



## REVIEW PAPER

# The strength of plants: theory and experimental methods to measure the mechanical properties of stems

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## Abstract

From the stems of agricultural crops to the structural trunks of trees, studying the mechanical behaviour of plant stems is critical for both commerce and science. Plant scientists are also increasingly relying on mechanical test data for plant phenotyping. Yet there are neither standardized methods nor systematic reviews of current methods for the testing of herbaceous stems. We discuss the architecture of plant stems and highlight important micro- and macro-structural parameters that need to be controlled and accounted for when designing test methodologies, or that need to be understood in order to explain observed mechanical behaviour. Then, we critically evaluate various methods to test structural properties of stems, including flexural bending (two-, three-, and four-point bending) and axial loading (tensile, compressive, and buckling) tests. Recommendations are made on best practices. This review is relevant to fundamental studies exploring plant biomechanics, mechanical phenotyping of plants, and the determinants of mechanical properties in cell walls, as well as to application-focused studies, such as in agro-breeding and forest management projects, aiming to understand deformation processes of stem structures. The methods explored here can also be extended to other elongated, rod-shaped organs (e.g. petioles, midribs, and even roots).

**Keywords:** Bending, biomechanics, cell wall, metrology, phenotyping, stem, stiffness, strength, structure–property relations, tensile properties

## Introduction

A recent special issue on ‘Plant Biomechanics and Mechanobiology’ in the *Journal of Experimental Botany* (2013, 64, 4617–4827) conveyed that there is increasing interest amongst biologists to examine the effects of mechanical forces on biological systems, including those from the phylum *Plantae*. In living plants, the multiscale interplay of growth, morphology, and external (i.e. biotic and abiotic interactions) and internal mechanics continues to fascinate scientists. Improvements in the field of mechanical metrology (i.e. science of mechanical measurement), particularly at smaller length scales (e.g. cell wall and down to macromolecules), now enable closer inspection of plant development from a

biomechanics perspective, including in an evolutionary and materials science framework (i.e. structure–property–function relationships) (Smith, 1950; Niklas, 1992; Spatz *et al.*, 1997; Spatz *et al.*, 1998; Gibson, 2005; Fournier *et al.*, 2013). Mechanical characterization is also an important and now frequently used tool for phenotyping of plants, in the wider quest for crop improvement, as well as basic plant research (Pieruschka and Lawson, 2015). The inherent mechanical properties of the plant cell wall and stem are relevant to agro-breeding studies (Kokubo *et al.*, 1989; Robertson *et al.*, 2015) aiming to tackle issues such as crop loss due to lodging by uprooting or stem breakage (Crook and Ennos, 1996;

Skubisz, 2002). This even applies to larger tree trunks, which are susceptible to damage from wind loads (e.g. wind-throw and wind-snap), a major cause of concern in forest management (Hale *et al.*, 2012). With regards to harvest and processing of plant stems and their derivatives for eventual use as materials, knowledge of the bulk deformation behaviour of the stem and its interaction with machines (due to mechanical loads exerted by the latter) is critical in process optimization and yield efficiency (Leblicq *et al.*, 2015). In this context, the force response of stems not only has economic implications, but may also provide insights into the evolution of land plants, as force response must be a driver in natural selection (Smith, 1950; Rowe and Speck, 2005). Reliable testing of plant stem structures is critical for both commerce and scientific research.

However, the result (and accuracy) of a mechanical measurement is only as good as the quality of the measurement method. It is noteworthy that conventional measurement tools and engineering concepts are not always entirely applicable to testing biological systems, including plant stem structures, without modification, given the complexities in external and internal morphologies of the stem (e.g. irregular cross-sections, presence of nodes and branches), conditions during testing (e.g. turgor pressure, moisture content), as well as response (e.g. anisotropic, non-linear, time-dependent mechanical behaviour). These specifications become particularly important when comparing results between studies, and more so between studies from different research groups. Some form of standardized practices and accepted methods would help in ensuring reliability and repeatability of measurements.

A number of diverse materials are derived from plant stems: fibres, yarns, paper, and textiles, reinforced polymer composites, as well as wood and wood products. There are established standards (primarily international, American ASTM, and European EN), if not well-accepted methods, for testing microscale elementary fibres and fibre bundles extracted from the plants (e.g. ISO, 1974, 1995), processed textile yarns and fabrics (e.g. ISO, 1988, 1989, 2013; ASTM, 2013), and macroscale plant-based materials, such as wood products and fibre-reinforced polymer composites (e.g. ISO, 2003, 2015; ASTM, 2012). In contrast, despite there being numerous studies in the plant science literature on the mechanical testing of whole, herbaceous plant stems, there are no standardized testing methods, with the exception of those for tree stems, known as roundwood (e.g. BSI, 2005).

Here, we critically review the literature to evaluate mechanical testing methods for plant stem structures. We highlight important factors based on stem morphologies and other structural properties that require consideration when designing tests for plant stems. We then describe best practice from our own experience and that of others. Furthermore, we envisage that these discussions may also extend as useful guidance for the mechanical inspection of any elongated, rod-shaped plant organ, such as petioles, mid-ribs, and roots.

Box 1 and Figs 1 and 2 describe and define key mechanical engineering terms that will be used throughout this review. These lists are not exhaustive, and the readers are referred to

## Box 1. Key terms from the fields of structural engineering and materials science

**Loading.** Forces in a material may act to extend the material in the direction of the force—denoted **tension**—or to shrink the material in the direction of the force—denoted **compression**. A **shear force** causes sliding of parallel planes in the material relative to one another. When a structural element is subjected to **bending** forces, this induces tension at one extreme of the cross-section, compression at the other, and a variation between the two throughout the cross-section. This variation in stresses requires shear in the cross-section as well. A stem in bending may therefore fail in either tension, compression, or shear. Table 1 summarizes the tests methods used to apply these forces, and the properties they can be used to estimate.

**Elasticity.** The theory of elasticity describes how materials deform under the application of forces, and is founded on the work of Hooke (1678). The force per unit initial area in a material is denoted **engineering stress**, and **engineering strain** is its extension per unit original length. While **true stress** is determined based on the true, deformed cross-section area at that load (rather than on the initial, undeformed cross-section area), we principally refer to the engineering stress as ‘stress’, and engineering strain as ‘strain’. **Stiffness** is the ratio of force to displacement, and is a property of a structure, with stiffer structures deflecting less for a given load. The stiffness of a structure is affected by the material properties and the geometry of the structure. In experimental work, we commonly use the stiffness measured in a test to estimate the elastic modulus, a property of the material being tested, as shown in Fig. 1. The **elastic modulus**, or **Young’s modulus**, is the ratio of the stress applied to a material to the linear strain it exhibits. Stems are made from a combination of materials formed into cells, and a combination of different cell types combined into tissues. The elastic modulus of the stem material, therefore, reflects the combination of properties contributed by the different materials. Methods for using the properties of individual components to describe the behaviour of the whole stem are discussed in the text. The inverse of stiffness is **compliance**.

**Failure.** The failure of a material may be by either yield or fracture. At **yield**, a stress is reached in the material at which its stiffness greatly reduces, so it deforms substantially for a small increase in stress. In **fracture**, a crack, beginning at a flaw in the material, grows until it prevents the material from carrying load. A system which exhibits large deformations is referred to as a **mechanism** rather than a structure. Once part of the stem yields or fractures, it may form a mechanism causing the stem to collapse, known as a **failure mechanism**. Stems have evolved strategies to avoid particular failure mechanisms,

as described in the text. **Strength** is the maximum total stress a material can withstand before failure. Because bending tests are such a common way of estimating structural properties, there is a specific term for the strength measured in this test. The **modulus of rupture** is, therefore, the estimated peak stress for a stem at failure, as measured using a bending test such as three-point bending.

**Buckling.** When a compressive force is applied to a slender structure such as a plant stem, it does not fail by pure crushing of the material. Before the force necessary for pure crushing is reached, any lack of straightness (however small) will cause an initially straight stem to bend. The bending deformation increases the bending forces on the stem, and eventually the stem will become unstable, and fail in bending. The force at which this happens is called the **buckling** load. This process can occur in entire stems, as illustrated in Fig. 2, but also in individual cells, if they are sufficiently long and slender. In hollow stems, the **Brazier effect** (Brazier, 1927) may occur, in which, as it bends, the stem cross-section becomes more oval, reducing its ability to resist bending and further reducing the buckling load. Research into buckling of plant stems is described in the text.

**Fibre-reinforced composite.** Slender fibres can have extremely high strength and stiffness. In compression, however, these fibres alone do not exhibit their full strength because they are susceptible to buckling. If the fibres are instead used as reinforcement in a **matrix** of material capable of restraining against buckling and suitable to distribute the load around the fibres, then the strength and stiffness of the fibres can be effectively used. Stems may be described as fibre-reinforced composites at two scales. At the cellular scale, the ‘fibres’ may be long slender sclerenchyma with thick cell walls with low microfibril angle, in a ‘matrix’ of shorter, stockier, and softer parenchyma. Within the secondary cell wall, cellulose fibril aggregates may be the aligned ‘fibres’, in a matrix of other polysaccharides. Often, the matrix has **isotropic** material properties, while the fibres and the resulting aligned-fibre composite exhibit **anisotropy**.

*Journal of Experimental Botany*’s special issue (cited above) and (Mouliia, 2013) for a more comprehensive list.

## Stem architecture and its relationship to mechanical properties

Many of the factors governing stem mechanics are based on the stem architecture, which manifest at both the microstructure and the macrostructure scales. These factors need to be accounted for when designing testing methodologies, and understood to explain the observed mechanical behaviour. In addition, knowledge of the stem structural hierarchy may be useful in inferring material properties (at the tissue and cell wall level) from measured properties of the stem structure.

