undergone larger strain i.e. the difference between the resting length $l_0^{(2)}$ and the actual length *l*). In other words, given the same stress, BD can stretch more than AC. Plastic spring growth allows the spring to stretch further under the same stress (in practice, models usually impose constant turgor *T* and hence constant stress, rather than equal *l*; the element BD would consequently stretch further than depicted). Energy balance is also at play, as turgor *T* pushing the outside elements causes the springs to strain (the inside elements, e.g. D, are pushed from all sides equally). The stretch produces an elastic force which opposes the turgor. The position of the vertices are ones that balance the turgor forces and elastic forces.

written in eq. (4), where the pressure is balanced with the effect of stretching and plastic growth. The energy should then be minimised, as physical systems will always favour a state that requires the least energy (e.g. a ball in a bowl prefers to stay in the bottom where its gravitational potential energy is minimised). This model is able to replicate observed microtubule orientations in different experimental scenarios, for example primordium growth. These studies show that, firstly, microtubules are highly important in stress sensing, and secondly, they have regulatory effects on the geometry of a tissue. 621 The models we have described in this section suggest a fundamental role of mechanical cues in tissue mechanics, provide evidence that the interplay between mechanics and signalling is 622 key to determining observed behaviour, and support the view that genetic regulation alone 623 cannot account for observed phenotypes (Bassel et al. 2014). Questions remain, for example 624 the effect of spatial variations of mechanical properties on cell behaviour, specifically in the 625 SAM, is not yet understood (Truskina and Vernoux 2018). It has also been noted that the 626 feedback loop between microtubules and stress cannot always completely explain how cells 627 develop such a complex geometry in the first place (Kierzkowski and Routier-Kierzkowska 628 629 2019). Tissue stress origins are also still elusive (Baskin and Jensen 2013). Moreover, the complex relationship between anisotropies and microtubules has not been fully investigated; 630 631 we will discuss further in section 4.

- 632
- 633

634 4 Outlook

635

Whilst there have been many success stories where mechanical models have had significant
impact on our understanding of plant growth, there are still many exciting future directions
for mathematicians and biologists alike to explore.

639

The roles and mechanisms of enzyme and protein action in wall loosening have not been 640 641 fully understood (Cosgrove 2016). Expansins are an important group of non-enzymatic proteins that cause wall stress relaxation, enabling cell wall creep. How they interact with the 642 linkages between microfibrils is unknown. For example, α -expansins have the apparently 643 contradicting effects of inducing creep while maintaining wall strength (Yuan et al. 2001, 644 Wang et al. 2008). Xyloglucan endotransglucosylase/hydrolase (XTH) also affects the cell 645 wall but its action of cutting and rejoining xyloglucans does not necessarily cause an increase 646 in extensibility of the wall (Cosgrove 2016). It also does not seem to affect growth in plants 647 where XTH expression is suppressed (Cosgrove 2005). It could be interesting to explore how 648 expansin/XTH function relates to cell wall structure, as there is the possibility that expansin 649 can cut biomechanical hotspots, that XTH may be ineffective due to a possible inability to 650 access the xyloglucan, or that XTH could control elongation or strengthening by affecting 651 xyloglucan length (Cosgrove 2005, 2014). Endoglucanase expression is also said to have 652 potential to cause wall loosening (Cosgrove 2014). The interactions between different pectin 653

654 methylesterases (PMEs) are still elusive and it has been proposed that unlocking PME action 655 could help examine pectin's role in the cell wall (Levesque-Tremblay et al. 2015). Models 656 similar to those in section 3.1 might be helpful, where one could examine not just which 657 molecules hold the stress but test possible wall-loosening mechanisms (e.g. expansins 658 targeting hotspots) against experiments.

