Integrative Mechanobiology of Growth and Architectural Development in Changing Mechanical Environments

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Abstract Mechanosensitive control of plant growth is a major process shaping how terrestrial plants acclimate to the mechanical challenges set by wind, self-weight, and autostresses. Loads acting on the plant are distributed down to the tissues, following continuum mechanics. Mechanosensing, though, occurs within the cell, building up into integrated signals; yet the reviews on mechanosensing tend to address macroscopic and molecular responses, ignoring the biomechanical aspects of load distribution to tissues and reducing biological signal integration to a "mean plant cell." In this chapter, load distribution and biological signal integration are analyzed directly. The *Sum of Strain Sensing* model S^3m is then discussed as a

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synthesis of the state of the art in quantitative deterministic knowledge and as a template for the development of an integrative and system mechanobiology.

1 Introduction

From the instant cells first formed at the onset of life on Earth, they have been facing tough mechanical constraints linked to fluctuations in their osmotic environment (see chapter "Osmosensing"). Multicellularity and cell differentiation then added another source of mechanical stresses called tissue tensions or "autostresses" (see Hejnowicz 1997; Moulia 2000; Moulia and Fournier 2009, chapter "Mechanics of the Meristems"). This sparked the development of cellular systems for mechanosensing and subsequent control of mechanical cell properties very early on in the realm of evolution, and all the living cells that have been observed display mechanosensing systems (e.g., Haswell et al. 2008, see chapters "Introduction: Tensegral World of Plants" and "Mechanics of the Cytoskeleton").

However, land colonization and the evolution toward an erect habit under selective pressure for light competition and propagule dispersal has created three very challenging changes in the mechanical environment of plants (Niklas 1998).

First, the large mass density ratio between plant tissues and air (approximately 10^3) means land plants display very little buoyancy (compared to water plants) and thus get only negligible support from surrounding fluid. The vertical position can therefore become unstable over erected growth due to global Euler buckling. This sets a potential gravitational limit for self-supportive vertical growth on land (beyond that limit, additional growth in length or weight will actually reduce rather than increase the height of the stem (McMahon 1973; Jaouen et al. 2007; also see Moulia and Fournier-Djimbi 1997; Niklas and Spatz 2004 for critical reviews). Growth at low buoyancy sets a second instability in the erected form, as the continuous deposition of cell wall layers in growing cells fixes any long-lasting deformation, meaning deformation increases over time (see reviews in Moulia et al. 2006; Moulia and Fournier 2009; Almeras and Fournier 2009). This slow instability (compared to the instantaneous Euler buckling) is especially important for stems undergoing secondary growth (Fournier et al. 2006; Almeras and Fournier 2009).

The second change due to the mechanical in-land environment is the large fluctuations in the availability of water in the atmosphere (and in soils). As photosynthesis requirements have led to very high surface-to-volume ratios, the strong transpiration flows may induce losses of turgor and rigidity in hydrostatic tissues, despite the various control mechanisms plants have evolved (Niklas 1992; Tardieu 2003; also see chapter "Hydraulics of Vascular Water Transport"). Such changes induce broad variations in the mechanical stresses internal to plants (Chapters "Mechanics of the Cytoskeleton" and "Mechanical Force Responses of Plant Cells and Plants").

Last but not least, the very low kinematic viscosity of air and the existence of climatic air temperature and pressure gradients induce frequent and potentially

strong winds (Stull 2007). Winds are major factors of land climate and thus of the physical environment of the plant. Therefore, any aerial organ in a terrestrial plant is submitted to fluctuating, intermittent but recurrent wind loads (de Langre 2008), apart from the tiny, well-protected shoot apical meristems. However, the extent of wind loads varies extensively over time and geographical space, depending on meteorology and local topography. Moreover, as plants grow erect, and display their foliage, wind drag and lever arms increase, producing a huge increase in wind-induced mechanical loads (Delus et al. 2004). Note that the vibrational excitability of a plant subjected to wind turbulence is also dependent on variables undergoing major changes during growth (Rodriguez et al. 2008).

It was been understood that the challenges set by wind drag and buckling instability may play an important role in the height growth of terrestrial plants (see Moulia and Fournier-Djimbi 1997 for a critical review), but the picture has become sharper over the last decade. It has become clear that growing plants have to contend with both static mechanical challenges (increased mean wind drag and risk of buckling instability, as well as the slow instability due to growth itself) and dynamic mechanical challenges related to the spectrum of their dynamic excitability in response to turbulent wind loads (Gardiner et al. 2008; Sellier et al. 2008; Rodriguez et al. 2008).

It has also become clear that these mechanical challenges depend very strongly on whether the plant is growing isolated or within a canopy. Plants growing isolated are submitted to a continuously increasing static and dynamic wind drag challenge. In contrast, plants growing within an existing canopy (like tree saplings) first experience a phase of low wind drag. But the low understory light resources force them to grow in height with little diametric growth, which generates a significant risk of buckling instability (Jaouen et al. 2007). As they get close to the canopy top, they become submitted to increased wind drag, but with a very intermittent gust regime produced by "honami" eddies peaking in a restricted waveband (Py et al. 2006; Dupont and Brunet 2008). This may induce resonant oscillations in plants that can be damaging, despite the lower mean static drag force compared to isolated plants (Sellier et al. 2008; Sellier and Fourcaud 2009; Gardiner et al. 2008). Finally, if the plant overreaches well beyond canopy height (as is the case with trees in savanna), it becomes an almost isolated plant.

It has been argued that the sheer diversity of mechanical challenges and the fact that they all change in quality and intensity during growth actually prevent a genetically fixed mechanical design from being competitive (except species colonizing a very specific niche; Fournier et al. 2006; Moulia et al. 2006). Therefore, a phenotypical developmental plasticity has been selected that includes (1) acclimation of the plant's load-bearing structure through thigmomorphogenesis, and (2) a process of active recovery and posture control through bending motors (Moulia et al. 2006; Barbacci et al. 2009). As the integrative mechanobiology of tropisms and postural controls has recently been reviewed in detail (e.g., Moulia et al. 2006; Moulia and Fournier 2009; Almeras and Fournier 2009), this chapter focuses on the integrative mechanobiology of thigmomorphogenetic acclimation.

Thigmomorphogenesis was first demonstrated by submitting plants to (artificial) mechanical bending. A syndrome of responses is then observed in a large number of species, involving (1) a reduction in longitudinal stem growth, (2) a stimulation of secondary radial stem growth (if a cambium is present), possibly with differentiation of a more flexible but stronger "flexure wood," and (3) a reallocation of biomass to the root system (see chapter "Mechanical Force Responses of Plant Cells and Plants," Telewski 2006; Moulia et al. 2006 and Coutand 2010 for reviews from complementary standpoints). This mechanosensitive control of growth allometries results in stunting and anchoring the shoots, while conserving most of the capacity for wind drag reduction through reconfiguration made possible by the more flexible wood. The thigmomorphogenetic syndrome thus seems to improve plant acclimation to the effects of static wind drag. Indeed, isolated plants submitted to high winds display a morphology that is reminiscent of the thigmomorphogenetic response (Jaffe et al. 2002). However, the fact that broad-scale thigmomorphogenetic responses were obtained with very small loads (e.g., one slight bending per day) cast doubt on the significance of thigmomorphogenesis in natural conditions, as plants responding as they did in laboratory experiments should not grow at all in natural windy settings. Furthermore, in some species and growth stages, thigmomorphogenesis seemed to be outweighed by photomorphogenesis (Holbrook and Putz 1989). However, studies on different dense stands, including a 15-m-high lodgepine forest (Meng et al. 2006) and a 0.7-m-high dense alfalfa stand (Moulia and Combes 2004) have shown that thigmomorphogenesis is a major response in many plant canopies. By comparing free-swaying stands to a control treatment with limited wind-induced motion, Moulia and Combes (2004) demonstrated that in the range of moderate, chronic winds ($U < 30$ km h^{-1}), in situ thigmomorphogenetic effects could range from a 40% decrease in stand height with a 65% decrease in aerial biomass down to no effect at all when little wind occurred. Thigmomorphogenesis was thus found to be a major process in the control of plant canopy growth in the ecological range of natural chronic winds. Further insight into the significance of thigmomorphogenesis for wind acclimation was recently provided by analysis of the dynamic excitability of trees (Rodriguez et al. 2008). It was found that throughout development, trees tend to tune their allometric growth to reduce changes in their resonance frequencies and compartmentalize their dynamic bending energy.

The question of how a coordinated thigmomorphogenetic syndrome of responses can be achieved through mechanoperception and signal integration within the plant is thus a major issue. Reviews on thigmomorphogenesis and mechanosensing (e.g., chapter "Mechanical Force Responses of Plant Cells and Plants," Braam 2005; Telewski 2006; Monshausen and Gilroy 2009) usually present the global thigmomorphogenetic syndrome and then zero in to detail mechano-induced genes in herbs (Lee et al. 2005) or trees (Leblanc-Fournier et al. 2008; Martin et al. 2009). However, there is little analysis of the link between the two levels of description (macroscopic growth responses and gene expression patterns in cells and tissues).

Mechanosensing occurs at cell level (see chapters "Introduction: Tensegral World of Plants," "Micromechanics of Cell Wall," "Mechanics of the Cytoskeleton,"

"Mechanics of the Meristems," and "Mechanical Force Responses of Plant Cells and Plants," for more in-depth analysis of mechanosensitive cell biology), yet mechanics stimulations apply mechanical loads at whole-plant level, acting either at its boundaries (as in the case of bending through wind drag) or across its full volume (as in the case of weight or inertial forces). Therefore, the links between the loads and the changes in mechanical state of tissue elements and cells that triggers cell mechanosensing depend not only on the load but also on the mechanical structure of the plant.