### Microstructure: at the tissue and cell level

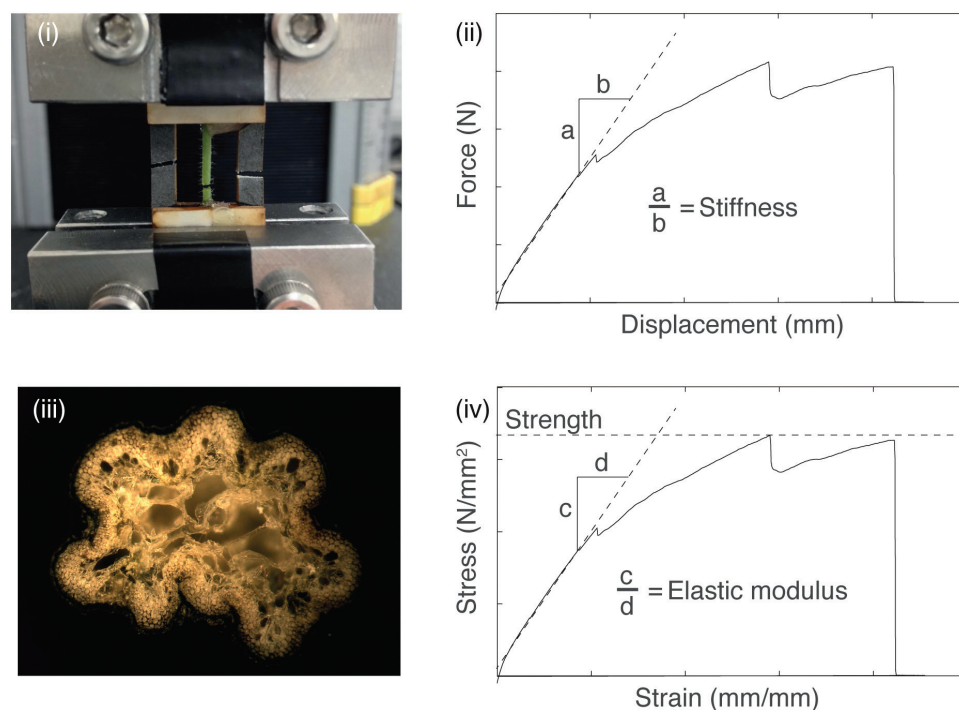
Stems and roots are the two main structural axes of all vascular plants: a group which includes gymnosperms, angiosperms, and ferns. Ferns typically lack vertical, overground stems. All gymnosperm stems are woody, and they tend to form near cylindrical, solid stems. Angiosperms can be further categorized as (i) herbaceous monocots including grasses such as bamboo; (ii) herbaceous dicots such as flax and the model plant *Arabidopsis thaliana*; and (iii) woody dicots including trees (Table 2). Angiosperms display a wide variety of strategies for structural resistance, both in the arrangement of stiffer and more flexible cells, and in their global geometry, as depicted by the schematics in Table 2. While upward, primary growth, mediated by the shoot (apical meristem), is common for vascular plants, dicots also have the ability for secondary growth, which means that their stems can get thicker. The proliferative activity of the vascular cambium, a group of dividing cells located between and giving rise to xylem (water-conducting tissue positioned towards the inside of the cambium) and to phloem (tissue responsible for transfer of nutrients positioned on the outside of the cambium), governs ‘wood’ formation (Ramage *et al.*, 2017). Here, we focus on herbaceous stems, while also drawing relevant knowledge from existing work on mechanical characterization of woody stems.

At the microstructure scale, all stems have the same three primary tissue types (Table 2): (i) dermal tissue as a protective surface layer; (ii) ground tissue, which typically makes up the largest fraction of a stem’s volume, for photosynthesis, storage, and support; and (iii) vascular tissue for water and nutrient transport, but also structural support (Speck and Burgert, 2011; Gibson, 2012; Davison *et al.*, 2013). Each tissue type is composed of various cell types, with the structure of the cells having evolved for specific functions (Table 2).

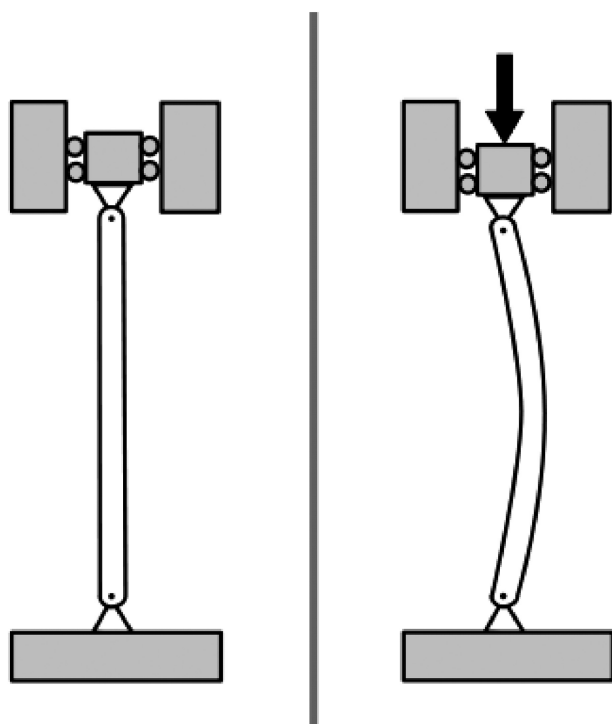
Parenchyma cells in the ground tissue have a soft, thin, flexible primary cell wall. The primary cell wall layers are typically lignin deficient and contain a low content of stiff cellulose fibrils. Through cell pressure probes and mechanical (tensile or bending) tests on parenchymatic tissue, such as plugs of potato tubers (Niklas, 1992) and *Caladium* petioles (Caliaro *et al.*, 2013), the reported elastic modulus of parenchyma cells falls in the range of 0.001–4 GPa (Karam and Gibson, 1994). The presence of large vacuoles in these thin-walled cells implies that turgor pressure has a substantial effect on its measured mechanical response (Niklas, 1992; Leroux, 2012). Collenchyma cells in the ground tissue have unevenly thickened primary cell walls with higher cellulose content, and therefore can offer some rigidity to young stems (Leroux, 2012). Typically, the principal structure-supporting cells are specialized sclerenchyma fibre cells found to some extent in the ground tissue, but primarily in the vascular tissue. In addition to primary cell walls, these elongated cells have thick, lignified, cellulose-rich secondary cell wall layers.

Vascular cells with secondary cell walls, such as in the xylem as fibres and vessels (e.g. in hardwood) or tracheids (e.g. in softwood), and in the phloem as fibres, sieve elements (e.g. in softwood), or tubes (e.g. in hardwood), also offer substantial structural support. These cells have elastic moduli, measured





**Fig. 1.** Tensile testing a senesced, dry stem of *Arabidopsis thaliana*: force and displacement are measured during the test (i), from which stiffness of the structure can be calculated (ii). Measurement of the cross-sectional area (iii), in this case by microscopy post-testing, allows the force to be converted to stress, and the material strength and elastic modulus to be calculated (iv).



**Fig. 2.** An initially straight slender member, such as a stem, bends under vertical load, a process known as buckling; the direction in which it bends depends on the initial lack of straightness of the member

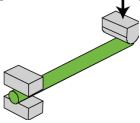
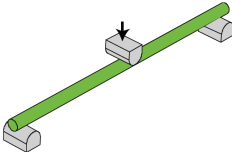
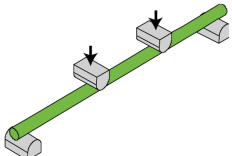




typically through tensile tests, in the range of 10–35 GPa (the higher modulus being that of wood cell walls) (Karam and Gibson, 1994), but even up to 70 GPa in the dry state (such as in flax phloem fibres) (Shah, 2013). For comparison, the

elastic moduli of native cellulose, hemicellulose, and lignin, which are the principal polymer constituents in plant cell walls, are estimated using computational models to be in the range of 74–168, 7–8, and 2–4 GPa, respectively (Shah, 2013).

While measuring the exact properties of the different tissue and cell types is difficult (Karam and Gibson, 1994), an appreciation of their relative contributions to the mechanical properties of a stem has been reflected in experimental investigations. For example, testing grasses with a dense outer shell (comprising sclerenchyma, vascular bundles, and collenchyma) and a pithy core (comprising mainly parenchyma, but also some collenchyma and vascular bundles), Karam and Gibson (1994) measured the stiffness ratio of the shell to the core to a range between 10 and 100. Similarly, Kohler and Spatz (2002) separated the outer tissues and inner core of the herbaceous dicot *Aristolochia macrophylla*, and measured the tensile stress–strain curve of each of these for comparison with that of the complete stem, as shown in Fig. 3. Their results show the outer strengthening tissues (made up of collenchyma, parenchyma, and sclerenchyma) to have an elastic modulus and strength approximately four times higher than the core tissues (phloem, xylem, interfascicular parenchyma, and pith). Niklas and Paolillo (1997) also demonstrate that the mature epidermis (inclusive of an underlying layer of collenchyma cells) of monocot tulip flower stems are an important stiffening agent in turgid stems, contributing as much as 50% to overall stem bending stiffness. All these studies validate the idea that collenchymatous and sclerenchymatous tissues are the principle structure supporting cells against tension and bending loads [note that parenchymatous core cells, much like foam cores in sandwich-structured composites, provide resistance to

**Table 1.** Mechanical test types typically used on plant stems

Example references are also provided which readers can access to explore methods and protocols.

Test type		Properties measured	Examples
Cantilever bend		Bending strength, elastic modulus	Henry and Thomas (2002); Caliaro <i>et al.</i> (2013)
Three-point bend		Bending strength, elastic modulus	Skubisz (2001, 2002); Petutschnigg and Katz (2004); Green <i>et al.</i> (2006); Lim <i>et al.</i> (2011); Christoforo <i>et al.</i> (2012); Ampofo <i>et al.</i> (2013); Slater and Ennos (2013); Lemloh <i>et al.</i> (2014)
Four-point bend		Bending strength, elastic modulus	Goubet <i>et al.</i> (2009); Robertson <i>et al.</i> (2015)
Tension		Tensile strength, elastic modulus	Spatz <i>et al.</i> (1998); Ryden <i>et al.</i> (2003); Cavalier <i>et al.</i> (2008); Abasolo <i>et al.</i> (2009)
Buckling		Critical buckling load	Niklas (1998); Spatz <i>et al.</i> (1998); Frese and Blass (2014)
Compression		Compressive strength, elastic modulus	Niklas (1998); Wright <i>et al.</i> (2005); Frese and Blass (2014)
Short-beam shear and torsion		Shear strength, shear modulus	Vogel (1992, 1995); Niklas (1997a); Spatz <i>et al.</i> (1997); Skubisz (2001); Goodman <i>et al.</i> (2002)

buckling of the stem (Gibson, 2005, 2012)]. In woody dicots, Onoda *et al.* (2010) found that while most species have a thick bark (including outer bark, phloem, and cambium), the contribution of bark to stem stiffness was small.

Figure 4 illustrates the hierarchical and multiphase nature of stems. From the view of micromechanics, stems can be analysed as laminated fibre-reinforced composites (Speck and Burgert, 2011, Gibson, 2012, Hofstetter and Gamstedt, 2009, Faisal *et al.*, 2013) or as cellular solids (Karam and Gibson, 1994; Gibson, 2005, 2012).

*Analysing plant stems as composite materials*

As fibre-reinforced composites, one can simplistically consider vascular tissue bundles (e.g. fibres and tracheids) as the primary load-bearing elements in a monocot stem, and that mechanical properties (e.g. elastic modulus, *E*) of the stem are dominated

by the properties of these vascular bundles (*E<sub>V</sub>*) and their differential distribution across the stem (*V<sub>V</sub>*) (Equation 1; Fig. 4 of a monocot bamboo stem) (Ruggeberg *et al.*, 2010, 2008).