659

Pectins in the cell wall have not been thoroughly investigated, despite their making up over 660 30% of the primary cell walls in most higher plants (Levesque-Tremblay et al. 2015). 661 662 Although their role has been considered in pollen tubes (Rojas et al. 2011, Fayant et al. 2010), in other models their effect has been included as a generic isotropic term. This 663 approach could give an accurate description of pectin's effect (Huang et al. 2015), however it 664 neglects the potential influence of pectin-cellulose interactions, or that de-methylesterified 665 pectin could affect the porosity of cellulose-xyloglucan networks, thereby influencing 666 enzyme action (Cosgrove 2016). Inhibition of PME activity is known to prevent the 667 formation of primordia at the meristem, showing that pectins influence wall extensibility, and 668 that their spatial regulation of methylated and demethylated aids morphogenesis (Höfte et al. 669 670 2012, Braybrook et al. 2012, Braybrook and Peaucelle 2013) with pectin asymmetry in the 671 hypocotyl epidermis shown to aid anisotropy (Bou Daher et al. 2018). Moreover, even though de-esterified pectin is found in vitro to be stiffer than methyl-esterified pectin, regions of de-672 673 esterified pectin can give rise to softer cell walls in the meristem, which is an as-yet unexplained phenomenon (Cosgrove 2016). 674

675

676 There is evidence that microtubules are highly influential in anisotropy/morphogenesis (see 677 section 3.3), although this relationship is in no way straightforward. It has been found that cellulose fibres in the outer epidermal wall of most stems are orientated axially (Baskin and 678 679 Jensen 2013), and that anisotropic tissues are not necessarily made up of anisotropic cells (Bou Daher et al. 2018). A related question is how cellulose orientation is decided, because 680 the influence of microtubules on cellulose direction is questionable in some situations 681 (Cosgrove 2014, Bou Daher et al. 2018). Cellulose can also passively re-orientate as the cell 682 grows (Anderson et al. 2010), and although cells may have a net orientation of cellulose 683 fibres, they are deposited in a variety of orientations between lamella layers in the cell walls 684 (Zhang et al. 2016). These features are not often included in models. By incorporating the 685 686 differences between cells into tissue expansion models, we might be able to identify the possible origins of tissue stress. In addition, models could test different possible mechanismsfor cellulose alignment and tissue anisotropy.

689

Models which exploit the geometry of growing plant tissues, for example using shell theory, 690 691 lead to significant reductions in complexity, giving more tractable models and interpretable results. However, some features are often neglected. For example, shell models often assume 692 a straight centreline and thus cannot be used to model steering, twisting or bending. 693 Similarly, emergent anisotropy arising as a tissue-wide phenomenon is often neglected. 694 695 Indeed, internal layers do contribute to morphogenesis such as the vasculature (Hamant and Haswell 2017). There is therefore a growing need to progress from 2D to 3D models 696 697 (Kroeger et al. 2008, Fozard et al. 2013).

698

Finite-element models can become unstable and less accurate when simulated cell growth causes the elements to increase in length (Fozard et al. 2013). This requires the system to be re-meshed, which can be computationally expensive (Chickarmane et al. 2010). It has also been pointed out that the implementation of finite-element methods in iterations implies that growth occurs in small discrete steps (Fayant et al. 2010). Depending on the number of nodes, this could lead to subtle shape changes, even though a fine mesh density could possibly ensure the errors are small.

706

707

708 5 Summary

709

In this review we have demonstrated that mathematical models can help unravel mysteries of how the cell wall structure allows controlled growth, what causes intricate tissue morphologies and how stress feedback works across whole tissues. For mathematicians, plant biomechanics is a truly exciting field in which great opportunities beckon, with numerous fascinating questions and opportunities for impactful work.

715

Some of the best models have resulted from multidisciplinary collaborations between mathematicians and biologists, which allow modelling and experimentation to be adapted to each other in real time. It is therefore vital for the progress of the field that mathematicians and biologists work together to produce models which are experimentally verifiable, can explain biological phenomena mechanistically, and can raise important new questions aboutthe elusive nature of plant biology.

- 722
- 723

724 6 Acknowledgments

725

We would like to thank Joanna Chustecki, James Tyrrell and Clare Ziegler for their helpful
inputs. RJD and ETS thank EPSRC for funding via grants EP/M0015X/1 and EP/N509590/1.
ETS thanks the QJMAM fund for facilitating attendance at the Plant Biomechanics
Conference 2018. JL thanks the University of Birmingham for Postdoctoral Fellowship
funding. We would also like to thank the reviewers for their constructive feedback.