Analyzing and modeling the integrative biology of mechanosensing and thigmomorphogenetic response thus involves two phases (1) biomechanical analysis of how external mechanical loads on the plant are distributed over the constitutive plant tissue and cells, and (2) integrative mechanobiological modeling of local mechanosensing and how the plant integrates it. The aim of this chapter is to review the issue of plant integrative biomechanics and mechanobiology, from the load on the plant to tissue elements and cells, and then from mechanosensitive gene expression to global thigmomorphogenetic responses. The issue of the adaptive value of these responses will be then briefly discussed. Although the mechanical challenges of an erect habit on a terrestrial environment apply to any terrestrial taxa, our focus will mostly remain limited to the stems of terrestrial vascular seed plants. These phyla have evolved some of the largest and most perennial living erect structures on Earth – modern trees. A more general and complete view of the evolutionary aspects of mechanical design can be found in chapter 14.

2 From Whole-Plant Loads to Tissue Element Loads: Integrative Plant Biomechanics

2.1 Some Basics of the Mechanics of Deformable Materials (fluids and solids), Including Biomaterials

Before entering into biomechanics and mechanobiology, it is useful to first share a few concepts, principles, and tools of solid and fluid mechanics (definitions complementary to those given in chapter "Micromechanics of Cell Walls," see also Boudaoud 2010).

Mechanics is usually defined as the science that deals with the movements of bodies under the influence of forces (Mechanics 2010). For a given material domain within the body (characterized by its amount of matter, i.e., its mass), these movements can be split into (1) *global motion* of the domain, characterized by the motion of a central point – the center of mass – and possible rotations of the body around this point, and (2) the relative movement of the other parts of the domain with respect to this central point and altering its shape and/or size, called *deformation*. Motion can be measured by *velocity* (in meters per second) and deformation by the

strains (relative change in lengths in all directions of a domain around a position within the body).

Any body compelled to change its global velocity of motion and/or to deform needs a *force* to do so (unit Newton, N). *Newton's second law* states that change of velocity (acceleration) is proportional to the force delivered and inversely proportional to the mass of the body. Reciprocally, any acceleration of a body of mass m (such as during wind-induced oscillations) will produce an additional force, called the *inertial force*.

A body cannot be completely at rest unless all forces and all moments acting on it are balanced. Once a complete balance exists, the body is said to be in *static equilibrium*. Note that the origin of some forces involved in static equilibrium is not directly observable. *Reaction forces* have to be taken into account. For example, when you are standing on the floor, the compressive force due to your weight is counterbalanced by a reaction of the floor. Although the mechanisms explaining the onset of such distant reaction forces are highly intuitive, they are sometimes misunderstood in the biological literature. These mechanisms are related to the requirements of internal mechanical equilibrium for all the domains within the body, and to the existence of physicochemical bonds within the materials. When subjected to the action of forces, a deformable body will strain. This deformation will stretch bonds and slide/shear internal elements, allowing internal reaction forces to set up until an internal and external mechanical static equilibrium is achieved (or quasi-static equilibrium if slow internal sliding occurs). A measure of the density of these internal reaction forces is given by the amount of internal force per unit area, called *stress* (unit: Pascal, Pa $=$ N/m^2). These internal reaction stresses are transmitted from place to place up to the sites of external constraints, where they generate external reaction forces. If the solid is *stiff* or *rigid*, then little strain will be necessary. However, if it is *compliant* or *flexible*, then large strains are necessary before a static equilibrium can be achieved with the applied loads. Note that there is a limit at which the internal stresses may overcome the *strength* of the material, leading to *fracture* (see Niklas 1992 and Moulia and Fournier-Djimbi 1997 for a more complete review on these topics). As stresses are a derived quantity, they are not directly observable and have to be inferred from kinematic measurements (strains and/or accelerations).

Finally, when there is no static equilibrium, accelerated movements take place. The notion of static equilibrium can then be extended to a *dynamic equilibrium* by taking into consideration inertial forces in addition to the static forces (d'Alembert's principle). The reaction forces, particularly stresses, may increase accordingly.

2.2 Mechanical Modeling as an Integrative Structure–Function Tool for Plant Biomechanics

It is virtually impossible for the human brain to directly and simultaneously process all the above-described effects from external loads through to internal bond

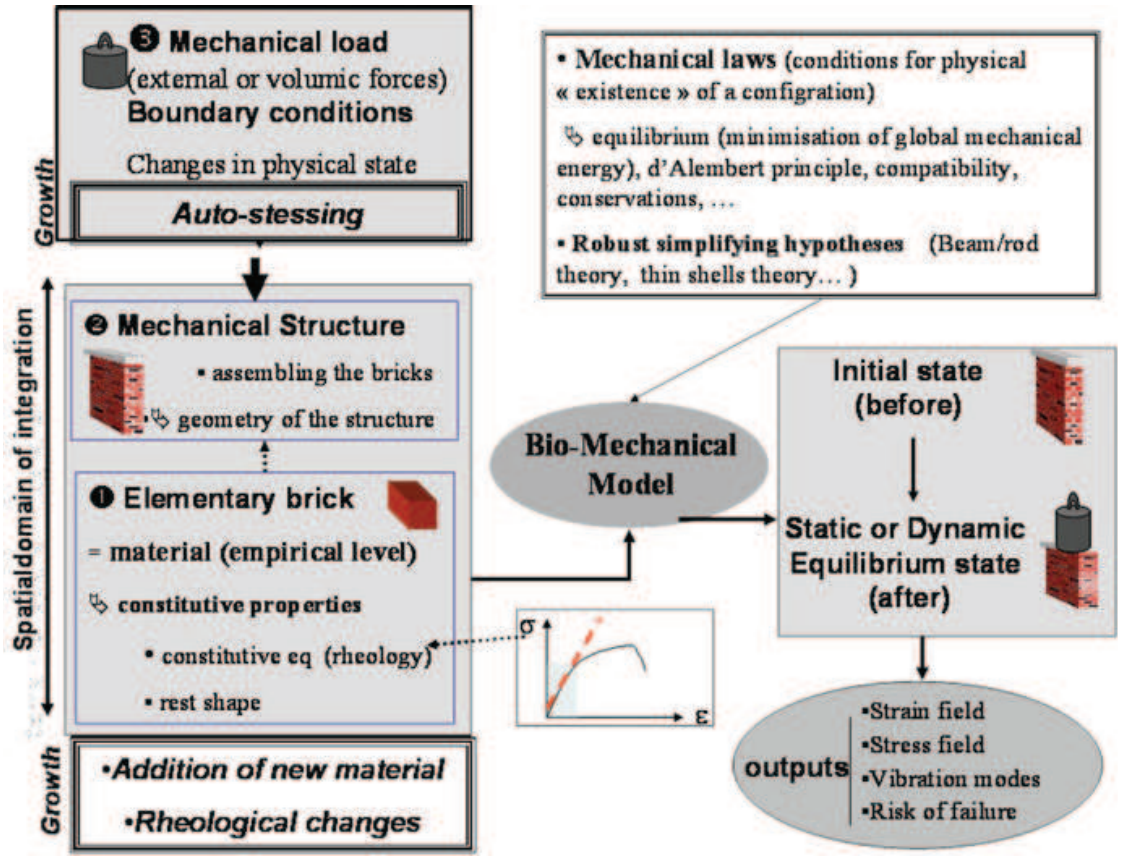

Fig. 1 The structure of a ISM Model for use in Plant Biomechanics. ISM models consider (at least) two scales in the system: a scale of phenomenological empiricism called the material scale, and a scale of mechanistic spatial integration, the mechanical structure. The internal and boundary loads (inputs) result in a change in mechanical state that can be calculated using mechanical principles and robust simplifying theories. ISM models can produce various outputs characterizing mechanical state or dynamics, such as strain (ε) and stress (σ) fields, vibration modes, or risk factors versus rupture

straining. We therefore employ dedicated tools called models, which can be produced through a scientific method called Integrative Structural Mechanics (ISM) modeling. It is useful to gain a general view of ISM models (Fig. 1) as (1) ISM modeling is central to understanding the link between external loads and the mechanical stimulus on cells (integrative plant biomechanics), and (2) it can serve as a paradigm for integrative mechanobiology (and more generally sensing biology), as we will see later.

Figure 1 sketches the general structure of an ISM model. The first step is to define the scale of the material, which can be viewed as the basic building block of the integrative model (the smallest level of organization taken into account). The mechanical properties of this material (its capacity for building stresses when reacting to a deformation of its "bonds," called its *rheology*) are defined by an empirical phenomenological equation, called the *constitutive equation*. This equation describes the relation between stresses (reaction) and strains or strain rates in each material element. For example, if the material can be modeled as *linear*

elastic, the stresses will be proportional to the strains, and will vanish when the load disappears. But the constitutive equation also requires the definition of the at-rest configuration of the building block (its shape in a mechanically isolated state).

Next, the structure is defined by assembling the material elements together (i.e., defining the toplogy of the way they bind together and the resulting geometry of this assemblage). This structure defines the domain of integration of the model.

Finally, the load itself is modeled (how much, in what directions, where in the structure?). This includes external forces, for example, a wind-drag force distributed over the plant, but also boundary constraints giving rise to reaction forces. As mentioned in the Introduction section, multicellular plants also display significant autostresses (internal loads) linked to their growth and turgor or to cell wall differentiation (Hejnowicz 1997; Moulia 2000; Moulia and Fournier 2009).