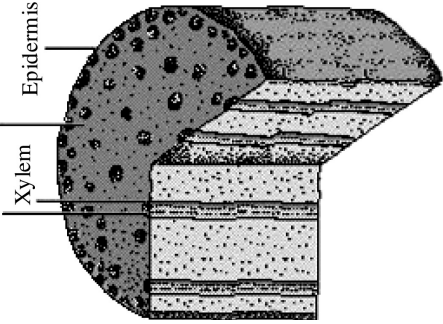
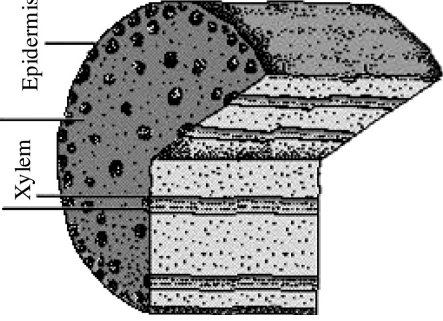
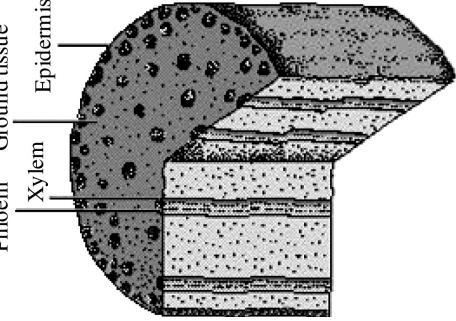
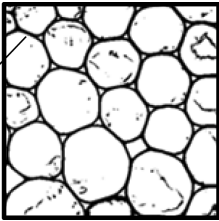
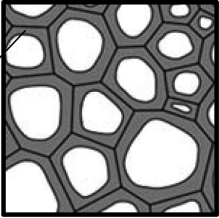
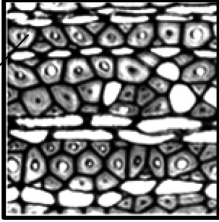
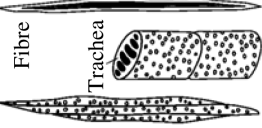
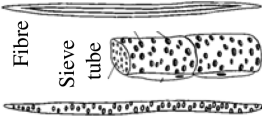
$$E = E_V V_V + E_G V_G \approx E_V V_V \tag{1}$$

where *E* represents the elastic modulus, *V* represents the (solid) volume fraction, and the subscripts V and G denote vascular and ground tissue, respectively.

Alternatively, one can consider the plant stems to have a multilamellar composite structure (Gibson, 2012; Leroux, 2012), with the different cell types composed of multiple primary and/or secondary cell wall laminae (Fig. 4). Each lamina is then regarded as a fibre-reinforced composite composed of helically wound cellulose microfibrils, oriented at specific angles to the cell axis, embedded in a

**Table 2.** The structure of three principal categories of angiosperm plant stems (at a macroscale), and the functions of principal tissue and principal cell types that exist in stems

Schematics of plant stems are adapted from Kirkby (1996). Schematics of cells are adapted from (Taiz and Zeiger (2010).

Stem types	Herbaceous monocot	Herbaceous dicot	Woody dicot
Schematic			
Description	Bundles of vascular tissue (phloem and xylem) are scattered throughout the ground tissue.	Bundles of vascular tissue (phloem and xylem) form a ring, separating the ground tissue into cortex and a central pith.	Epidermis is replaced by periderm (bark and cork). Ring of vascular tissue (phloem and xylem) develops secondary growth, and separates the ground tissue into cortex and pith.
Example plants	Grains and grasses, bamboo	Arabidopsis, flax	Trees (poplar, oak)
Tissue types	<b>Dermal tissue</b>	<b>Ground tissue</b>	<b>Vascular tissue</b>
Function	Protection and support; reduce water loss	Photosynthesis; food storage; regeneration; support	Transport (of food, water, minerals); support
Cell types in tissue	Epidermal cells (guard cells, parenchyma)	Parenchyma, collenchyma, sclerenchyma (fibres)	Parenchyma, sclerenchyma, xylem and phloem tissues (fibres, vessels, tracheids)
Cell types	<b>Parenchyma</b>	<b>Collenchyma</b>	<b>Sclerenchyma</b>
Schematic			
Description	Living cells with a thin primary cell wall and low axial aspect ratio	Elongated cells with non-uniformly thickened primary cell walls	Dead cells with thick, lignified secondary cell walls. Exist as sclerids and fibres
			<b>Xylem</b>
			Tracheid
			
			<b>Phloem</b>
			Sieve cell
			



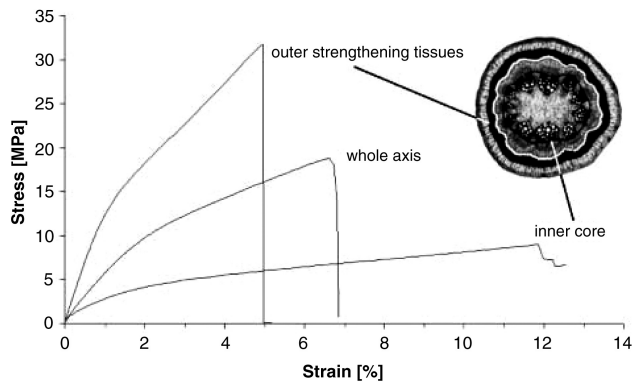
pectin–hemicellulose (in primary cell walls) or lignin–hemicellulose (in secondary cell walls) matrix (Bledzki and Gassan, 1999; Gassan *et al.*, 2001; Leroux, 2012; Placet *et al.*, 2012). Cellulose fibrils are substantially (up to 40 times) stiffer than pectin, hemicellulose, and lignin (Shah, 2013). In sclerenchymatous cells, since the S2 cell wall layer, which is the second sublayer of the secondary cell wall, is typically substantially thicker than all other layers combined, one could simplify this model (Gassan *et al.*, 2001; Pickering, 2008; Shah *et al.*, 2012a). The principal governing factors in such a model (Equation 2) are the cellulose content and microfibril orientation in the S2 layer (Bledzki and Gassan, 1999; Shah, 2013).

$$E = E_C V_C \cos^2(\theta) + E_{NC} V_{NC} \quad (2)$$

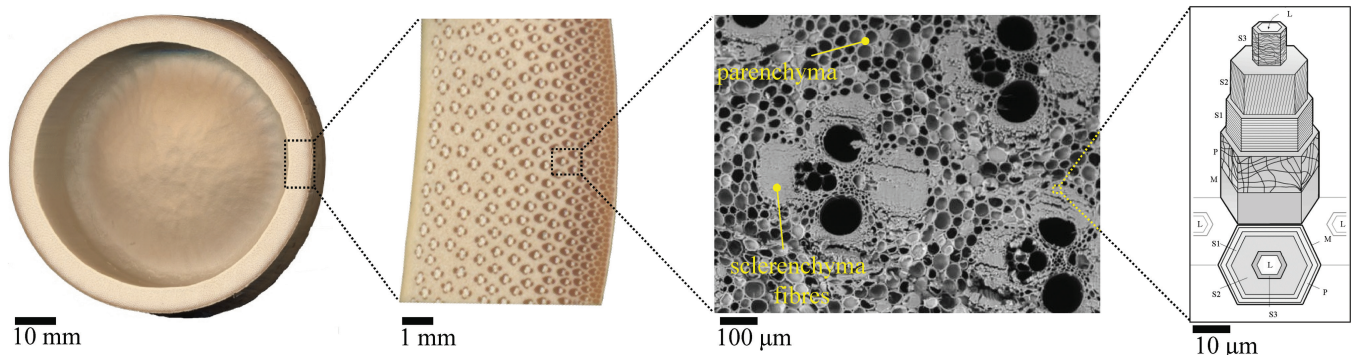
where  $E$  represents the elastic modulus,  $V$  represents the volume fraction, the subscripts C and NC denote cellulosic and non-cellulosic solid components in the cells, and  $\theta$  denotes the microfibril angle in the S2 cell wall layer.

#### Analysing plant stems as cellular materials

On the other hand, given the cellular and (in some cases) porous nature of plant stems, describing their behaviour through cellular solid micromechanics is attractive



**Fig. 3.** Stress–strain curves for parts of a stem of *Aristolochia macrophylla* (from Planta, Micromechanics of plant tissues beyond the linear–elastic range. 215, 2002, 33–40. L Kohler and HC Spatz (© Springer-Verlag) with permission of Springer).



**Fig. 4.** Plant stems have a hierarchical structure (*Phyllostachys pubescens* bamboo, a herbaceous monocot, as an example). The stem comprises multiple cell types and therefore can be analysed as a cellular solid. Stems may also be considered as multiscale composite structures, with sclerenchyma fibres in a matrix of parenchyma cells, and the fibres themselves as multilayered cellulose fibril-based composites.

(Fig. 4c) (Karam and Gibson, 1994; Gibson, 2005, 2012). Sclerenchymatous cells are tube like (long, slender, and often tapered), forming a honeycomb-like structure, while parenchymatous cells are box like (short and flat), forming a polyhedral, closed-cell foam-like structure. Their mechanical properties can be described by the properties of the solid material and the relative density of the cell (Equation 3) (Gibson, 2005, 2012; Karam and Gibson, 1994). The relative density of the cell  $\rho/\rho_s$  (ratio of basic density to absolute density) is representative of the cell geometry and proportion of solid material in the cell.

$$E = K E_s \left( \frac{\rho}{\rho_s} \right)^n \quad (3)$$

where  $E$  represents the elastic modulus,  $\rho$  represents density, the subscript S denotes solid material, and  $K$  and  $n$  are constants dependent on cell geometry and loading configuration (Gibson, 2005, 2012).

#### Macrostructure: at the stem level

At the macrostructural level, the key parameters that affect stem mechanical properties and behaviour fall broadly into three categories: (i) composition; (ii) geometry; and (iii) structural features.

#### Effects of composition

Basic dry density (dry mass per unit of fresh volume) is a useful indicator of composition in biological systems. The density of a plant stem increases with the solid fraction (i.e. a larger proportion of thicker cells with secondary growth), as well as cellulose content and cellulose crystallinity (Shah, 2013). Apparent through Equation 3, there is a strong, positive correlation between density and mechanical properties, both stiffness and strength (Fournier *et al.*, 2013). Indeed, density is considered as an easy-to-measure property for structural timber, and therefore it is used as one parameter to segregate timber into structural grades, based on extensive empirical data (Forest Products Laboratory, 2010; Ridley-Ellis, 2011).