731

732 **REFERENCES**

- Ali O, Mirabet V, Godin C, Traas J. 2014. Physical models of plant development. Annual
- Review of Cell and Developmental Biology **30**, 59–78.
- 735 Ali O, Traas J. 2016. Force-driven polymerization and turgor-induced wall expansion.
- 736 Trends in Plant Science **21**, 398–409.
- Anderson CT, Carroll A, Akhmetova L, Somerville C. 2010. Real-time imaging of
 cellulose reorientation during cell wall expansion in *Arabidopsis* roots. Plant Physiology 152,
 787–796.
- 740 Anderson P. 1972. More is different. Science 177, 393–396.
- 741 Arfken G, Weber H. 1995. Mathematical Methods for Physicists. Ch. 2, pp. 126-154, 4th
- 742 edn, Academic Press, London.
- 743 Armezzani A, Abad U, Ali O, et al. 2018. Transcriptional induction of cell wall remodelling
- genes is coupled to microtubule-driven growth isotropy at the shoot apex in Arabidopsis.
- 745 Development doi: 10.1242/dev.162255
- 746 Astarita G, Marrucci G. 1974. Principles of Non-Newtonian Fluid Mechanics. Ch. 1, pp. 1-
- 747 41, McGraw-Hill, Maidenhead.
- 748 Barbacci, A, Lahaye M, Magnenet. 2013. Another brick in the cell wall: biosynthesis
- dependent growth model, PLOS ONE doi: 10.1371/journal.pone.0074400
- 750 Baskin TI. 2001. On the alignment of cellulose microfibrils by cortical microtubules: a
- review and a model. Protoplasma **215**, 150–171.

- Baskin TI. 2005. Anisotropic expansion of the plant cell wall. Annual Review of Cell and
 Developmental Biology 21, 203–222.
- **Baskin TI, Jensen OE**. 2013. On the role of stress anisotropy in the growth of stems. Journal

of Experimental Botany **64**, 4697–4707.

756 Bassel GW, Stamm P, Mosca G, de Reuille PB, Gibbs DJ, Winter R, Janka A,

Holdsworth MJ, Smith RS. 2014. Mechanical constraints imposed by 3d cellular geometry

and arrangement modulate growth patterns in the Arabidopsis embryo. Proceedings of the

- 759 National Academy of Sciences doi: 10.1073/pnas.1404616111
- 760 Beauzamy L, Louveaux M, Hamant O, Boudaoud A. 2015. Mechanically, the shoot apical
- 761 meristem of *Arabidopsis* behaves like a shell inflated by a pressure of about 1 mpa. Frontiers
- 762 in Plant Science doi: 10.3389/fpls.2015.01038
- Beauzamy L, Nakayama N, Boudaoud A. 2014. Flowers under pressure: ins and outs of
 turgor regulation in development. Annals of Botany 114, 1517–1533.
- 765 Bidhendia AJ, Geitmann A. 2018. finite element modeling of shape changes in plant cells.
- 766 Plant Physiology doi: 10.1104/pp.17.01684
- **Bidhendi A, Geitmann A.** 2019. Geometrical details matter for mechanical modeling of cell
- 768 morphogenesis. Developmental Cell, in press.
- 769 Bidhendi A, Geitmann A. 2019. Methods to quantify primary plant cell wall mechanics.
- 770 Journal of Experimental Botany, in press.
- 771 Bidhendi, AJ, Altartouri B, Gosselin F, Geitmann A. 2019. Mechanical stress initiates and
- sustains the morphogenesis of wavy leaf epidermal cells. bioRxiv doi: doi:
 https://doi.org/10.1101/563403
- **Bird R, Armstrong R, Hassager O**. 1987. Dynamics of Polymeric Liquids. Volume 1: Fluid
- 775 Mechanics, Ch. 4, pp. 169-236, 2nd edn, John Wiley and Sons, New York.
- 776 Boudon F, Chopard J, Ali O, Gilles B, Hamant O, Boudaoud A, Traas J, Godin C. 2015.
- A computational framework for 3d mechanical modeling of plant morphogenesis with
 cellular resolution. PLOS Computational Biology doi: 10.1371/journal.pcbi.1003950
- Bove J, Vaillancourt B, Kroeger J, Hepler PK, Wiseman PW, Geitmann A. 2008.
 Magnitude and direction of vesicle dynamics in growing pollen tubes using spatiotemporal
 image correlation spectroscopy and fluorescence recovery after photobleaching. Plant
 Physiology 147, 1646–1658.
- Bozorg B, Krupinski P, Jönsson H. 2014. Stress and strain provide positional and
 directional cues in development. PLOS Computational Biology doi:
 10.1371/journal.pcbi.1003410