Once the material structure and load have been defined, the model can be built, using the principles of mechanics (such as static or dynamic equilibrium, or the compatibility of strains between adjacent material elements), plus any simplifying hypotheses needed, such as those defining rods and beams theory. This model can then be solved either analytically (e.g., Jaouen et al. 2007) or numerically (e.g., Moulia et al. 1994; Rodriguez et al. 2008). In many cases, the model cannot be solved, making simulation studies over time (i.e., numerical experiments) the only achievable option (e.g., Dupont and Brunet 2008). Depending on the objectives of the model, many outputs can be computed, such as displacement velocity of the top of the plant, its bending rigidity (e.g., Speck et al. 1990; Moulia and Fournier 1997), the resonance modes of its dynamic excitability (Spatz and Speck 2002; Sellier et al. 2008; Rodriguez et al. 2008), the risk of global buckling instability (Jaouen et al. 2007), or even the full distribution of the stresses and strains (e.g., Coutand and Moulia 2000; Sellier and Fourcaud 2009).

It should be noted that plants are open systems in which amount of material and eventually rheology change through growth. The combination of these factors with autostressing has deep mechanical implications and forge the specificity of biomechanical models (see Fournier et al. 2006). Moreover, as we will see later, the growth and autostressing processes are under the control of mechanosensing (see also Moulia and Fournier 2009).

ISM models can be built to integrate several scales, from cell wall component to cell wall (as, for example, in cell growth models; see chapter “Intracellular Movement. Integration at the Cellular Level as Reflected in the Organization of Organelle Movements”), from cell wall element to tissue behavior, or from tissue through to whole plant level. But it is difficult and potentially misleading to span the whole range of scales in a plant. The choice of the relevant scales is an important aspect of the art of mechanical modeling. It is also important to remember that models are essentially a quantitative formalization of a set of hypotheses, which means they have to be validated before being used as a tool for analyzing plant biomechanics or mechanoperceptive experiments (e.g., Moulia and Fournier 1997). Once validated, though, they can then be exploited to analyze the distribution of stresses and strains in tissue elements in experimental conditions. Using controlled stem bending to study thigmomorphogenetic responses, Coutand and Moulia (2000) employed

a validated composite-beam model of plant organs (Moulia and Fournier 1997) to assess the distribution of strains and stresses in the tissue elements. Other models can then be used to analyze the state of strains and stresses in a plant submitted to wind (e.g., Sellier et al. 2008), and thereby assess the ecophysiological range of mechanostimulation. Finally, models also make it possible to assess the functional performance of a given mechanical structure in terms of wind-induced fracture risks in plants (Gardiner et al. 2008) or buckling risks in saplings (Jaouen et al. 2007).

Extensive biomechanical modeling of plants has produced a small set of general features that can be usefully recalled here.

First of all, the intensity of stresses and strains within a plant organ is highly heterogeneous, both across the organ and across the component tissues. This heterogeneous spatial distribution does not depend solely on the location and intensity of mechanical loads. Geometry (length, diameter, tapering) and anatomy have major influences on stress and strain distribution across plant tissues within a plant organ (although acting in different combinations for stresses and strains; see Speck et al. 1990; Niklas 1992; Moulia and Fournier 1997; Coutand 2010; Boudaoud 2010 for details). This spatially heterogeneous distribution has profound consequences for the location of fracture risks in plants (Niklas 1992), as well for mechanosensing (Coutand and Moulia 2000, see Sect. 3). Note that the combination of load geometry and plant structure can lead to nonintuitive distributions. In pine trees under wind load, Ancelin et al. (2004) computed in many cases two maxima of bending stresses: one at the tree collar (as expected from lever arm effects) and one just below the crown. The ratio between the two peaks essentially depended on trunk taper.

Second, in a slender structure such as erect plant organs, both self-weight and wind loads acts mostly through organ bending. From a numerical example on a 38-m-high conifer tree (*Picea* sp.), Esser showed 65 years ago that static bending stresses overcome static compression stresses for a mean trunk inclination of only half a degree (!) or a mean wind velocity exceeding the low limit of 3.3 m s^{-1}, i.e., 12 km h^{-1} (see Moulia and Fournier 1997 for details on this calculation).

Focusing on plant morphology, the slenderness $S = L/R$ and the tapering of radius R along the length are probably the major mechanical characteristics of plant structures (Moulia and Fournier 1997; de Langre 2008; Sellier and Fourcaud 2009; Rodriguez et al. 2008; Jaouen et al. 2007; Almeras and Fournier 2009). For the same dynamic wind load, a 33% decrease in the trunk base diameter of an adult pine tree translates into a 60% higher stress at the stem periphery, whereas a change in height changes the distribution of stresses throughout the trunk (Sellier and Fourcaud 2009).

3 The Mechanosensing System in Plants: Integrative Sensing and Growth Control

As stated in the Introduction, very few studies have dealt with quantitative integrative mechanosensing. Moulia and coworkers (Coutand and Moulia 2000; Coutand et al. 2009) tackled the issue by producing the first integrative model of the

mechanosensing system in plants, the Sum of Strains Sensing Model. We will study this model in more detail. This is not meant to lend it an absolute value, for as with all models they can be proved, improved, or disproved. However, this model does, in its current state, builds up a synthesis of our understanding of mechanosensitive integration, and it defines the minimal baseline modules that have to be considered in any model.

3.1 From Plant Loading to Cell Sensing and Gene Expression

The smallest complete unit of mechanosensing is a cell. Subcellular structures such as mechanosensitive channels (Haswell Peyronnet et al. 2008) and cytoskeleton (Baluška et al. 2003) are obviously involved, but the complete processing of mechanical signals can only be achieved at whole-cell level or, due to cell-to-cell coupling (through plasmodesmata for example), at the level of a small cluster of cells, called a *tissue element*. As detailed earlier, the links between loads and the changes in mechanical state of tissue elements triggering cell mechanosensing are dependent on the mechanical structure of the plant. As most plant organs are made of tissues with diverse mechanical stiffness, the distribution of stresses across and organ and within a population of organs does not parallel the distribution of strains (Moulia and Fournier 1997; Coutand and Moulia 2000), making it critical to identify which of the two mechanical state variables is involved in mechanosensing.

3.1.1 Stress or Strain Sensing?

Taking advantage on the natural variability of elastic tissue stiffness and stem diameters to decorrelate bending strains from bending stresses, Coutand and Moulia (2000) were able to show that neither stress-based, force-based, or energy-based criteria could explain the 1:10 variability in thigmomorphogenetic inhibition of longitudinal growth generated through controlled stem bending in the elastic (i.e., reversible) range. However, strain-based criteria could explain up to 76% of this variability. Strain sensing is also further supported by observations that animal tissues with stiff extracellular matrix also respond to local tissue strains (e.g., bones; see Ehrlich and Lanyon 2002; Moulia et al. 2006). Consistently with a central role for strain sensing in plants, transgenic tobacco plants with xylem of reduced stiffness undergo enhanced xylem development and attain overall stem stiffness and thus strain levels comparable to wild type (Hepworth and Vincent 1999). Therefore, local strain of the tissue element is the proper variable for assessing local mechanical stimuli, at least in tissues with stiff extracellular matrix such as plant tissues (Coutand and Moulia 2000, reviewed in Braam 2005; Telewski 2006; Baskin 2006; Moulia et al. 2006; Coutand 2010; Boudaoud 2010).

This point needs to be emphasized, and discussed carefully.

- First of all, the semantics of the concept of "stress" can generate epistemological confusion. Linguistically, the noun "stress" derives from distress. It has since been adopted in mechanics, biology, and psychology, probably vectored by the common human experience that distress causes somatic internal tensions (muscular autostresses). However, while stress then received a strict definition in mechanics with a clear dual opposition with strain, the definitional situation has not yet been cleared up in biology, as illustrated by the definition of stress as "any strain that disturbs the functioning of an organism" (Stress 2010). This situation has, for many biologists, fostered the implicit assumption that (mechanical) stress is the sensed variable.
- Second, the results by Coutand and Moulia (2000) demonstrate that mechanical strain is the local tissue variable making it possible to ascribe a local mechanical stimulus to a group of cells. This does not mean that stresses *within* the cell are not involved, but each cell is enveloped in a cell wall that is much stiffer than the cell itself, with the result that cell-wall stresses dominate the rheological behavior of the tissue element (see Niklas 1992 and even Hamant et al. 2008 on the thin-walled meristematic cells). If the mechanosensory system of the cell is unchanged but cell wall stiffness is doubled, then it will take twice as much force and wall stresses to bring the cell elements to the same level of stretching (strain) and internal reaction (stress *in* the cell microstructure).
- Reports of correlations between the intensity of the response and the applied force (and stresses) based on a large variation in the applied force and a low variation in the load-bearing mechanical structure (e.g., Jaffe et al. 1980; Mattheck and Bethge 1998) have failed to discuss the issue of stress versus strain sensing. Indeed, force stresses and strains then covary, making it is impossible to decipher their individual influences. The demonstration in Coutand and Moulia (2000) is more than correlative. It stems from a mechanical (and hence mechanistic) dissection of the mechanosensing process, combining biomechanical modeling with experimental assessment.