Moreover, density affects both longitudinal and transverse mechanical properties, and therefore anisotropy: in

comparison with dense stems, light stems tend to have a lower transverse strength relative to longitudinal strength (i.e. higher anisotropy). Consequently, when bent, stems of lower density fail by local buckling in the compression zone, while stems of higher density break by tensile fracture, exhibiting longitudinal cracks (Ennos and van Casteren, 2010). This explains why plant stems grown in shaded environments, having lower density, fail by buckling in the compression zone, while stems of the same species grown in artificially adjusted light, having higher density, break via catastrophic longitudinal crack propagation (Ludwig *et al.*, 2009). Notably, this serves as an example of how a difference in growing conditions and habitats may have a non-trivial impact on the mechanical behaviour of plant stems. Indeed, the relationship between growth and density is more complex. Softwoods with very wide growth rings have a lower density than softwoods with small growth rings. In ring-porous hardwoods, it is vice versa.

Living plant stems are dynamic structures, and this makes mechanical measurements more challenging, including in comparison with senesced or 'dead' stems, as well as isolated and processed stem material (e.g. timber). In fresh stems in particular, not only the effects of turgor pressure and water content, both of which are discussed in more detail later, but also the molecular and cellular responses to mechanical loads, in the form of changing cell wall compositions, biochemical properties, and water influx/efflux, can have a marked effect on measured properties. These effects, of course, tend to be larger in longer running and time-dependent tests, such as creep and cyclic loading. Consequently, such tests can be very informative in revealing important information of cell/stem growth and morphogenesis (Cosgrove, 1993, 2010; Park and Cosgrove, 2012).

Within a certain range, the moisture content and drying/moisture regain of stems have been shown to have a strong effect on mechanical properties. In wood science, it is well known that 'green wood' (i.e. the unprocessed stem of a tree) has moisture content above the 'fibre saturation point'. The fibre saturation point refers to the point in the drying/moisture regain process below which there is no free water in the pores of cells (only bound water in the cell walls), and above which there is both free and bound water (Ramage *et al.*, 2017). In wood, the fibre saturation point is ~25–30 weight%

moisture content (ratio of mass of moisture in the material relative to its dry weight) (Forest Products Laboratory, 2010). The fibre saturation point also defines the point above which any change in moisture content has little effect on mechanical properties, but below which strength and stiffness increase substantially with decreasing moisture content (Forest Products Laboratory, 2010). The existence of a fibre saturation point in other plant stems is expected, although yet to be investigated in detail, and humidity and temperature (and consequently moisture content) would therefore be expected to influence mechanical properties. While a fresh or living stem would be above the fibre saturation point, once cut for testing, moisture content may drop rapidly, reaching equilibrium moisture content typically below the fibre saturation point. Systematic stem tests would require control of these moisture-influencing factors, including dwell times between stem preparation and testing, and conditions during preparation, storage, and testing.

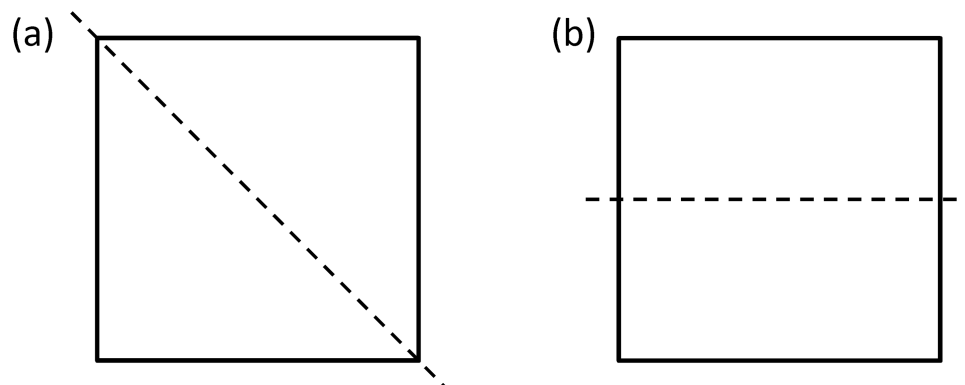
An important consequence of the living nature of plant stems, and the dependence of growth, morphology, composition, and properties on environmental conditions, is that any analysis of stem mechanical properties, and resulting statistical analysis of the data, should be based on several repeats (at least 10) over a number of biological replicas (at least three).

#### Effects of geometry

Geometric factors, such as the stem cross-section shape and dimensions, can affect stress development within a stem, and consequently its behaviour and properties.

There is a large diversity in stem sectional shape: stems can have circular and elliptical (as in *Euphorbia peplus*), polygonal (including triangular as in *Carex pensylvanica*, square as in *Mentha piperita*, hexagonal as in *Dipsacus sylvestris*, octagonal as in *Sericocarpus asteroides*), and even non-polygonal cross-sections (Smith, 1950).

For solid, isovolumetric stems, the orientation of load (and the neutral axis as a result; see Fig. 5) determines which cross-section shape is more efficient in bending (i.e. will deflect less upon load application). For example, while square cross-section stems with the neutral axis through opposite vertices (i.e. load applied on an edge; Fig. 5a) would be less rigid than circular cross-section stems, square cross-section stems with



**Fig. 5.** Schematic of a square cross-section stem with the neutral axis (a) passing through diagonal vertices and (b) bisecting the sides of opposite cells at the corners, to support bending stresses that arise when the neutral axis passes through opposite vertices (as in a) (Smith, 1950).



the neutral axis perpendicular to the surfaces (Fig. 5b) would more rigid than circular cross-section stems (Smith, 1950). Consequently, stems with square cross-sections often have most of the strengthening material (i.e. thicker collenchyma and sclerenchyma).

It has been experimentally shown that non-circular stems are characterized by higher twist-to-bend ratios (Niklas, 1992), which is a strategy against typical stresses, such as buckling and bending (Vogel, 1995, 1992, 1997; Etnier and Vogel, 2000; Etnier, 2001). The 'twist-to-bend ratio' is a ratio of flexural rigidity to torsional rigidity, which indicates the relative resistance of a stem to bending versus twisting; a higher ratio implies that the stem twists more readily than it bends. Specifically, higher ratios, as in triangular cross-section stems of sedges, enable the stems to shed wind loads by twisting readily into low-drag configurations (i.e. facing down-wind), thereby reducing the possibility of damage (Etnier and Vogel, 2000).

Other than the cross-section shape, which is usually irregular and can change with factors such as moisture content (e.g. dry stems can collapse), cross-section dimensions are important in determining mechanical properties. The effect of inaccurate dimension measurements on the second moment of area would be even more substantial (due to a fourth power effect), leading to grossly incorrect bending stiffness and strength.

Upon experiencing mechanical loading, such as during wind flexing, plants actively modify growth and development processes; this phenomenon is well known as thigmomorphogenesis. Thigmomorphogenesis significantly influences stem mechanical behaviour (Badel *et al.*, 2015). Not only do modifications in cell differentiation and wood tissue anatomy, such as formation of 'flexure wood' and increase in microfibril angle (Telewski, 1989), change the properties of the stem material, in most cases, the over-riding influence on stem rigidity arises from large increases in radial growth (and therefore second moment of area of the stem cross-section), as well as local ovalization (Badel *et al.*, 2015). Stems may also have a taper in their cross-section with increasing height. In general, in the absence of critical defects and constant material properties, mechanics would dictate failure to occur where stress is maximum, namely at the smallest cross-section. The extent of taper would dictate ideal gauge lengths to use during testing.

A critical aspect of stem sectional properties is their hollowness ratio. Evidence suggests that hollow stems can grow 26% taller than the equivalent solid stem (Niklas, 1992). This is a logical solution when biomass production is a constraint. While not all plant stems have a central cavity (e.g. tree trunks), many stems are naturally hollow (e.g. bamboo culm), or have a pithy core (e.g. *A. thaliana*) that does not contribute to mechanical properties and even hollows out when the plant matures or reaches senescence. The hollowness ratio represents the ratio of rind thickness to stem diameter. This ratio determines whether the stem is thin walled or thick walled, thereby governing its failure mechanism (Wegst and Ashby, 2007). For example, when subjected to compressive or bending stresses, thin-walled stems (with a rind thickness to stem diameter ratio  $<0.15$ ; Mattheck, 1995) break due to

Brazier buckling (Brazier, 1927; Niklas, 1992), whereas thick-walled stems fail due to material yield or fracture (Mattheck, 1995; Wegst and Ashby, 2007; Leblicq *et al.*, 2015). The presence or lack of a core (or a 'core-rind' structure), therefore, also affects the failure behaviour. The core is a foam-like, parenchymatous, compliant material, whereas the dense, sclerenchymatous outer shell (rind) is stiff. Despite being compliant, the core provides buckling resistance under axial compression and resistance to kinking failure under bending load. The improvement in buckling and bending capacity of the stem is related to the hollowness ratio and the ratio of the elastic modulus of the core material to the rind material (Gibson, 2005).

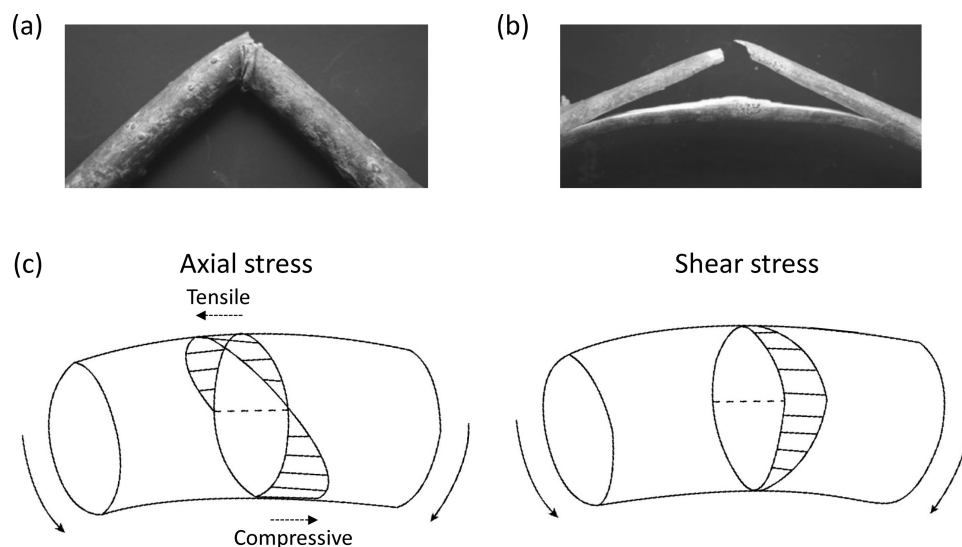
### *Effects of structural features*

Stems can improve both local and global properties by incorporating and modifying structural features, such as nodes and preferential distribution of stiffening fibres to the outermost parts of the stems.