- Braybrook SA, Hofte H, Peaucelle A. 2012. Probing the mechanical contributions of the
 pectin matrix: insights for cell growth. Plant Signaling & Behavior 7, 1037–1041.
- 788 Braybrook SA, Peaucelle A. 2013. Mechano-chemical aspects of organ formation in
- 789 Arabidopsis thaliana: the relationship between auxin and pectin. PLOS ONE doi:

790 10.1371/journal.pone.0057813

- 791 Bruce DM. 2003. Mathematical modelling of the cellular mechanics of plants. Philosophical
- 792 Transactions of the Royal Society of London B: Biological Sciences **358**, 1437–1444.
- 793 Brujan EA. 2011. Cavitation in Non-Newtonian Fluids: with Biomedical and Bioengineering
- Applications. Ch. 1, pp. 1-43, Springer, Heidelberg.
- 795 Cameron C, Geitmann A. 2018. Cell mechanics of pollen tube growth. Current Opinion in
- 796 Genetics & Development **51**, 11–17.
- 797 Chebli Y, Geitmann A. 2007. Mechanical principles governing pollen tube growth.
 798 Functional Plant Science and Biotechnology 1, 232–245.
- 799 Chickarmane V, Roeder AH, Tarr PT, Cunha A, Tobin C, Meyerowitz EM. 2010.
- 800 Computational morphodynamics: a modeling framework to understand plant growth. Annual
- 801 Review of Plant Biology **61**, 65–87.
- 802 Cosgrove DJ. 1993. How do plant cell wall extend? Plant Physiology 102, 1–6.
- 803 Cosgrove DJ. 2005. Growth of the plant cell wall. Nature Reviews Molecular Cell Biology
 804 6, 850–861.
- 805 **Cosgrove DJ**. 2014. Re-constructing our models of cellulose and primary cell wall assembly.
- 806 Current Opinion in Plant Biology 22, 122–131.
- 807 Cosgrove DJ. 2016. Catalysts of plant cell wall loosening. F1000Research doi:
 808 10.12688/f1000research.7180.1
- 809 Bou Daher F, Chen Y, Bozorg B, Clough JH, Jönsson H, Braybrook SA. 2018.
- 810 Anisotropic growth is achieved through the additive mechanical effect of material anisotropy

and elastic asymmetry. eLife doi: 10.7554/eLife.38161

- de Reuille PB, Routier-Kierzkowska AL, Kierzkowski D, et al. 2015. MorphoGraphX: a
- platform for quantifying morphogenesis in 4d. eLife doi: 10.7554/eLife.05864
- **Dumais J.** 2007, Can mechanics control pattern formation in plants? Current Opinion in
- 815 Plant Biology **10**, 58–62.
- **Dill E**. 2007. Continuum Mechanics: Elasticity, Plasticity, Viscoelasticity. Ch. 5, p. 213 ff.,
- 817 CRC Press, Boca Raton, FL.