The stress versus strain sensing debate has recently experienced a revival following the analysis of mechanosensitive microtubule reorientation in shoot apical meristems by Hamant et al. (2008) (see also Boudaoud 2010 for more insights). By combining mechanical modeling and imaging, the authors elegantly demonstrated that microtubule orientation in shoot apical meristems was driven by tissue mechanics. They also claimed that microtubule direction was better explained by principal tissue stresses than by principal tissue strains (especially at the hinge between the meristem and the primordial). This may result from the peculiar profile of apical meristematic cells, in which the cell wall is so thin and flexible that the stiffness of the cytoskeleton may be no longer negligible. However, this is at odds with the mechanical model developed to estimate stress and (auto)strain fields, which completely neglects the microtubules as a load-bearing cytoskeleton (see note S1 in Hamant et al. 2008, supplementary online data), and the phase-transition dynamics of these polymerizing–depolymerizing structures (Trepat et al. 2007;

Foethke et al. 2009). A better explanation may stem from the hypothesis that different sensing pathways may be involved, with different mechanical relations with the cell wall. Mechanosensing of external loads is thought to involve mechanosensitive ionic channels that sit in the soft cellular membranes, and are gated by membrane tensional stresses (Peyronnet, et al. 2008). They thus cannot sense wall stresses, but are just stretched following cell wall strains. On the contrary, direct adhesion domains exist where the cytoskeleton elements link to the cell wall (see Baluška et al. 2003; Coutand 2010). These linkers are partially embedded into the cell wall, and hence cell wall stresses are directly transmitted to them. At last, it should also be noted that tissue wall strains and stresses were computed by Hamant et al. (2008) through a complex model incorporating several other strong hypotheses on the rheology of cell wall and water fluxes and the processes governing anisotropic deposition of cellulose microfibrils and cell division. All these hypotheses have yet to be validated, and are all crucial for the definition of the rest shape of cell elements, and thus of their strain. The argument by Hamant et al. (2008) is thus more indirect than the one by Coutand and Moulia (2000) and should be confirmed by more studies. Anyhow, leaving the particular issue of the mechanosensing of growth-autostresses anisotropy by cytoskeleton in the very thin wall apical cells behind and coming back to thigmomorphogenetic responses to external loads, tissue strains have been found to be the relevant variable for measuring the internal mechanical stimulus applied to cells in the nonmeristematic plant tissues (also see Coutand 2010).

Once the mechanosensed local tissue variable has been established, the next step is to understand the mechanosensing integrative process. One approach has been to proceed by analogy with structural modeling in mechanics (as presented in Fig. 1). It was thus necessary to define (1) the local strain-sensing function of a cell (equivalent to the *constitutive equation* in mechanics), and (2) an integration mechanism within the perceptive structure of the plant that calculates the plant's total sensing activity, S_i. As the focus of interest includes the thigmomorphogenetic responses of the plant, plant response modules have been added relating S_i with primary growth (Coutand and Moulia 2000), secondary growth (Coutand et al. 2009), and with the quantitative expression of primary mechanosensitive genes (Coutand et al. 2009).

3.1.2 The Local Mechanosensing Function of a Cell

Among the mechanisms involved in mechanosensing, mechanosensitive ionic channels have attracted the most detailed quantitative studies through the patch–voltage–pressure–clamp technique on protoplasts (plant cells enzymatically stripped of their cell wall, e.g., Ding and Pickard 1993; Peyronnet et al. 2008). By altering turgor pressure, thereby inducing strains (and tensional stresses) in the plasma membrane, and monitoring ionic current after clamping the voltage, it becomes possible to quantitatively characterize the response of mechanosensitive channels. The general shape of these response curves is sigmoidal and can easily be linearized

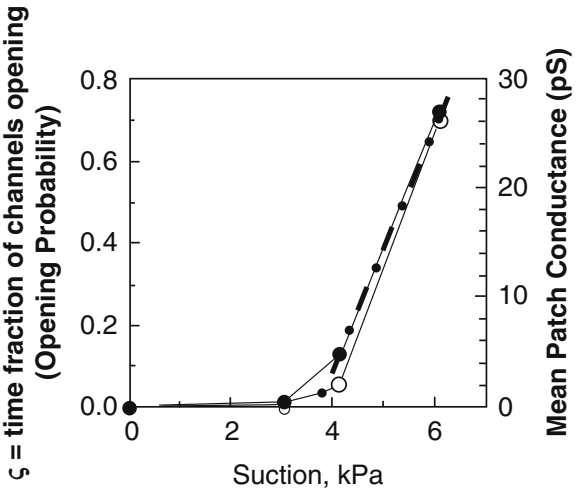

Fig. 2 Opening probability of mechanosensitive channels (MSCs) and mean patch conductance as a function of patch depression (and hence membrane tension and MSC strain) *open circle*, *filled circle* two replicates, *dashed dotted line* linear fit; modified from Ding and Prickard, 1993. Copyright © 1993, The Plant Journal, John Wiley and Sons

in the range of small strains (Fig. 2). Based on these data, Coutand and Moulia (2000) made the assumption that in *the range of small tissue strains*, the local mechanosensitive function of a tissue element could be approximated through a linear function over a threshold, i.e.,

$$\mathrm{d}S_\mathrm{i} = k_\mathrm{s}(\varepsilon - \varepsilon_0)\mathrm{d}V \quad \text{if } \varepsilon > \varepsilon_0, \text{ else } \mathrm{d}S_\mathrm{i} = 0, \tag{1}$$

where $\mathrm{d}S_\mathrm{i}$ is the local signal in the cell (in Fig. 2, $\mathrm{d}S_\mathrm{i}$=dI, where I is the ionic current), k_s is a mechanosensitivity factor ($k_\mathrm{s} = 0$ translates as insensitive tissue, while higher k_s equates to more sensing), ε is the local mechanical strain of the tissue element, ε_0 an eventual strain threshold or minimal effective strain ($\varepsilon_0 \geq 0$) (see Moulia et al. 2006 for a review), and dV is the volume of the tissue element.

Equation (1) assumes that only tensile strains are sensed ($\varepsilon > \varepsilon_0 \geq 0$), but also extends straightforwardly to the case where both tensile and compressive strains are sensed proportionally to their absolute value, as observed in animal bone tissues (Schrieffer et al. 2005).

Using a model for mechanosensing integration (to be discussed later), Moulia and coworkers have shown that this hypothesis could quantitatively explain the variation of thigmomorphogenetic responses for both primary growth (Coutand and Moulia 2000) and secondary growth (Coutand et al. 2009). Moreover, they were recently able to directly assess equation (1) experimentally (Coutand et al. 2009) by measuring the expression of a primary mechanosensitive gene coding for a zinc finger protein, ZFP2, with probable transcription factor function (Leblanc-Fournier et al. 2008; Martin et al. 2009). The expression pattern of *ZFP2* makes it a good marker for assessing the mechanosensing function. Indeed, it is very quickly and transiently overexpressed (detected as early as 5 min after tissue straining), and only in the strained tissues (probably in a cell-autonomous manner). The response of the cell mechanotransduction pathway (from primary reaction in the cytoplasm to primary gene expression in the nucleus) could thus be assessed by measuring the relative quantitative abundance of *ZFP2* transcripts, Q_r, using Quantitative Real-Time PCR (Coutand et al. 2009, in *Populus tremula*alba* (*Pta*)).

Q_r is the ratio between the content of *Pta ZFP2* transcripts in the strained tissue elements $n_t(\varepsilon)$ and the content of an unstrained control $n_t(\varepsilon = 0)$ (McMaugh and Lyon 2003; an eventual correction for similar volume in both samples is achieved through a multiplicative dilution prefactor, estimated through the assessment of the reference gene(s), not shown here). If the model in (1) holds for the entire mechanotransduction pathway, then it predicts that the increment of the content of *Pta-ZFP2* transcript in a strained tissue element should be:

$$n_{t(\varepsilon)} - n_{t(0)} = k_{mt}(\varepsilon - \varepsilon_0)\mathrm{d}V,$$

$$\text{i.e., } n_{t(\varepsilon)} = k_{mt}(\varepsilon - \varepsilon_0)\mathrm{d}V + C_0\,\mathrm{d}V, \tag{2}$$

where k_{mt} is the sensitivity of the local mechanotransduction pathway, and C_0 is the transcript concentration in the unstrained control (or baseline concentration), meaning the predicted Q_r, noted $\hat{Q}_r$, should lead to

$$\hat{Q}_r = \frac{n_{t(\varepsilon)}}{n_{t(0)}} = \frac{k_{mt}}{C_0}(\varepsilon) - \left(\frac{k_{mt}}{C_0}\varepsilon_0 - 1\right). \tag{3}$$

As the stems were strained though bending, the strains were not uniform across the cross-section. Moreover, the volume of stem collected for QPCR was small but not infinitesimal (typically 200 mm^3) meaning volume-averaged strains have to be considered. Thus, (3) cannot apply directly. If we call $N_t(\varepsilon)$ the total number of transcripts in the volume of strained tissue analyzed, and $N_t(\varepsilon)$ the content of an unstrained control $N_t(\varepsilon)$ over the same volume V, then both are the sum of the contents of all the tissue elements over the volume V

$$N_{t(\varepsilon)} = \iiint_V k_{mt}(\varepsilon - \varepsilon_0)\mathrm{d}V + \iiint_V C_0\,\mathrm{d}V. \tag{4}$$

If we assume that the mechanosensitivity of the cells, k_{mt}, and the baseline transcript concentration, C_0, are constant within the segment of organs under study (tissues of same age, and same history), then

$$Q_{rorgan} = \frac{N_{t(\varepsilon)}}{N_{t(0)}} = \frac{k_{mt}}{C_0}\left(\frac{\iiint_V \varepsilon\,\mathrm{d}V}{\iiint_V \mathrm{d}V}\right) - \left(\frac{k_{mt}}{C_0}\frac{\iiint_V V\varepsilon_0\,\mathrm{d}V}{\iiint_V V\mathrm{d}V} - 1\right)$$

$$\text{i.e., } Q_{rorgan} = \frac{k_{mt}}{C_0}\bar{\varepsilon} - \left(\frac{k_{mt}}{C_0}\bar{\varepsilon}_0 - 1\right). \tag{5}$$

Indeed, the experimental relation between measured Q_r and volume-averaged strain $\bar{\varepsilon}$ was found to be linear (Fig. 3), with (5) explaining 77% of the 1:500 variation of Q_r. The results in Fig. 3 thus validate the local model of (1). They also give the first *in planta* measurement of the mechanosensitivity of the mechanotransduction

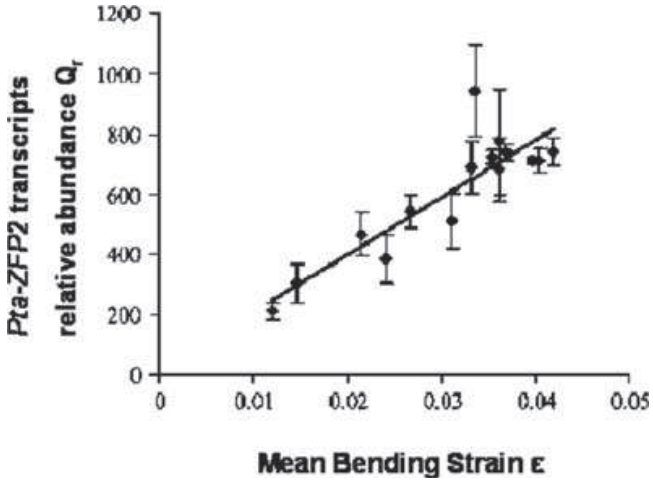

Fig. 3 Measured relative transcript abundance, Qr, of the primary mechanosensitive gene *Pta ZFP2* (assessed by Q-RT-PCR) and the volume-averaged strain in the bent stem-segment (i.e., Sum of the strain normalized by the volume of the bent tissue; Coutand et al. 2009). © 2009 Plant Physiology, American Society of Plant Biologists

pathway in a plant tissue. Under the conditions of this experiment (young poplars sitting in a controlled growth cabinet with minimal mechanical stimulation before the experiment), a 1% strain induces a (transient) 200-fold increment in the number of transcripts of the primary mechanosensitive gene *Pta-ZFP*2!