If stems are analysed purely on the basis of their overall bending stiffness, then locating stiff fibres towards the outer edge of the stem is advantageous, as tensile and compressive stresses under bending load increase away from the central axis (Fig. 6). Schulgasser and Witztum (1997) showed, however, that this encourages failure by local compressive buckling or kinking, both of which involve the buckling of groups of fibres within the stem, rather than the stem as a whole. This can be shown by the theory of composite materials to occur because the shear strength of the outer material does not increase as rapidly as its compressive strength (Schuerch, 1966). Local compressive buckling may be particularly prevalent in hollow stems such as reed and bamboo (Niklas, 1998). Such stems have often developed to be septate, where diaphragms at nodes act to restrain the wall against this form of failure (Robertson *et al.*, 2015). Nodes are a common feature in many hollow stems, particularly grasses, that prevent local buckling and provide transverse reinforcement. Nodes can also act as spring-like joints to store and release energy when subjected to axial or transverse forces (Niklas, 1997b, 1998). However, stress and strain gradients at the interface of nodes and antinodes results in these being likely sites for failure when subjected to axial or bending loads. The stress and strain gradients are a result of changes in microstructure (e.g. cellulose microfibril angle) and physical properties (e.g. density), and therefore mechanical properties.

Junctions and nodes (e.g. stem-leaf, stem-petiole, and stem-branch) are also likely points of failure in stems. This is well known in wood science, where knots (stem-branch junctions in trees) are counted to assess the quality of the wood. Knots have a local increase in microfibril angle and density to improve local fracture toughness and density (by promoting mixed modalities in failure) (Speck and Burgert, 2011). However, once again, stress/strain gradients at the vicinity of these defects result in failure upon loading at the junctions.

Some herbaceous stems are supported, at least in part, by 'hydro-skeletons' (Niklas, 1992). This refers to a system where turgor pressure from internal fluids withstands compressive loads, while the sclerenchymatous rind supports tensile loads.



**Fig. 6.** Typical failure of stems in bending: stems may (a) kink and buckle on the compressive surface (typically for low-density, thin-walled stems) or (b) exhibit 'green-stick failure' where a transverse crack originates on the tensile surface and propagates longitudinally along the plane of maximum shear stress (typically for high-density, thick-walled stems). (c) Bending results in the development of a combination of axial (compressive and tensile) and shear stresses. Bending moment is represented by the solid curved arrows at the beam ends. Adapted from [Ennos and van Casteren \(2010\)](#).

A drop in turgor pressure, visually observed as the wilting of a stem when a plant is not watered, leads to buckling. Turgor pressure can also pre-stress and stretch the internal cell walls of the stem ([Karam and Gibson, 1994](#)).

When measuring the mechanical properties of stems that do rely on turgor pressure for support, it is important to control the turgor pressure of the system, or account for it when analysing the data. For example, [Paul-Victor and Rowe \(2011\)](#) conducted turgor tests on *Arabidopsis* stems prior to mechanical testing. Excised stems were tested for bending properties at regular intervals to examine the effect of loss of turgor pressure on mechanical properties. They observed a 10% drop in stiffness after 15 min, extending to 25 min when the segment ends were sealed with wax. Sealing segment ends, and either rapid testing or significantly delayed testing segment extraction ([Robertson \*et al.\*, 2015](#)) are recommended ways to minimize the effect of changing turgor pressure. Some researchers have also submerged plant stem tissues into osmotic solutions to adjust the turgor pressure before testing ([Falk \*et al.\*, 1958](#); [Niklas and Paolillo, 1997](#); [Caliaro \*et al.\*, 2013](#)). There are also methods to predict and back-calculate the properties of the solid cell wall material for any turgor pressure ([Nilsson \*et al.\*, 1958](#); [Karam and Gibson, 1994](#)). In general, the models suggest that the relative stiffness ratio between the cell walls and the stem is proportional to the ratio between the internal turgor pressure and the stem stiffness. For reference, turgor pressure varies between 0.1 MPa and 2 MPa for a range of stems, leading to a ratio between turgor pressure and stem stiffness of the order of  $10^{-3}$  to  $>10^{-2}$  ([Karam and Gibson, 1994](#); [Caliaro \*et al.\*, 2013](#)).

## Approaches to testing plant stems

**Table 1** lists typical tests that stems are subjected to for mechanical property measurement. In nature, plant stems

most commonly fail due to a bending moment by either yield or local buckling on the compression edge ([Fig. 6a](#)) or combined tensile and interlaminar shear failure ([Fig. 6b](#)). Both of these failure types originate from the axial stress (dashed straight arrows in [Fig. 6c](#)) and associated shear stress which develop due to the bending moment (solid curved arrows) in the stem. Consequently, flexural testing has become an important tool to assess stem mechanical properties.

A pure bending test does not fully replicate conditions in nature, however, where failure may be due to a combination of axial overload, from the weight of the plant and anything supported by it (e.g. snow), and adverse bending loads resulting from weather conditions (e.g. wind loading and rain beating) ([Niklas, 1992](#)). An understanding of the response to combined loads may be gained through modelling or calculation based on the fundamental properties of the stem material.

In flexural tests, specimens are subjected to mixed-mode conditions, with tensile and compressive stresses arising linearly on opposite sides of the neutral axis, and shear stresses increasing to a maximum towards the neutral axis ([Fig. 6c](#)). It can be difficult, therefore, to identify the fundamental mechanical properties of the stem material from such a test. Axial load testing has therefore been used to measure the elastic modulus (or stiffness) and tensile strength (or failure load) of stem tissue material (or stem structures), which may be different in tension and compression.

Furthermore, plant materials exhibit time-dependent, viscoelastic behaviour, as they are based on polymeric building blocks (i.e. cellulose, hemicellulose, and lignin). The full mechanical characterization of stems, therefore, requires tests which include a time component of load. This can be in the form of creep (sustained constant loading), stress relaxation (sustained constant deformation), or cyclic loading (as in oscillation due to wind loading).

Here, these various possible testing methods are discussed and evaluated. Recommendations are also given in designing suitable test procedures.

### Cross-section morphometry

To estimate stresses in the material due to a force applied to the structure, some geometric properties of the stem cross-section are required. As discussed earlier, the accuracy of these cross-section properties can have a significant impact on the accuracy of stress estimations.

In flexural tests, the second moment of area of the cross-section is required, so not only the transverse area, but also the shape of the cross-section must be measured or assumed. In shear, the cross-section shape also affects the distribution of stress. In the literature, it is most common to assume a cylindrical stem, and measure its diameter (or width) and possibly rind thickness (if hollow), at one or more points along its length (Skubisz, 2002; Lemloh *et al.*, 2014). Microscopy may be used to justify the assumed section shape after testing (Lemloh *et al.*, 2014). A more direct assessment of second moment of area, and of the contribution of the different tissues to bending stiffness through image analysis, is possible (Moulia and Fournier, 1997). We recommend this to become more common practice.

Under axial loading, only the cross-sectional area is required, which may be determined either indirectly or directly. There are two principal indirect methods. One approach is to determine the total volume of a stem segment of known length by a volume displacement technique (Skubisz, 2001); this, alongside a known mass of the segment, would yield an estimate of the average cross-section area. Another indirect measurement technique, borrowed from textile engineers (Shah *et al.*, 2012b), is to use the 'mass per unit length' of the stem segment as an indication of the area of material resisting the applied load. This, combined with either a measured density of solid cell wall material (using gas pycnometry, for instance) or an assumed density, would give an estimate of the average cross-section area of solid cell wall material resisting the applied load (neglecting the hollow lumen) (Cosgrove, 2011; Shah *et al.*, 2012b). Direct measurement techniques, in the case of small

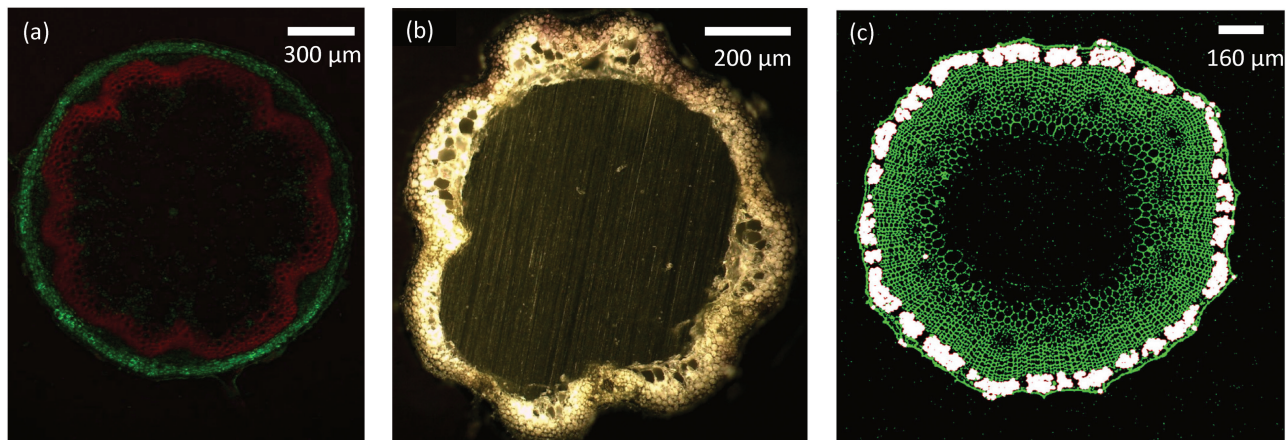
stems (less than a few millimetres in diameter), include the use of X-ray micro-computed tomography (CT) scanning prior to testing (Zeng *et al.*, 2015), or optical microscopy post-testing. The latter can be done by mounting stems vertically onto card frames and even casting these into resin blocks which are then polished for imaging (Thomason *et al.*, 2011) (Fig. 7). For larger stems (e.g. bamboo culms), section properties are easier to measure using callipers, but it may also be possible (for accuracy) to scan the surfaces (e.g. using high-resolution X-ray) (Masselter and Speck, 2008; McElrone *et al.*, 2013; Brereton *et al.*, 2015; Charra-Vaskoua *et al.*, 2016).

### Flexural bending tests

#### Cantilever bending

Flexure can be induced in a stem by any applied load which includes a pair of loads forming a couple. This is most simply done using a cantilever fixed at one end and loaded at the other. Cantilever, or two-point, bending can be carried out on *in vivo* stems in a pot (Caliaro *et al.*, 2013) and even on leaves (Moulia *et al.*, 1994). This is particularly useful where turgor pressure is expected to play a significant part in the mechanical resistance of the stem, as was the case for the *Abutilon theophrasti* tested by Henry and Thomas (2002) and *Caladium bicolor* by Caliaro *et al.* (2013). The American standard test method for flexural testing of tree stems is a cantilever test (ASTM, 2012).