- 818 Dumais J, Shaw SL, Steele CR, Long SR, Ray PM. 2006. An anisotropic-viscoplastic
- 819 model of plant cell morphogenesis by tip growth. The International Journal of Developmental
- Biology **50**, 209–222.
- **Dupuy L, Mackenzie J, Rudge T, Haseloff J**. 2007. A system for modelling cell–cell
 interactions during plant morphogenesis. Annals of Botany 101, 1255–1265.
- **Dyson RJ, Band L, Jensen O**. 2012. A model of crosslink kinetics in the expanding plant cell wall: yield stress and enzyme action. Journal of Theoretical Biology **307**, 125–136.
- **Dyson RJ, Jensen O**. 2010. A fibre-reinforced fluid model of anisotropic plant cell growth.
- Journal of Fluid Mechanics **655**, 472–503.
- 827 Dyson RJ, Vizcay-Barrena G, Band LR et al. 2014. Mechanical modelling quantifies the
- 828 functional importance of outer tissue layers during root elongation and bending. New
- 829 Phytologist **202**, 1212–1222.
- **Edelstein-Keshet L**. 2005. Mathematical Models in Biology. Ch. 4, pp. 126-128, SIAM,
- 831 Philadelphia, PA.
- 832 Elman H, Silvester D, Wathen A. 2005. Finite Elements and Fast Iterative Solvers: with
- Applications in Incompressible Fluid Dynamics. Ch. 7, p 313 ff. and Ch. 8, p 341 ff., OUP,Oxford.
- Eringen A. 1980. Mechanics of Continua., Ch. 2, pp. 89-91, 2nd edn, Robert E. Krieger,
 Melbourne, FL.
- 837 Evans G, Blackledge J, Yardley P. 2000. Numerical Methods for Partial Differential
- 838 Equations. Ch. 6, p. 165 ff., Springer-Verlag, London.
- **Fawcett TW, Higginson AD**. 2012. Heavy use of equations impedes communication among
- biologists, Proceedings of the National Academy of Sciences **109**, 11735–11739.
- Fayant P, Girlanda O, Chebli Y, Aubin C-É, Villemure I, Geitmann A. 2010. Finite
- element model of polar growth in pollen tubes. The Plant Cell **22**, 2579–2593.

843 Fozard JA, Lucas M, King JR, Jensen OE. 2013. Vertex-element models for anisotropic growth

- of elongated plant organs. Frontiers in Plant Science doi: 10.3389/fpls.2013.00233
- **Geitmann A, Dyson R**. 2013. Modeling of the primary plant cell wall in the context of plant
- development. Cell Biology doi: https://doi.org/10.1007/978-1-4614-7881-2_8-1
- 847 Geitmann A, Ortega JK. 2009. Mechanics and modeling of plant cell growth. Trends in
- 848 Plant Science **14**, 467–478.
- **Goodier J, Hodge P**. 1958. Elasticity and Plasticity. John Wiley and Sons, New York.
- Hamant O, Haswell ES. 2017. Life behind the wall: sensing mechanical cues in plants.
- BMC biology doi: 10.1186/s12915-017-0403-5

- Hamant O, Heisler MG, Jönsson H et al. 2008. Developmental patterning by mechanical
 signals in *Arabidopsis*. Science 322, 1650–1655.
- 854 Hervieux N, Dumond M, Sapala A, Routier-Kierzkowska A-L, Kierzkowski D, Roeder

AHK, Smith RS, Boudaoud A, Hamant O. A Mechanical Feedback Restricts Sepal Growth

and Shape in *Arabidopsis*. Current Biology **26**, 1019-1028.

- 857 Huang R, Becker AA and Jones IA. 2015. A finite strain fibre-reinforced viscoelasto-
- viscoplastic model of plant cell wall growth. Journal of Engineering Mathematics 95, 121–
 154.
- Jarvis MC. 2009. Plant cell walls: supramolecular assembly, signalling and stress. Structural
 Chemistry 20, 245–253.

Kevorkian J. 2000. Partial Differential Equations: Analytical Solution Techniques. Ch.8, pp.

863 525-576, 2nd edn, Springer-Verlag, New York.