It was surprising that the bending strain range in which this linear mechanosensing model holds true goes up to (at least) 5%, i.e., quite large strains, way beyond the range of elastic strains in cell walls. This probably reflects the much greater flexibility of cell components compared to the cell wall (see Sato et al. 2005).

3.2 *Quantifying Global Thigmomorphogenetic Responses*

At this point, to properly set the problem of integrated mechanosensing, we now need to consider the global growth responses of the plant in greater depth. This was made possible by using a quantitatively controlled bending device while continuously monitoring primary elongation (Fig. 4a) or secondary thickening (Fig. 4b) using Linear Voltage Displacement Transducers (LVDT; Coutand et al. 2000). Figure 4 clearly demonstrates that an elastic bending restricted to the basal part of the stem induced a thigmomorphogenetic response in the distant primary growth zone, so that a long-range internal secondary signal S_i traveled from the bent tissues to the responding primary tissues (Coutand et al. 2000; also see Brenner et al. 2006). In contrast, the secondary growth response seems local to the bent zone (Coutand Martin et al. 2009), as previously argued by Mattheck and Bethge (1998). In both cases, the early response is that growth stops for one to a few hours, then restarts and eventually recovers the control rate. For primary growth, recovery time is highly dependent on the amount of bending strain, typically ranging from 100 to 1,000 min. From then on, no compensatory growth is observed, meaning that the final length of the plant is decreased (by -2 mm/bending in Fig. 4c). Secondary growth, though, shows clear and long-lasting growth stimulation after the initial inhibition, with growth rate increasing over 3 days then relaxing to the control rate for 3–4 more

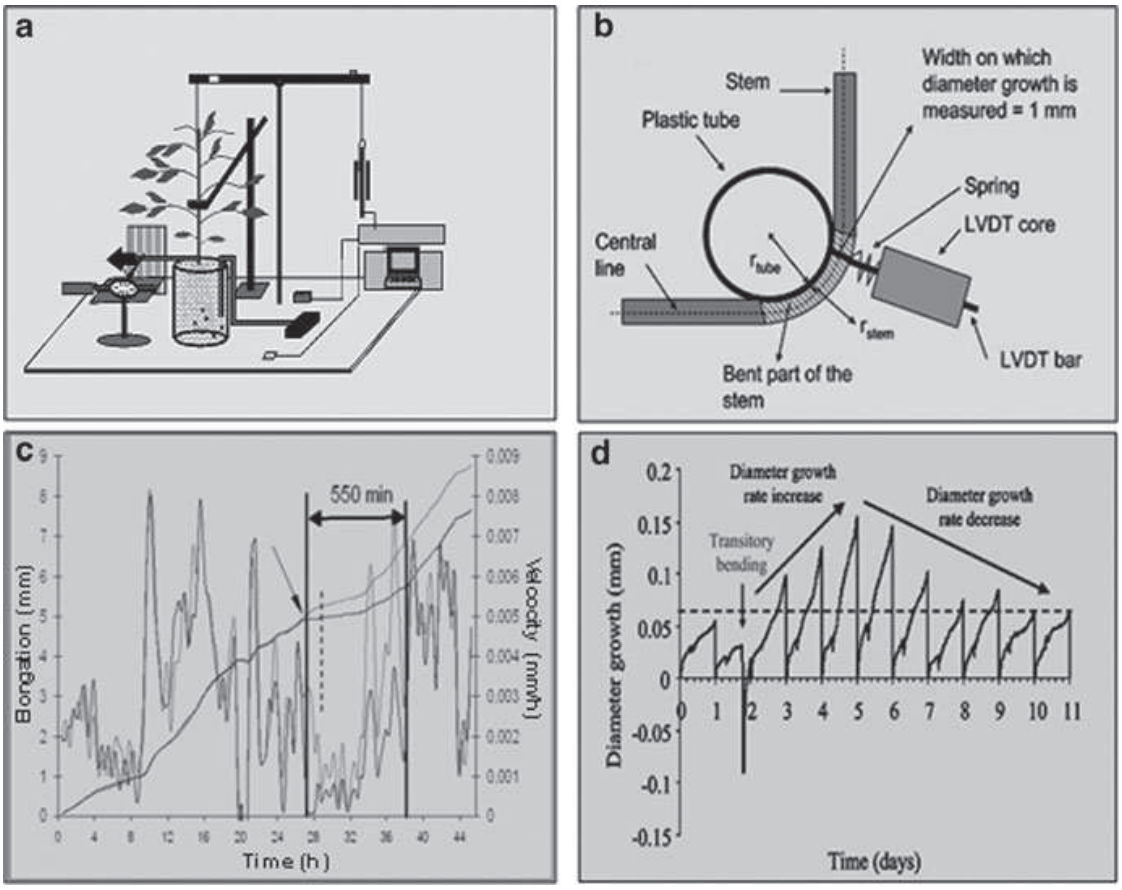

Fig. 4 Experimental setup for applying controlled, localized, and quantified bending strains while continuously monitoring growth using Linear Voltage Displacement Transducers (Coutand et al. 2000, 2009; Coutand and Moulia 2000 reproduced by kind permission of Journal of Experimental Botany and Oxford University Press). (**a**) Controlled elastic bending of the basal part of the stem (distant from the primary growth zone) while continuously monitoring primary elongation, r; (**b**) Controlled bending of the basal part of the stem, while continuously monitoring secondary radial expansion growth at the bend site; (**c**) Chart of primary elongation growth after one transient bending of the stem base *black*= bent plant *gray*= control plant; (**d**) chart of secondary radial growth after one transient bending of the stem base (as the responses lasted several days, LVDT was reset to zero daily to enable easy comparison of daily growth increments)

days. The effect of this stimulation of secondary growth (+0.35 mm/bending in Fig. 4d) was approximately 30 times higher than the effect of the initial inhibition, resulting in an overall stimulation of radial growth. In contrast with primary growth, the timing of the response seemed to be much less dependent on the level of bending strain than the peak (and total) increment in growth rate (Coutand et al. 2009, 2010).

3.3 Integrating Local Mechanotransduction into Plant Mechanosensing: The Sum of Strain Sensing Model (S^3m)

It is a huge task to integrate all the physiological factors and genetic regulations involved in thigmomorphogenesis. Transcriptomic studies in *Arabidopsis thaliana*

revealed that some 760 genes (over 3% of the genome) had their expression regulated 30 min after a mechanical stimulation (Lee et al. 2005). Even considering leaves only, Fluch et al. (2008) found at least 192 genes under transcriptional regulation in poplar. Besides the size of the transcriptomic regulation network, the complexity of long-distance signaling is also a factor (e.g., Brenner et al. 2006).

When dealing with a structural mechanics model applied to the bending of a plant organ (e.g., Moulia and Fournier 1997; Sellier and Fourcaud 2009, see Fig. 1) there are billions of molecules and bounds involved. However, it takes just a handful of equations to (1) capture the change in scale between the mechanical behavior of a tissue element and of the whole organ, and (2) to understand the effect of the plant's mechanical structure on its mechanical function. Indeed, a useful mechanistic integrative model is not meant to integrate every aspect of real-world settings but only those necessary to explain a given phenomenology. It remains just a tool for testing a set of interacting hypotheses against experimental data.

Working from this idea, Moulia and coworkers intended to build a *minimal model* of mechanosensing integration, from the level of the strained tissue element up to the thigmomorphogenetic growth responses in the entire stem (Coutand and Moulia 2000). This model is designed to chart the effects on the global thigmomorphogenetic responses of both the mechanical structure and the *mechanoperceptive structure* (the anatomical distribution of mechanosensitive tissues within the plant) of the organ. It is built in analogy with the process of integrative modeling in structural mechanics (ISM, see Fig. 1).

The starting point was equation (1) which quantifies local mechanosensing in a tissue element. As mechanostimulation sparks signals to move out of the cell, it was assumed that the secondary signal output by each cell, $\mathrm{d}S_\mathrm{o}$, is directly proportional to the mechanotransduction signal over an eventual threshold – and hence to $\mathrm{d}S_\mathrm{i}$ (hypothesis H1), and can thus be written as:

$$\mathrm{d}S_\mathrm{o} = k \cdot \mathrm{d}Si = k_\mathrm{o}(\varepsilon - \varepsilon_0)\mathrm{d}V \tag{6}$$

(with $k_\mathrm{o} = k \times k_\mathrm{i}$). We have seen previously that the timeframe for long-distance signal propagation was very short compared with growth response. It was thus assumed that for our purposes, the details of the signal propagation, especially signal damping, could be neglected. The simplest model for the integration of the mechanical sensing is then that the output signals, $\mathrm{d}S_\mathrm{o}$, of all the mechanosensitive cells simply sum up into a global secondary internal signal S_i (hypothesis H2). In short, the more cells are strained, the higher the S_i.