The drawback of this technique is that the largest shear stress occurs in the same area as the largest bending stresses, making it difficult to isolate the two effects to estimate the fundamental properties of the material under test. In addition, elementary beam theory is only applicable for small deflections; as a rule-of-thumb, this is when tip deflections (for end-loaded stems) are <10% of their length. For large deflections, more complex equations and computational methods may be required to determine the mechanical properties (Morgan and Cannell, 1987; Vogel, 1992). This is because the equilibrium equations based on the undeformed shape are no longer valid after substantial deformation. Alternatively, paired measurements of (change in) curvature and bending moment can be made (Moulia *et al.*, 1994); this approach has



**Fig. 7.** Direct measurement of stem cross-section area can be done by confocal microscopy (a) (of a fresh *Arabidopsis* stem), optical microscopy (b) [from Shah *et al.* (2015) of a senesced, dried *Arabidopsis* stem], or X-ray micro-CT scanning (c) [from Zeng *et al.* (2015) of a flax stem].



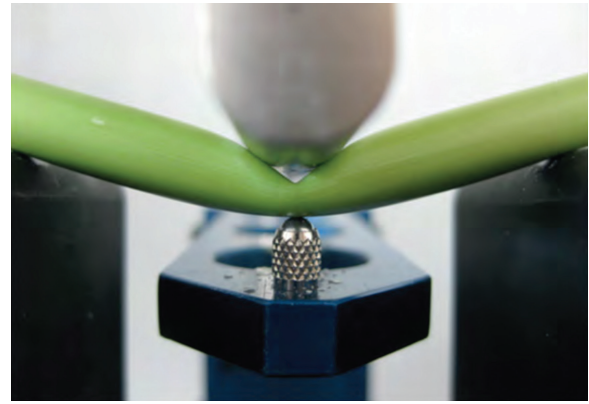
the advantage of accounting for sagging and initial curvature. The latter arises from self-weight and tip-load of some stems.

### Three-point bending

In three-point bending, the largest bending deflection is at mid-span, and the highest shear near the supports. This is the most common form of mechanical test carried out on stems (Skubisz, 2001, 2002; Lim *et al.*, 2011; Christoforo *et al.*, 2012; Ampofo *et al.*, 2013; Slater and Ennos, 2013; Lemloh *et al.*, 2014; Robertson *et al.*, 2015). The deformation is characterized by a single displacement measurement (at mid-span), and no clamping is required: the stem rests on two simple supports, and is subject to a point load. This test set-up has been applied for spans from a few millimetres (Lemloh *et al.*, 2014) up to several metres (Lim *et al.*, 2011).

The stem segment to be tested must be sufficiently slender that the contribution of shear to the measured deflection is small. Shear leads to an underestimation of strength and elastic modulus (van Casteren *et al.*, 2012). Standards for tests on small timber specimens use a span-to-depth ratio of 14 (BSI, 1957). The span-to-depth ratio (i.e. where span is the distance between supports, and depth is representative of a diameter or thickness of the stem) may, however, depend on the hollowness ratio of a stem. For example, for solid stems, researchers have suggested a minimum span-to-depth ratio of 20 (van Casteren *et al.*, 2012), while for hollow stems (such as in the reed *Arundo donax*) a minimum span-to-depth ratio of 10–15 may be appropriate (Spatz *et al.*, 1997). In a mechanical study of 42 grass species (which included hollow and solid stems), Evans *et al.* (2007) suggest that a span-to-depth ratio of 10 would make the contribution of shear negligible. Other studies have suggested that, for example when testing the stems of the herbaceous dicot *A. thaliana*, a span-to-depth ratio of 35–40 should be used (Paul-Victor and Rowe, 2011). We recommend that, if an initial pilot study to test the effect of span length is not feasible, a minimum span-to-depth ratio of 20 should be used. Of course, longer span-to-depth ratios would further minimize cross-section deformation from shear (Robertson *et al.*, 2015), but this may be limited by the presence of nodes, for example, or the sagging of a non-rigid stem. Note that the four-point bending method may be used to eliminate the contribution of shear deformation altogether.

A drawback of the three-point bending method is the stress concentration at the loading point, which may lead to a local bearing failure of the stem, or a local deformation of the section which in turn leads to a change in cross-section during the test. An example of this can be seen in Fig. 8, where a kink has formed at the loading point. The resulting ovalization and buckling reduce stiffness significantly and need to be accounted for (through the use of cumbersome equations) when determining mechanical properties, as described by Leblicq *et al.* (2015). In septate stems, where a diaphragm across a node reinforces against local buckling, stems are shown to be stronger in these bending tests when loaded at the node (Robertson *et al.*, 2015). Increasing the span-to-depth ratio as well as selection of an appropriate loading anvil shape (e.g. a rounded anvil is preferred over a knife-edge anvil) may help in reducing stress concentrations (Robertson *et al.*, 2015).



**Fig. 8.** Three-point bending test on a stem of *Sorghum bicolor* (Lemloh *et al.*, 2014).

### Four-point bending

Four-point bending results in a region between the two loading points where there is no shear in the specimen and the bending moment is constant. Any failure in this region can therefore be classed as a bending failure at a known bending moment. The absence of shear stresses enables assessment of the longitudinal elastic properties. However, this method requires more instrumentation than three-point bending to isolate the component of deflection that occurs in between the two loading points. Robertson *et al.* (2015) use the region of constant bending to observe variations of stiffness in septate stems, showing that there is no clear reduction in stiffness at the nodal points.

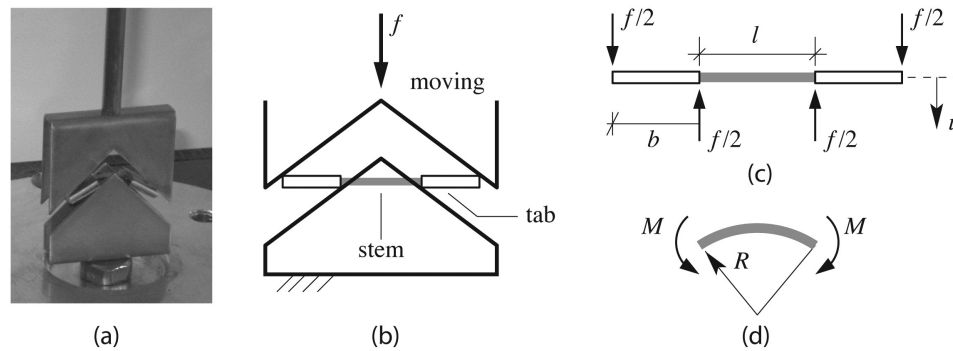
Tree stems, referred to as roundwood, have been widely tested in four-point bending (Ross *et al.*, 2001; Vestøl and Høibø, 2010). This is one area of stem testing where standard methods have been developed in Europe (BSI, 2005). The taper and ovality of the stem are taken into account in calculating elastic moduli and strength from bending tests. Standards also recommend a shaped loading head to reduce local crushing (BSI, 2005; ASTM, 2012).

For small stem specimens, displacement measurement may require bespoke approaches. A modified four-point bending method is used by Goubet *et al.* (2009) to investigate the effect of various genetic modifications on the strength and stiffness of stems of *A. thaliana*. They encase the stem outside of the loading points in steel tabs, as shown in Fig. 9, so that the deflection measured at the loading points is due to the bending of the stem between them.

As stem materials are viscoelastic and have rate-dependent properties (particularly strength and stiffness), the rate of loading is controlled. Standard tests on wood commonly require failure to occur after 5 min of loading (BSI, 1957, 2005). Rate dependency is an important factor to consider when comparing the literature with dissimilar loading rates.

### Axial loading

A major advantage of bending tests is that specimen clamping is not required. However, as they induce mixed modes of stress across the stem cross-section, one cannot obtain fundamental material properties, for example the distinct tensile and



**Fig. 9.** Test set-up for four-point bending of *Arabidopsis thaliana* stems (from Goubet F, Barton CJ, Mortimer JC, Yu X, Zhang Z, Miles GP, Richens J, Liepman AH, Seffen K, Dupree p. 2009. Cell wall glucomannan in *Arabidopsis* is synthesised by CSLA glycosyltransferases, and influences the progression of embryogenesis. *The Plant Journal* 60, 527–538 with permission of Wiley).

compressive elastic modulus. Axial loading tests are therefore attractive to isolate these properties. In addition, unlike bending tests, axial tests do not require the measurement of the second moment of area to determine geometry-normalized properties. Rather, they rely on transverse cross-section area, which is easier to determine accurately. Axial loads can be applied in either tension or compression.

#### Tensile testing

In tensile tests, a key component of the experimental method is the way in which the specimen is held at each end, namely specimen gripping. While some researchers do grip stem segments without any specimen preparation (Usherwood *et al.*, 1997), it should be noted that clamping points may initiate failure in the specimen, resulting in a lower measured strength. This is of particular concern in (hollow) stems which may resist substantial loads along their length, but crush at much lower loads across the stem.

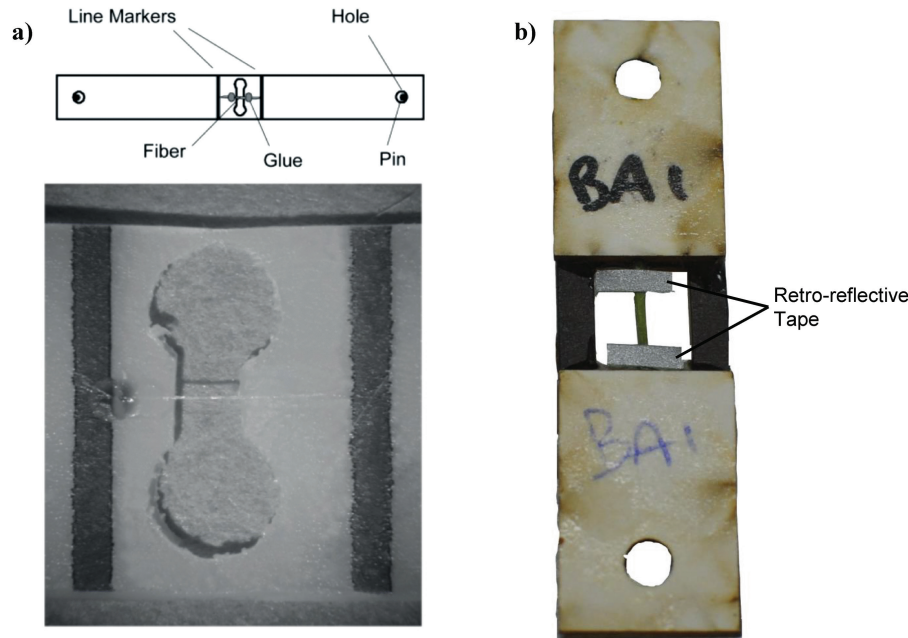
Some researchers choose to test rectangular strips of the stem wall (Spatz *et al.*, 1997, 1998; Ruggeberg *et al.*, 2010), assuming it to be representative of the mechanical properties of the stem wall material. Testing of strips is not uncommon in wood science and technology. In such an approach, it is possible to remove layers of the stem wall (e.g. scrape off parenchymatous cells) to test the contribution of different tissue types (Spatz *et al.*, 1998).