- 864 Kierzkowski D, Nakayama N, Routier-Kierzkowska A-L, Weber A, Bayer E,
- 865 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.
- Kierzkowsk D, Routier-Kierzkowska A-L. 2019. Cellular basis of growth in plants:
 geometry matters. Current Opinion in Plant Biology 47, 56–63.
- Kroeger JH, Geitmann A. 2012. Pollen tube growth: getting a grip on cell biology through
 modelling. Mechanics Research Communications 42, 32–39.
- 871 Kroeger JH, Geitmann A, Grant M. 2008. Model for calcium dependent oscillatory growth
- in pollen tubes. Journal of Theoretical Biology **253**, 363–374.
- 873 Krupinski P, Bozorg B, Larsson A, Pietra S, Grebe M, Jönsson H. 2016. A model
- analysis of mechanisms for radial microtubular patterns at root hair initiation sites. Frontiers
- in Plant Science doi: 10.3389/fpls.2016.01560
- 876 Levesque-Tremblay G, Pelloux J, Braybrook SA, Müller K. 2015. Tuning of pectin
- methylesterification: consequences for cell wall biomechanics and development. Planta 242,
 791–811.
- **Lockhart JA**. 1965. An analysis of irreversible plant cell elongation. Journal of Theoretical
 Biology 8, 264–275.
- 881 Majda M, Grones P, Sintorn I-M et al. 2017. Mechanochemical polarization of contiguous
- cell walls shapes plant pavement cells. Developmental Cell **43**, 290–304.
- 883 Mattheij R, Rienstra S, ten Thije Boonkkamp J. 2005. Partial Differential Equations:
- 884 Modeling, Analysis, Computation. Ch. 2, pp. 13-29 and Ch 16, p 501 ff., SIAM,
- 885 Philadelphia, PA.

- 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.
- 888 Nili A, Yi H, Crespi VH, Puri VM. 2015. Examination of biological hotspot hypothesis of
- primary cell wall using a computational cell wall network model. Cellulose **22**, 1027–1038.
- **Ogden R**. 2013. Non-Linear Elastic Deformations, Ch. 4, pp. 204-222, Dover, Mineola, NY.
- 891 O'Malley R. 2014. Historical Developments in Singular Perturbations. Ch. 2, pp. 27-31,
- 892 Springer, Cham, Switzerland.
- Paolucci S. 2016. Continuum Mechanics and Thermodynamics of Matter. Ch. 5, pp. 191194, CUP, Cambridge.
- Park YB, Cosgrove DJ. 2015. Xyloglucan and its interactions with other components of the
 growing cell wall. Plant and Cell Physiology 56, 180–194.

897 Parker D. 2003. Fields, Flows and Waves: An Introduction to Continuum Models. Ch. 6, p.

- 898 101 ff., Springer-Verlag, London.
- 899 Peaucelle A, Braybrook S, Höfte H. 2012. Cell wall mechanics and growth control in
- plants: the role of pectins revisited. Frontiers in Plant Science doi: 10.3389/fpls.2012.00121.
- 901 Pietruszka M. 2011. Solutions for a local equation of anisotropic plant cell growth: an
 902 analytical study of expansin activity. Journal of The Royal Society Interface 8, 975–987.
- 903 **Prusinkiewicz P**. 2004. Modeling plant growth and development. Current Opinion in Plant Biology
- **904 7**, 79–83.
- Ptashnyk M, Seguin B. 2016. The impact of microfibril orientations on the biomechanics of
 plant cell walls and tissues. Bulletin of Mathematical Biology 78, 2135–2164.
- 907 Renteln P. 2014. Manifolds, Tensors, and Forms: An Introduction for Mathematicians and
- 908 Physicists. Ch. 2, pp. 30-48, CUP, Cambridge.
- **Rojas ER, Hotton S, Dumais J**. 2011. Chemically mediated mechanical expansion of the
 pollen tube cell wall. Biophysical Journal 101, 1844–1853.
- 911 Sampathkumar A, Krupinski P, Wightman R, Milani P, Berquand A, Boudaoud A,
- 912 Hamant O, Jönsson H, Meyerowitz EM. 2014. Subcellular and supracellular mechanical
- 913 stress prescribes cytoskeleton behavior in *Arabidopsis* cotyledon pavement cells, eLife
 914 doi:10.7554/eLife.01967.
- **Sandler S**. 1999, Chemical and Engineering Thermodynamics, Ch. 3, pp. 89-97, 3rd edn, John
- 916 Wiley and Sons, New York.
- 917 Sapala A, Runions A, Routier-Kierzkowska A-L et al. 2018. Why plants make puzzle
- 918 cells, and how their shape emerges. eLife doi: 10.7554/eLife.32794