However, the domains of mechanosensitive integration seem to differ for the responses of primary and secondary growth zones. Subapical primary growth responds to distant sensing throughout the stem volume V_s, whereas distributed cambial growth only seems to be affected by strain-induced signals propagating radially in the cell layer of the cross-section A_s. The internal signal propagated axially along the whole stem and controlling the response of primary growth $S_{\mathrm{i},1}$ can then be written as (Coutand and Moulia 2000):

$$S_{i,1\,(\varepsilon)} = \iiint_{V_s} k_{o(\varsigma,y,z)}\left(\varepsilon_{(\varsigma,y,z)} - \varepsilon_0\right)\mathrm{d}V, \tag{7}$$

where ζ is the distance from the apex and (y, z) describes the position of the tissue elements across the cross-section of the stem. By analogy, the internal signal propagating along the stem radius and controlling secondary growth, $S_{i,2}$, in the cambium at a position ζ on the stem thus becomes (Coutand et al. 2009):

$$S_{i,2(\varepsilon,\varsigma)} = \iiint_{\mathrm{lc}A_s(\varsigma)} k_{\mathrm{o}(\varsigma,y,z)}\left(\varepsilon_{(y,z)} - \varepsilon_0\right)\mathrm{d}x\,\mathrm{d}y\,\mathrm{d}z = lc \iint_{A_s(\varsigma)} k_{\mathrm{o}(\varsigma,y,z)}\left(\varepsilon_{(y,z)} - \varepsilon_0\right)\mathrm{d}y\,\mathrm{d}z, \tag{8}$$

where l_c is the typical length of an initial cell in the cambium (or, in a more practical way, the longitudinal length over which radial growth is measured, i.e., the size of the LVDT pad sitting on the stem in Fig. 4b, which was 1 mm in that particular case; Coutand et al. 2009).

Note that the distribution of mechanosensitive tissues defining the mechanosensitive structure of the plant (of volume V_s and cross-sectional area $A_s(\zeta)$ at position ζ on the stem) does not span the whole stem volume but only the mechano-competent tissues. More precisely, the mechanosensitive structure of the plant (at a given time point) is given by the geometrical description of mechanosensitivity $k_o(\varsigma, y, z)$ and threshold $\varepsilon_o(\varsigma, y, z)$, just as the mechanical structure of the plant is given by the spatial distribution of the mechanical properties (e.g., the longitudinal Young's modulus $E_L(\varsigma, y, z)$ and a yield threshold $\sigma_0(\varsigma, y, z)$).

In a first approximation, Coutand and Moulia (2000) assumed that lignified tissues could be considered nonsensing, and that all living tissues had similar mechanosensitivity k_o. Using *Jr- ZFP2* in situ RNA hybridization as a marker of mechano-competence in walnut stems (*Juglans regia*), Leblanc-Fournier et al. (2008) found that the cortical and medullar parenchyma of stems (and to a lesser extent some phloem parenchyma cells) displayed marking. The stiffer epidermal cells, collenchyma, xylem, and sclerenchyma did not, nor did the meristematic cambium [this lack of marking in cambial and epidermal cells is another argument in favor of a nonautonomous mechanosensitive control of cell growth, as expressed in (7) and (8)]. However, comparative tests on the *Sum of Strain Sensing* model have shown that the model output only marginally depends on the detailed distribution of mechanosensitivity (Coutand and Moulia 2000), at least in the range of anatomical variability displayed by plants from the same cultivar at the same growth stage. The most determinant factor was the geometry of the stem. For simplicity purposes, more recent studies then took mechanosensitivity to be homogeneous over all tissues (e.g., Coutand Martin et al. 2009). If $k_o(\varsigma, y, z)$ and $\varepsilon_o(\varsigma, y, z)$ are constant, then they can be factorized in the spatial integrals, so that the model evolves to

$$S_{i,1(\varepsilon)} = k_o\left(\iiint_{V_s} \varepsilon_{(\varsigma,y,z)}\,\mathrm{d}V\right) - k_o\varepsilon_0\,V_s = k_o S_{1\,\mathrm{strains}} - \Sigma_0, \tag{9}$$

$$S_{i,2(\varepsilon)}(\varsigma) = k_{\mathrm{o}}\left(l_{\mathrm{c}} \cdot \iint\limits_{\mathrm{As}(\varsigma)} \varepsilon_{(\varsigma,y,z)}\,\mathrm{d}y\,\mathrm{d}z\right) - k_{\mathrm{o}}\varepsilon_0 l_{\mathrm{c}} A_{\mathrm{s}} = k_{\mathrm{o}} S_{2\,\mathrm{strains}}(\varsigma) - \Sigma_{0,2}(\varsigma). \quad (10)$$

This model thus predicts that the integrated signals are linearly dependent on integrals of the strain field over the domains of mechanosensitive integration for primary and secondary growth ($S_{1\mathrm{strains}}$ and $S_{2\mathrm{strains}}$, respectively). This is what prompted the original name for the "Sum of Strains" model. However, a more accurate name is the "*Sum of Strain Sensing*" *model* (S^3m), as it is not the strain that is summed but the output of strain sensing by cells.

This minimal model of mechanosensitive integration was tested quantitatively versus the corresponding growth responses described earlier (Fig. 4a, b) not only by varying the applied force but also by sampling the variability of plant structure (size, anatomy, distribution of stiff tissues, and mechanocompetent tissues). In this case, the prediction of the S^3model is not that the growth response should be linear with S_{istrains} (as it was for the relative abundance of transcripts) but that the growth responses should give a dose–response curve on the Sum of Strains. Indeed, if collecting more cells necessarily entails adding RNA to the sample (linearity), the biological thigmomorphogenetic response of growing tissues to the (candidate) integrated signal S_{i} may not be additive.

As shown in Fig. 5, a tight logarithmic relation was found between primary growth response (recovery time τ_{r}) and $S_{1\mathrm{strains}}$, explaining 75% of the 1:10 variation in the response (Coutand and Moulia 2000).

$$\tau_{\mathrm{recovery}} = a_1 \cdot \ln\left(\frac{S_{1\,\mathrm{strains}}}{S_{0_{1\mathrm{strains}}}}\right) \quad \text{for } \mathrm{S}_{1\,\mathrm{strains}} > S_{0_{1\mathrm{strains}}}. \quad (11)$$

Similarly, a relation was found between $S_{2\mathrm{strains}}$ and the response of radial growth, again explaining 75% of the 1:5 variation generated by varying stem bending and size (Coutand et al. 2009). A first experiment on poplar seemed to reveal that a linear relationship between radial growth response and $\mathrm{S}_{2\mathrm{strains}}$ was statistically slightly more significant than a logarithmic relation. However, further analysis on a

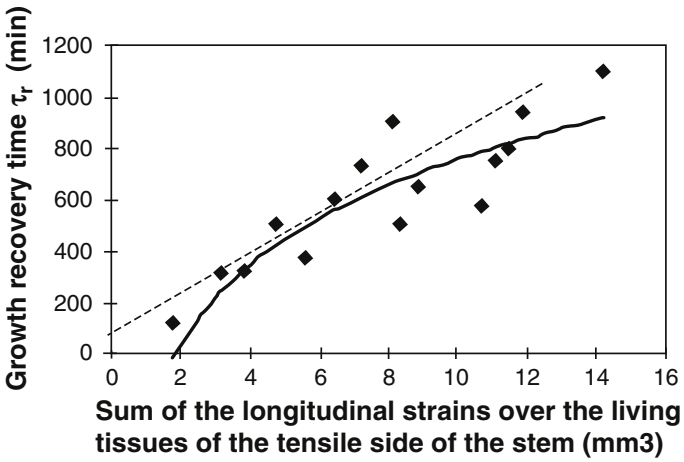

Fig. 5 Dose–response curve of the recovery time of the primary growth response to the Sum of Strains *dashed dotted line* linear fit, *block line* log fit, *filled diamond* experimental results (adapted from Coutand and Moulia 2000 reproduced by kind permission of Journal of Experimental Botany and Oxford University Press)

set of dicot tree species (Coutand et al. 2010) showed that the logarithmic relation is more generic, and thus to be preferred (also see Telewski 2006).

These logarithmic relations need to be discussed.