When testing the whole stem, it is advised to protect the stem at the grips. One possibility is to encapsulate the stem with a rigid, thermoset polymer block or tubing. Thereafter, the polymer block can be gripped on a tensile tester, with the load transferring from the block to the stem. Zeng *et al.* (2015), for example, use a polyurethane tubing at both clamp ends, which is bound to segments of flax stem using Araldite epoxy resin. To protect further the hollow stems from crushing and to avoid resin wicking through the hollow stem, Zeng *et al.* (2015) also insert 0.5–1.5 mm steel cores, as deep as the clamping length. Zeng *et al.* (2015) comment that this methodology prevented premature fibre damage in the clamping region and ensured failure in the gauge length. In a similar approach, Wright *et al.* (2005) prepared special end-grips that consisted of metal pins to fill the core of the hollow stems to protect from crushing, and a sleeve-like self-adhesive heat

shrink-wrap on the outside to protect the surface of the stem from damage. Yet another technique is to sandwich the stem ends between aluminium tags using a cyanoacrylate-like polymer (i.e. non-shrinking upon curing) or metal-filled epoxy-like gap-filling adhesive (Altaner *et al.*, 2014). Note that some polymers may require high-temperature curing; ambient-curing and low-exotherm adhesives are preferred, to avoid damaging the biological specimen through heat. Selecting a suitable adhesive also involves ensuring that slippage (i.e. debonding and subsequent sliding of the stem relative to the adhesive) does not occur during testing. The use of gap-filling glues and low cure-shrinkage adhesives may aid in ensuring intimate contact between the adhesive and the stem. Prior to gluing, cleaning stem ends (e.g. with water or alcohol to remove natural waxes on the surface) may also be recommended. To avoid physical gripping at the ends, one could even use end-tabs with holes; metallic pins through the holes can be used to align, hold, and load the specimen (Altaner *et al.*, 2014).

In contrast, some studies have used a more basic approach wherein stems are held by pneumatic clamps (at a prescribed pressure); the stem surface is protected by thick plastic and, to avoid slippage at the clamps, rough grinding paper is used (Kromer, 2009).

The use of mounting frames is attractive for thin stems with low stiffness, for example those which can deform substantially (and probably irreversibly) by the presence of metallic or polymeric inserts and sleeves at the ends. For example, etiolated hypocotyls of *A. thaliana*, producing <0.2 mm stems with exclusively primary cell walls, have been tested using microtensile test methods developed by Burgert *et al.* (2003) for single-cell-thick foils of wood, shown in Fig. 10a (Cavaliere *et al.*, 2008; Abasolo *et al.*, 2009). Foliar frames are used to set the gauge length, protect the specimen during installation in the loading machine, and to provide the connection to the loading head. Instead of foliar frames, one could use frames based on paper, cardboard, acetate sheets, or aluminium foil, depending on the stiffness of the frame required. For our tests on *Arabidopsis* stems, we are currently using 1 mm thick cardboard frames (Shah *et al.*, 2015) (Fig. 10b). The ends of the frames have 0.5 mm deep grooves to enable the stems to slot into them. The laser-cut grooves enable alignment, but more importantly ensure that the stem does not



**Fig. 10.** Tensile testing of stems may require the use of mounting systems. (a) Foliar frames, made from a single-cell-thick foils of wood, used in a microtensile test tester for testing etiolated hypocotyls (Burgert *et al.*, 2003). (b) Card frame, with laser-cut grooves at the end, used for testing genetically modified Arabidopsis stems (Shah *et al.*, 2015). Retro-reflective tapes are used for strain monitoring using a laser extensometer. (This figure is available in colour at JXB online.)

crush when the frame is externally gripped. In all frames, the specimen is attached to the frame by a polymer adhesive which is required to set rapidly, have gap-filling properties, and have high shear strength. Some adhesives that fit this specification include slow-setting epoxy (e.g. Araldite Precision), and a combination of glass ionomer luting cement (for gap filling) and cyanoacrylate (e.g. Loctite 454).

A particular challenge in testing specimens in tensile mode is the measurement of extension. On non-frame-mounted specimens, it is common simply to rely on cross-head extension. In that case, initial tests should be conducted to ensure that compliance of the test frame and grips has been accounted for. On frame-mounted specimens, slippage is another possibility which can skew the extension reading. For large stems, it may be possible to use contact methods (such as a clip-on extensometer) for strain measurement. However, special collars may be needed to be placed around the stem (in the gauge length) at the extensometer contact points, to provide attachment but also protect the stem from local transverse loads (Wright *et al.*, 2005). For thin, non-stiff stems, non-contact strain measurement methods are preferred. One approach is to use digital image processing. Burgert *et al.* (2003) track ink lines on their foliar frames, while Ryden *et al.* (2003) sprinkled the hypocotyls with coal dust to produce a high-contrast field for digital image tracking. In our methodology (Shah *et al.*, 2015), we are using a laser extensometer, which tracks the relative displacement of retro-reflective tape that is mounted onto the stems directly (Fig. 10).

Specimen test length and loading rate are important parameters that would affect the properties measured. Just as observed in single fibre tests (Shah, 2013), longer stem segments will show lower strength than shorter segments as the former have more defects. In addition, selection of an appropriate gauge

length to minimize effects of tapering, presence of junctions, and other structural features may be necessary. Higher test extension rates will show higher strength and stiffness, as viscoelastic materials tend to have rate-dependent properties.

#### Pure compression testing

As far as we know, stems in nature do not fail in pure compression. However, comparing tensile properties with compressive properties [obtained through compression tests at low length to diameter ratios, say 1:1 (Wright *et al.*, 2005), where bending does not occur] can help determine which of the modes (i.e. tensile or compressive) is more detrimental in bending, which is a mixed mode of loading. One should, however, be cautious when comparing compressive properties of one species with another, as loading conditions and specimen geometry have a significant effect on compressive behaviour. One such factor is the straightness of the specimen.

#### Compression buckling testing

Compressive buckling tests are useful in understanding the failure process of stems. Such tests also elucidate aspects of plant biomechanics, such as determining the tallest self-supporting stem length. Buckling tests have been carried out on tree stems of European chestnut (*Castanea sativa*), European oak trees (*Quercus robur*), and black locust (*Robinia pseudoacacia*) by Frese and Blass (2014). Their results highlight the importance of measuring the lack of straightness in the specimens, which reduces their failure buckling load.

The failure mode of stems in buckling depends on their geometry. In stocky stems, it is typically by ovalization and kinking of the wall of hollow stems, or microbuckling of fibres. This is corroborated by compression loading of hollow septate stems of the giant reed *Arundo donax*, at specimen



length to diameter ratios of  $\sim 50$ , where ovalization of the stem cross-section leads to longitudinal splitting of the stem wall (Spatz *et al.*, 1997). In slender stems, failure is by global buckling. For instance, Spatz *et al.* (1998) suggest that at length to diameter ratios of  $\sim 200$ – $250$ , living hollow stems of *Equisetum giganteum* fail by global buckling, unless they find external support.

The possibility of longitudinal splitting is higher in hollow stems than in solid stems (Spatz *et al.*, 1998). This is because circular cross-section hollow stems become elliptical when subjected to buckling loads. This ovalization and large decrease in the apparent diameter of the stem leads to a reduction in second moment of area, and, consequently, a drop in bending moment is observed even before critical strains of the material are reached. The three critical findings from any compression buckling test are (i) the elastic modulus in the linear region; (ii) the critical curvature; and (iii) the maximum bending moment/load. These can then be used to find useful information such as the critical compressive strain, which is often in the range of 0.5–1.5%, similar to that of the strengthening sclerenchymatous tissues in plant stems (Spatz *et al.*, 1998).

The presence of nodes can also alter the modes of failure in compression. It is well accepted that nodes operate as transverse struts and braces to reduce the possibility of Brazier wall buckling (Brazier, 1927) (refer to Box 1 for definition). However, due to the large stress–strain gradients at the vicinity of a node, nodes may also act as stress concentrators.

#### *Anisotropy and properties in transverse directions*

In its natural state, a stem predominantly resists forces along its axis and, as a result, it is far weaker in the direction orthogonal to the axis of the stem. Where the stem branches, there may be a component of force in this direction, and experimental methods have been developed to study the properties of that junction (Masselter *et al.*, 2011; Slater and Ennos, 2013). Robertson *et al.* (2015) tested corn, giant reed, and bamboo in compression in the orthogonal direction. Where whole stems are used or processed for other purposes, the mechanical properties in this direction may be important. An example of this is the use of full-culm bamboo in construction (Sharma *et al.*, 2013).

Plant materials, when subjected to uniaxial tensile or compressive loads, will not only extend or contract in that direction, but will also contract or expand in orthogonal directions. This phenomenon is captured by the ‘Poisson’s ratio’, which is a ratio of the negative transverse strain to the axial strain resulting from a load. Of course, multi-axial loading will lead to combined effects. The Poisson’s ratio can be used to inform other useful material properties such as bulk modulus, which is the resistance of a material to a change in volume when subjected to ‘hydrostatic loads’, or uniform loading from all directions. Hejnowicz and Sievers (1995) measured the Poisson’s ratio of tissues from herbaceous plants using microphotography, by measuring changes in transverse and longitudinal dimensions as a function of applied strain.

Plant stems, much like aligned fibre-reinforced composite structures, exhibit anisotropy. The anisotropy exists in morphology and structure [i.e. slender morphology of stems, aligned, high-aspect ratio (fibre) cells within the stem, and cellulose microfibrils within the cells], stress distribution, and growth and development (Baskin, 2005; Baskin and Jensen, 2013). Studies on anisotropy are therefore crucial in stem growth and morphogenesis. Anisotropy can be measured through a ratio in orthogonal directions of mechanical properties (such as elastic modulus in the transverse and longitudinal direction); this is common in wood science and technology (Forest Products Laboratory, 2010). It is also possible to compare measured stresses and strains in orthogonal directions (Silk and Abou Haidar, 1986; Hejnowicz and Sievers, 1995; Hejnowicz *et al.*, 2000; Baskin, 2005).

#### *Other forms of loading*

Shear properties of stems are commonly evaluated by torsion tests (Vogel, 1992, 1995b; Niklas, 1997a; Spatz *et al.*, 1997). In larger torsion tests, a metal bar may be screwed (Vogel, 1995) or glued (Niklas, 1997a) to the end of the horizontal stem at its centreline, and a torsion applied by an offset mass. In smaller stems, the ends of the stem may be mounted vertically, and glued into caps loaded by wires to apply torsion (Vogel, 1992).

Peel and tear tests, or short-beam shear tests (a three-point bending set-up with a small span-to-depth ratio) can be performed on stems to ascertain the interfacial or inter-laminar properties (Skubisz, 2001). These include interfaces between primary and secondary phloem tissue, and can even be monitored over the life cycle of a plant (Goodman *et al.*, 2002). Such tests reveal information on optimal harvest time, for example, as well as enabling monitoring of the effects of desiccation.