- Sapala A, Runions A, Smith RS. 2019. Mechanics, geometry and genetics of epidermal cell
 shape regulation: different pieces of the same puzzle. Current Opinion in Plant Biology 47,
 1–8.
- 922 Sassi M, Ali O, Boudon F et al. 2014, An auxin-mediated shift toward growth isotropy
- promotes organ formation at the shoot meristem in *Arabidopsis*, Current Biology 24, 2335–
 2342.
- Savaldi-Goldstein S, Peto C, Chory J. 2007, The epidermis both drives and restricts plant
 shoot growth. Nature 446, 199–202.
- 927 Scheller HV, Ulvskov P. 2010. Hemicelluloses, Annual Review of Plant Biology 61, 263–
 928 289.
- Soyars CL, James SR, Nimchuk ZL. 2016, Ready, aim, shoot: stem cell regulation of the
 shoot apical meristem, Current Opinion in Plant Biology 29, 163–168.
- 931 Spencer A. 2004. Continuum Mechanics, Ch. 5, pp. 44-59; Ch. 8, pp. 110-116; and Ch. 10,
- 932 pp. 136-142, Dover, Mineola, NY.
- 933 Steinrück H. 2010. Introduction to matched asymptotic methods, Asymptotic Methods in
- 934 Fluid Mechanics: Survey and Recent Advances (CISM Courses and Lecture, vol. 523),
- 935 CISM, Udine, Italy, pp. 1–22.
- **Truskina J, Vernoux T**. 2018. The growth of a stable stationary structure: coordinating cell
 behavior and patterning at the shoot apical meristem, Current Opinion in Plant Biology 41,
 83–88.
- Vandiver R, Goriely A. 2008. Tissue tension and axial growth of cylindrical structures in
 plants and elastic tissues. Europhysics Letters doi: 10.1209/0295-5075/84/58004
- 941 Veytsman BA, Cosgrove DJ. 1998. A model of cell wall expansion based on
 942 thermodynamics of polymer networks. Biophysical Journal 75, 2240–2250.
- 943 Vofély RV, Gallagher J, Pisano GD, Bartlett M, Braybrook SA. 2018. Of puzzles and
 944 pavements: a quantitative exploration of leaf epidermal cell shape. New Phytologist doi:
 945 10.1111/nph.15461
- Wang C, Wang L, McQueen-Mason S, Pritchard J, Thomas C. 2008. ph and expansin
 action on single suspension-cultured tomato (*lycopersicon esculentum*) cells. Journal of Plant
 Research 121, 527–534.
- 949 Yi H, Puri VM. 2012. Architecture-based multiscale computational modeling of plant cell
- 950 wall mechanics to examine the hydrogen-bonding hypothesis of cell wall network structure
- 951 model. Plant Physiology **160**, 1281–1292.

- 952 Yuan S, Wu Y, Cosgrove DJ. 2001. A fungal endoglucanase with plant cell wall extension
- activity, Plant Physiology **127**, 324–333.
- 954 Zhang T, Zheng Y, Cosgrove DJ. 2016. Spatial organization of cellulose microfibrils and
- 955 matrix polysaccharides in primary plant cell walls as imaged by multichannel atomic force
- 956 microscopy. The Plant Journal **85**, 179–192.
- 957 Zheng Y, Wang X, Chen Y, Wagner E, Cosgrove DJ. 2018. Xyloglucan in the primary cell
- 958 wall: assessment by fesem, selective enzyme digestions and nanogold affinity tags. The Plant
- 959 Journal **93**, 211–226.