- First, they are in agreement with the "Weber–Fechner law" widely (though not always) observed in human and animal sensory physiology (Weber's Law 2010), and in plant gravisensing (see Moulia and Fournier 2009). The Weber–Fechner law states that "the change in a stimulus that will be just noticeable is a constant ratio of the original stimulus." Indeed, differentiating equation (11) and noting the growth response, G, gives

$$\mathrm{d}G = a\left(\frac{\mathrm{d}S_{\mathrm{istrains}}}{S_{\mathrm{istrains}}}\right) = \frac{a}{S_{\mathrm{istrains}}}(\mathrm{d}S_{\mathrm{istrains}}). \tag{12}$$

  The increment in growth response upon an increment in the Sum of Strains signal is inversely proportional to the prevailing level of the signal, so that the apparent sensitivity of the response decreases hyperbolically with the prevailing level of mechanical stimulus. This tuning of the sensitivity of the response is termed "accommodation" (Schrieffer et al. 2005; Moulia et al. 2006), and it is likely to be of major adaptive value by avoiding overreactions to noise. Indeed, in wind, the standard error of wind velocity fluctuations increases proportionally to mean wind velocity (Stull 2007). Using a protocol of 0–80 repeated back-and-forth manual basal bending sways in *Ulmus americana*, Telewski and Pruyn (1998) also found a logarithmic relationship between change in height and number of sways (the amount of bending was uncontrolled but the number of sways is likely to correlate with the total Sum of Strains due to the sways). The logarithmic dose–response curve is thus likely to apply to repeated bending during wind sways (swaying frequency was not measured either, but experience tells us it was probably close to the fundamental modal frequency of the plant, which is much easier to achieve manually).
- Second, the $S_{0_{1\mathrm{strains}}}$ threshold should not be confused with the Minimal Effective Strain threshold ε_0 of the local sensing function. $S_{0_{1\mathrm{strains}}}$ is the threshold for reception of the global systemic signal reaching the growth zone, expressed using its Sum of Strain component. A more complete specification of the model using (9) and (11) should be

$$G = a'_1 \cdot \ln\left(\frac{S_{i,1(\varepsilon)}}{S_{0i,1}}\right) = a'_1 \cdot \ln\left(\frac{k_{\mathrm{o}} S_{1\,\mathrm{strains}} - \Sigma_{0,1}}{k_{\mathrm{o}} S_{0_{1\,\mathrm{strains}}} - \Sigma_{0,1}}\right). \tag{13}$$

  Data analysis showed that the strain threshold ε_0 (and its integral Σ_0) could be neglected for both the thigmomorphogenetic responses of primary and secondary growth under the conditions of the experiments, whereas $S_{0_{\mathrm{strains}}}$ threshold could not (but see further discussion in Sect. 3.5). Thus, $S_{0_{\mathrm{strains}}}$ is likely to depend on the receptor pathway in the growing tissues (and possibly on propagation from the sender mechanosensitive tissues to the receiver growing tissues).

- Third, the global thigmomorphogenetic sensitivity of a plant can be described quantitatively using only two parameters for primary growth response (a_1, $S_{0_{1strains}}$) (Coutand and Moulia 2000) and two for secondary response (a_2, $S_{0_{2strains}}$) Coutand et al. 2010). These two quantities are integrative "macro-characters" (Tardieu 2003). They include the whole in-plant signaling process but through explicit and validated mechanical and mechanosensitive integrations of the interactions between the mechanical and mechanoperceptive structures of the plant and its mechanical environment (load). Varying load and/or plant size and anatomy affects the $S_{1strains}$ value along the x-axis in Fig. 5, and thus the value of the response, but the relation expressed in (11) and (12) (and the corresponding log response curve) remain invariant. This relation and the corresponding parameters in (11) are thus independent of both load intensity and plant size/structure. They measure *intensive quantities*.
- Finally, (11) is not to be confused with a standard dose–response curve. Indeed, it involves an explicit integration of the effect of the mechanical and perceptive structures of the plant through the *Sum of Stain Sensing* model – a model that has been assessed experimentally. This is to be contrasted with purely correlative dose–response curves with an "arbitrarily chosen" measure of the stimulus (e.g., force; Jaffe et al. 1980).

3.4 S^3m, a Global Model of Thigmomorphogenesis?

The Sum of Strain Sensing model uses the distribution of tissue strains across the plant, $\varepsilon(x,y,z)$, called strain field, as an input. This distribution can be measured directly using kinematic methods (strain, contrary to stress, is an observable; see Moulia et al. 1994; Moulia and Fournier 2009). However, it is more informative to couple the S^3m model with a mechanical model which outputs the strain field as a function of the load, plant geometry, and plant tissue rheology (Fig. 6). Indeed, the filtering of environmental signals via the plant's mechanical structure and the consequences of multicellularity on the internal mechanical environment of the cells is then taken into account mechanistically. This one-way coupling (Mechanical model ⇨ $\varepsilon(x,y,z)$ ⇨ S^3m model) was realized by Coutand and Moulia (2000) to study primary growth response to controlled bending. This was actually the first attempt to experimentally assess the Sum of Strain Sensing model, and it also illustrates how an integrative model acts as an interpretative tool for an experiment.

An important step forward would be to achieve full coupling between a mechanic model and the S^3m model, namely to also implement the S^3m model(t) ⇨ growth response(t) ⇨ Mechanical model($t + 1$) ⇨ $\varepsilon(x,y,z,t + 1)$ ⇨ S^3m model $(t + 1)$ . . ., as described in Fig. 6. This would be the first attempt to time closure the model and explain thigmomorphogenetic acclimation over the plant growth and development time course (Moulia et al. 2006). Moreover, it represents an attempt to include mechanics and physics constraints into a very simple biological growth model (Fig. 6). This kind of fully coupled model could be characterized as a

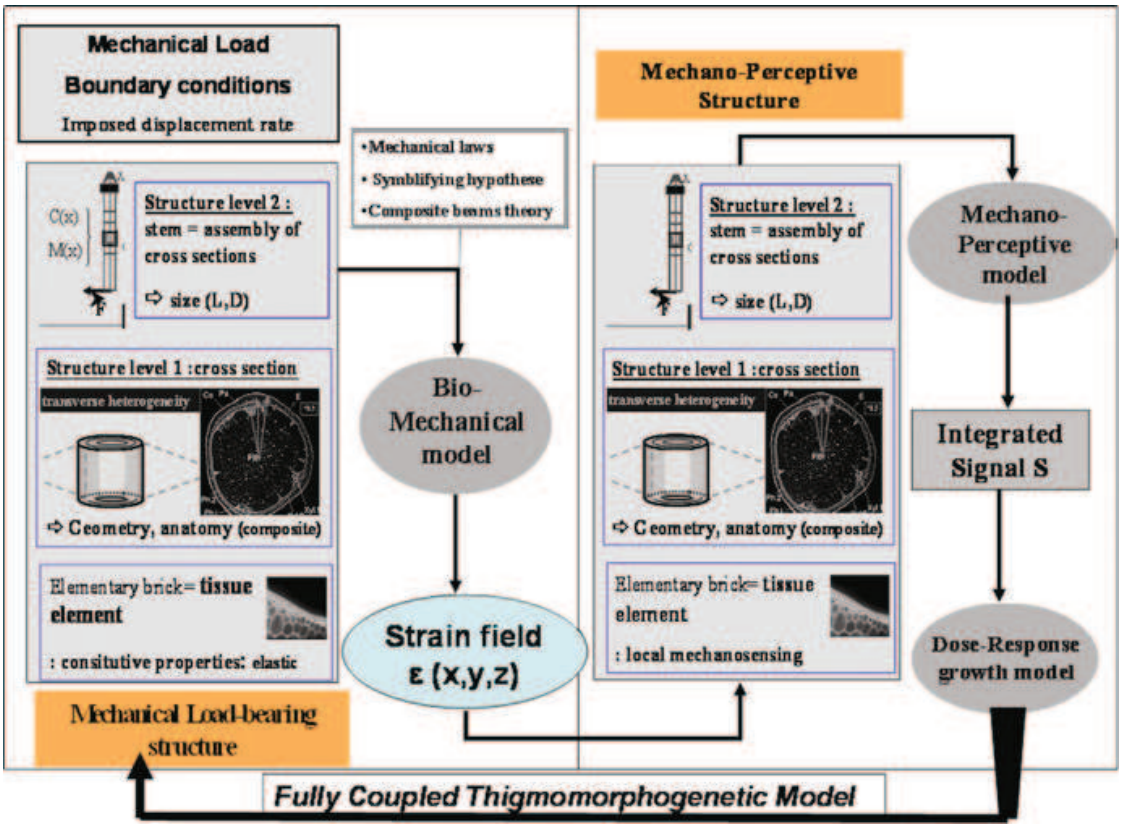

Fig. 6 Diagram of a fully coupled integrative dynamic model of thigmomorphogenesis. The ISM model of the mechanical load-bearing structure (*left*) was designed by Coutand and Moulia (2000) to analyze their bending experiments. It is based on a validated composite-beam model of plant organ flexion (Moulia and Fournier 1997). Its inputs are the curvature field, C, and the bending moment, M, along the stem (measured as in Moulia et al. 1994). Its parameters are (1) length, L, and diameters along the stem, $D(\zeta)$; (2) applied shear force, F; (3) anatomical cross-sectional images processed using the model by Moulia and Fournier (1997); and (4) estimates of tissue stiffness (longitudinal Young's moduli (Coutand and Moulia 2000). Like all models based on beam theory, this model defines two integration levels: the cross-section, and then the stem. The Mechanosensitive model is the model presented in Coutand and Moulia (2000). Its inputs are the strain field, $\varepsilon(x,y,z,t)$, in each stem, and the stem geometry factors L and $D(\zeta)$. Its output is the integrated signal S_i. It goes on to feed a model of thigmomorphogenetic growth responses (Coutand and Moulia 2000, Martin et al. 2009). In a fully coupled dynamic model of thigmomorphogenesis, the outputs of the thigmomorphogenetic growth response module can be used to update the size and geometry of the stem at the next time step, enabling time integration to be processed (or at least simulated)

dynamic model with dynamic structure (Prusinkiewicz and Rolland-Lagan 2006) performing the spatial and time integration of the thigmomorphogenetic control of growth, together with the integration of primary and secondary growth responses. Starting with initial conditions (the plant at an initial stage) and receiving external loading as an input, it may account for the complete thigmomorphogenetic syndrome of developmental acclimation over time. In particular, it could help explaining the phenotypical plasticity of stem allometries (slenderness and tapering) in response to different types of mechanical loads (artificial loads, isolated plant under wind, plants in canopy). This integration remains to be achieved and studied

mathematically and experimentally. It does, however, raise the question of time integration over longer periods and over repeated loads.