To assess mechanical properties at the cell wall level (micro- and nanoscale), methods such as nanoindentation, atomic force microscopy, and cellular force microscopy are commonly used. These nanomechanical tests have been reviewed by Burgert and Keplinger (2013) and Milani *et al.* (2013). Nanoindentation hardness testing involves penetration of an indenter tip with known geometry (e.g. Berkovich, spherical, and power-law indenters) of the sample surface, and simultaneous measurement of a load–displacement curve. From these measurements, estimates of surface hardness, a measure of resistance to plastic deformation from indentation loads, and elastic modulus in compression can be obtained. Atomic force microscopy measures properties at an even smaller scale, through the use of a tip that is mounted onto a cantilever-loaded spring. The force and deflection of the cantilever as it scans over the sample surface are measured to produce images based on mechanical property measurements. Both these nanomechanical testing methods rely on substantial sample preparation, particularly as flat surfaces are required. This often includes the embedment of samples into resins and subsequent surface polishing. Furthermore, these tests are often carried out on only a small region of a plant cell wall, and therefore conclusions on the properties of

a whole stem or even tissue, which are naturally variable and heterogeneous structures, should be drawn with care.

### *Time-dependent and cyclic-loading behaviour*

Tests on the time-dependent behaviour of stems have been used to investigate the processes responsible for growth of plants, and have therefore often been carried out on living plants (*in vivo*) or on plants submerged in water (*in vitro*). As has been expressed previously, living plants are especially dynamic structures with active cell walls. The turgor pressure produces the irreversible expansion of the cell wall which is part of the process of growth (Lockhart, 1965), although growth is not purely a mechanical process (Cosgrove and Jarvis, 2012). The extension and expansion of cells due to turgor pressure *in vivo* is time dependent and has been investigated using creep test methods, also referred to as well extension assays, particularly by Cosgrove *et al.* (Cosgrove, 1993, 2011; Park and Cosgrove, 2012). The deposition of the secondary cell wall occurs after this expansion is completed (Nishitani and Demura, 2015), and so the study of the irreversible deformation producing growth uses stems with predominantly primary cell walls, such as etiolated hypocotyls.

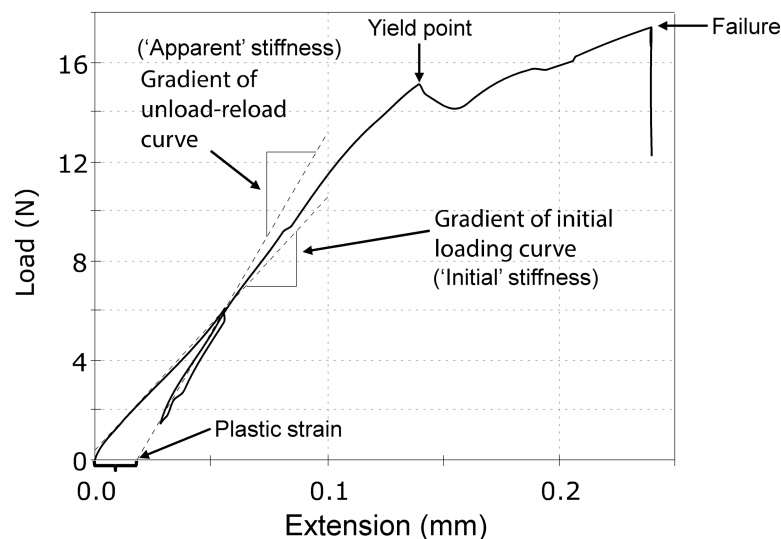
Creep under constant load in stems has been commonly measured, both *in vivo* and *in vitro*, with a set of masses suspended over a low-friction lever attached to the specimen (Kutschera and Schopfer, 1986; Kutschera and Briggs, 1987; Nonami and Boyer, 1990; Edelmann and Kohler, 1995; Suslov and Verbelen, 2006). The shape of the living plant may provide a convenient location for load application, such as the hypocotyl hook in soybean stems (Nonami and Boyer, 1990). For tests on epidermal strips, Kutschera and Briggs (1987) left the part of the stem to be clamped intact, while peeling the remainder to leave just the epidermis. Kutschera and Schopfer (1986) glued stem sections into hollow steel needles at either end, and the same method was followed by

Edelmann and Kohler (1995) and then Suslov and Verbelen (2006).

Tests under cyclic load show the recoverable and irrecoverable components of deformation, as well as hysteresis resulting from plastic and viscoelastic behaviour. There may therefore be differences in the paths on a force–displacement diagram for first loading and an unload–reload cycle, as shown in Fig. 11. Further cycles of loads may be applied at the same load (i.e. constant amplitude loading) or higher loads (i.e. progressive loading). The differences in properties extracted from the first and subsequent cycles of loading may highlight variations between plants; these differences would otherwise not be observed through monotonic/static tests.

Several researchers (Nolte and Schopfer, 1997; Kohler and Spatz, 2002; Abasolo *et al.*, 2009; Shah *et al.*, 2015) have considered cyclic loading of stems. Kohler and Spatz (2002) noted the increase in hysteresis under cyclic load in plants with reduced lignin in the secondary cell wall, using chemically modified *Aristolochia macrrophylla* and transgenic *A. thaliana*. Many plant materials show a higher stiffness on the second and subsequent cycles of load. Abasolo *et al.* (2009) tested etiolated hypocotyls of *A. thaliana* and noted the effect of altered pectin content on this stiffening. Studying the cyclic-loading behaviour of living plants in particular demonstrates that it is not just polymer remodelling that causes this type of differential material behaviours in cyclic loading, but also the cellular responses to alter the cell wall deposition and composition.

In modal testing, one can measure the dynamic response of a structure to an applied force, as a function of frequency. Such an analysis determines information such as the resonant (natural) frequencies of vibration, damping ratios, and mode shapes of a structure. Modal analysis of plants has been used to isolate the reversible elastic behaviour which results in a set of natural frequency. The branched systems of plants *in vivo* have a high modal density, making the identification of



**Fig. 11.** A typical load–displacement curve obtained from a single-cycle tensile test carried out on a basal stem section of *Arabidopsis thaliana*, showing an increase in stiffness and the development of irreversible ‘plastic strain’ after the first unload. The stress (or strength) at yield and failure can also be determined. Adapted from Shah *et al.* (2015).

individual modes challenging (Rodriguez *et al.*, 2008; Langre *et al.*, 2012). Der Loughian *et al.* (2014) found that the frequency of local modes, rather than global ones, was less sensitive to the size of the plant, and therefore may be more useful for phenotyping.

## Summary

Different test methods can reveal different properties of stems (Table 1). Three-point bending is the most common form of mechanical test carried out on stems, with its single biggest advantage being that no clamping is required, resulting in a simple test set-up. However, to ensure that shear contributions are not significant, a minimum span-to-depth ratio (of 20) is recommended. A four-point bend test is attractive as shear deformation is entirely eliminated, although relatively more instrumentation is required. Both three- and four-point bending result in large stress concentrations at the loading point, which lead to significant changes in cross-section (due to ovalization) and result in premature stem failure due to local crushing. The selection of an appropriate anvil shape (e.g. rounded anvil) is therefore recommended.

Although bending is a common mode of failure of stems in their natural environment, plants have developed structural strategies which balance twisting, shear, and local and global buckling. In buckling tests, specimen eccentricity and specimen length to diameter ratio (slenderness ratio) are important factors, particularly when comparing results from one study with another.

Even though it is not a natural mode of failure, tensile loading may give an insight into fundamental material properties in the stem. Furthermore, the error associated with determining cross-sectional area (for tensile properties) is much lower than that with determining the second moment of area (which is required for flexural properties), and therefore tensile properties can be more accurately measured. However, testing stems in tensile mode requires substantial specimen preparation. It is recommended that the stem ends are not clamped directly, as this is likely to result in premature failure at the grips. Rather, the use of frames (e.g. folial, or card) on which the stem is mounted for testing or other techniques to protect the ends from damage during gripping (e.g. encapsulating the ends in polymer blocks or tubing) is recommended. If mounting frames are being used, which are particularly attractive for small, non-stiff stems, adhesives with gap-filling properties, high shear stiffness, and low-exotherm ambient-curing properties should be utilized. The use of direct strain measurement techniques, such as laser extensometry, is also recommended, to ensure that extension upon load application is due to elastic/plastic deformation of the stem and not slippage at the clamps. Specimen gauge length needs to be specifically noted in tensile tests, and should be accounted for when comparing studies together.

Plant stems, like all materials made from polymeric building blocks, are viscoelastic in nature. Consequently, their properties are time and rate dependent. Therefore, in any

testing methodology, testing rates need to be appropriately controlled; as a rule of thumb, failure time should be ~60–90 s. In addition, the use of time-dependent loading regimes, such as in cyclic (low- and high-cycle fatigue) or creep/stress-relaxation tests, would inform us of the micromechanisms governing the viscoelastic behaviour of the stem material.

Finally, in mechanical testing, measurement and interpretation of geometry is required to ensure that test results and conclusions are meaningful. The use of imaging techniques, including optical microscopy (post-testing) and non-destructive X-ray scanning (prior to testing), would enable accurate assessment of geometry, particularly in the case of hollow and/or irregular cross-section stems. Parameters such as bulk and absolute density are also useful in evaluating the ratio of solid (structure-supporting) material in the cellular stems.

## Afterword

The field of experimental botany has become increasingly interdisciplinary, promoting dialogue and collaboration between biologists, engineers, physicists, and mathematicians. We ourselves, as architects, structural engineers, and materials scientists, engage with plant cell wall biochemists and chemists in across the scale research to understand and manipulate molecular organization within plant cell walls to inform the development of high-performance buildings in timber and bamboo for a sustainable built environment (Ramage *et al.*, 2017).

As such collaborations become increasingly common, offering new opportunities to obtain important experimental information that was not available before, there is a growing need to standardize, or at least offer guidelines for, the experimental testing and reporting of botanical structures. This methodological review was therefore written for a cross-disciplinary audience, particularly for biologists interested in measuring mechanical properties of their biological materials, for example for phenotyping (e.g. transgenic or cell wall mutant plants, database generation by core collection of genotypes), and engineers, who frequently test man-made materials, and are interested in studying biological materials, which however require specific considerations and even a re-think in measurement approach in comparison with man-made materials. We have tried to provide an overview on stem biology and mechanics, particularly how they relate to each other, and address the questions of how to test plant stems and what we can learn from such tests.

The principal discussions and recommendations in this review on appropriate methodology for mechanical metrology of plant stem structures can be further extended to any rod-shaped plant organ, such as petioles, midribs, and roots.

An appropriate test method, combined with an understanding of the structural strategies employed by a plant stem, can reveal opportunities for improved use of crops, biomimicry in man-made materials, the best use of materials derived from plant stems, and significant development in the field of plant sciences and cell wall research.



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