3.5 *Time Integration of Mechanosensing: The Problem of Slow Accommodation*

Mechanical loads in nature do not usually occur as a single bending (although postbuckling bending is an exception). Meteorological variations in wind usually result in windy weather alternating with quiet weather, in time patterns that follow climatic trends but are usually at days scale (Stull 2007). The effect of daily recurring chronic mechanical loads on plant mechanosensing has been studied by Martin et al. (2010) on poplar. The crucial point here was that the amount of Sum of Strain was kept almost constant along the nine successive daily bendings. As stem diameter necessarily changed over time, the bending to be applied to achieve an almost constant Sum of Strains was recalculated daily.

Secondary growth after one single bending reproduced the time course expected from previous studies (Fig. 7a). The effects of repeated daily bending were additive for the three first bendings but they then clearly departed from being additive. This was tested by comparing experimental results against two hypothetical models of time integration. In model 9x1B, the nine successive bendings are assumed to induce additive effects, making it a time extension of the logic of the Sum of Strain Sensing model, with successive strains also summing up over time (see also Coutand et al. 2010). In model 3x1B, only the three first bendings were assumed to have additive effects and were followed by complete desensitization. The experimental results clearly departed from 9x1B but were statistically very similar to the 3x1B model throughout the loading period, thus demonstrating a clear and intense desensitization after 3 days (the alternative explanation of growth saturation was ruled out; see Martin et al. 2010). This desensitization could be reversed, but only after 7–10 days without stimulation (similar behavior has been observed in mammal bones with even longer characteristic times at around 3 weeks; Schrieffer et al. 2005). This demonstrates a long-term accommodation of mechanosensitivity to daily repeated bending (after an initial additive behavior), which contrasts with the almost instantaneous logarithmic "Weber–Fechner" accommodation of the response. It would be interesting to vary the delay between the successive loads to monitor the shift between the two modes of accommodation. A candidate mechanism for long-term accommodation could be a slow increase in the Minimal Effective Strain threshold ε_0 after a strain stimulation, as observed in bones by Schrieffer et al. (2005). ε_0 would then range from almost zero after a long state of mechanical protection (as in Coutand and Moulia 2000; Coutand et al. 2009, and at the beginning of the experiment by Martin et al. 2010) to a much higher state after long-term recurring loads (Martin et al. 2010) – a change likely to be under transcriptional and/or translational regulation (e.g., variations in the cell density of mechanoreceptors and/or of molecular actors of the local cellular mechanosensory pathway).

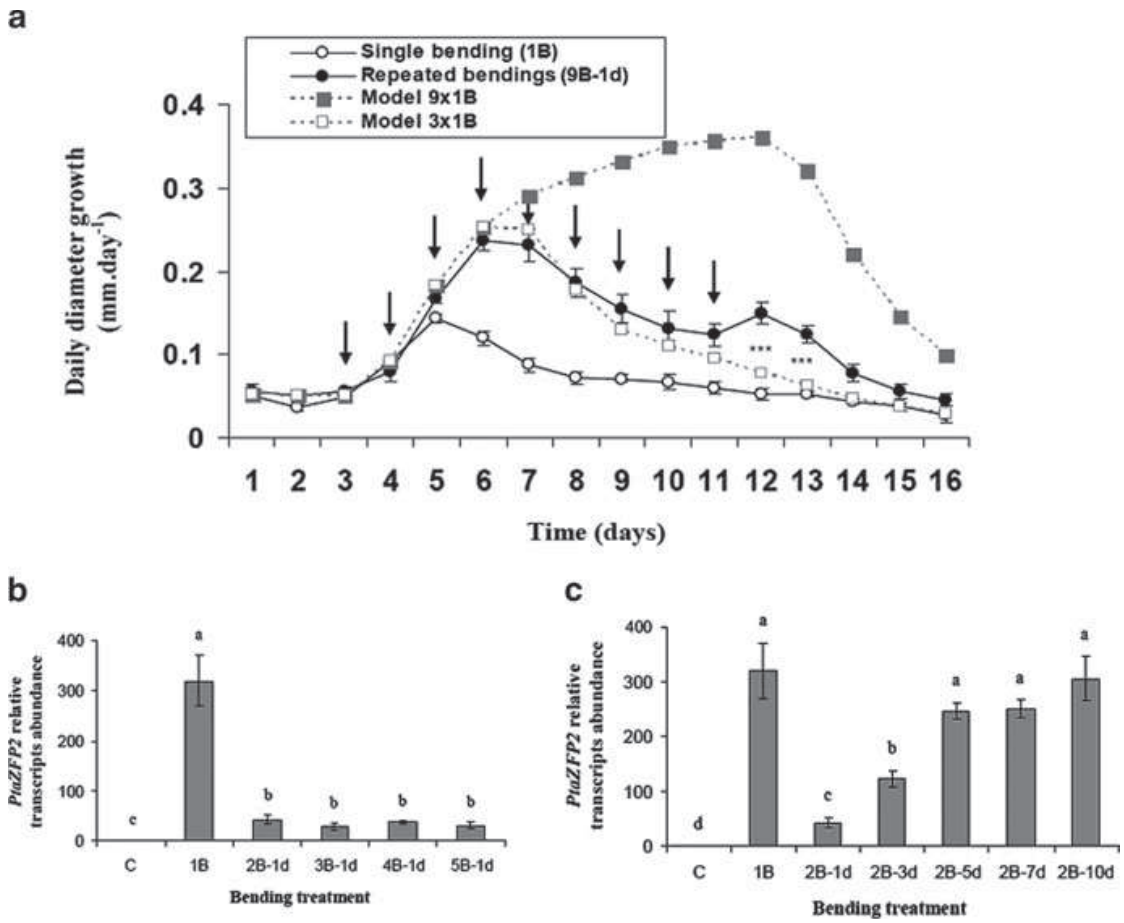

Fig. 7 Response of secondary growth (**a**) and *Pta-ZFP2* expression (**b**, **c**) to repeated daily bending (adapted from Martin et al. 2010 reproduced by kind permission of Journal of Experimental Botany and Oxford University Press). *Open circle* measured growth response to a single bending, *closed circle* measured growth response to nine successive bendings at 1-day intervals (9B-1d). *Dashed squared lines* model of additive effects (linear time integration) 9x1B, *open square* model with a sensitivity shift after 3 daily bendings (3x1B; accommodation). *C* control (no load); *1B* one single bending; *x*B–*y*d = *x* bendings each separated by *y* days; *Pta-ZFP2* Populus tremula*alba Zinc Finger Protein 2 gene

The putative adaptive significance of this slow accommodation process has not yet been studied in depth, but as windy weather involves repeated mechanostimulation, accommodation is visibly a major process governing plant response to wind loads in natural conditions. Slow accommodation also explains the quantitative discrepancy between the amount of response observed in the lab (where plants are usually left sitting for long periods under a very low levels of mechanical stimulation before experiments) and in natural conditions (as highlighted in the Introduction). Understanding and modeling the mechanisms and processes underpinning accommodation is therefore crucial to the analysis of wind acclimation in plants.

However, the detailed modeling of the time dynamics of mechanosensing from the analysis of growth response to repeated bending scenarios may well require a large series of many experiments. Understanding the gene regulation network underlying accommodation would be of great help. Moreover, mechanosensitive

genes provide additional observable variables for analyzing the accommodation process. For example, *Pta-ZFP2* relative transcript abundance (Q_r) was shown to undergo major desensitization after the very first bending (Fig. 7b), and relaxation to a fully sensitive state occurred within 5 days (Fig. 7c). Similar patterns were found for genes within the local mechanotransduction pathway (e.g., *Touch2 calmodulin*, a gene encoding *ACC synthase* from the ethylene pathway, Arteca and Arteca 1999; Martin et al. 2010). Conversely, the cell wall remodeling xyloglucan endotransglycosylase/hydrolase *TCH4-XTH*, probably more related to the growth response setting in the receptor zones, displayed distinct relaxation kinetics (Martin et al. 2010).

3.6 The Sum of Strain Sensing Model S^3m: A Template for the System Biology of Mechanosensing?

Although the process of slow accommodation after a few bendings remains to be analyzed and coupled with the Sum of Strain Sensing model (S^3m), S^3m does, in its present state, represent an initial foray into the integrative "system biology" of mechanosensing (e.g. Telewski 2006; Moulia et al. 2006; Baskin 2006). System biology is usually defined as a field seeking to converge knowledge on the structure and the dynamics of biological systems, and integrating recent insights on gene and gene product regulation networks (e.g., Alvarez-Buylla et al. 2007; Traas and Moneger 2010). This task is only achievable using modular models (Tardieu 2003). Our aim here is to show how S^3m can be used as a template tool for further mechanistic and integrative analysis of mechanosensing and thigmomorphogenesis.

3.6.1 From Plant to Genes and Back

The challenge involved in the integrative biology of mechanosensing can be described as (Moulia et al. 2006; Hamant et al. 2008):

1. Going from a plant exposed to its mechanical environment to the internal mechanical state of its mechanocompetent tissues and on to the subsequent local mechanotransduction process, the expression of mechanosensitive genes, and finally the production of the corresponding proteins and molecular mechanosensing machinery.
2. Then integrating local signaling and gene regulation in tissue elements into the overall syndrome of thigmomorphogenetic growth responses, and its adaptive relevance in terms of mechanical acclimation.

As this loop involves several organizational levels and scales plus a host of interactions, it cannot be handled without the help of specific tools called structure–function models (Godin and Sinoquet 2005), placing this challenge within the realm

of Systems Biology (Tardieu 2003; Moulia and Fournier 2009; Traas and Moneger 2010). The bottom line here is that System-Biology modeling and cross-comparison against the data produced through a suitable experiment makes it possible to assess a set of hypotheses. In cases where the combination of hypotheses cannot be worked out without calculus, modeling becomes an extension of the experimental method (Legay 1997).

3.6.2 A Template to Integrate More Detailed Modules

Although quite simple, the Sum of Strain Sensing model addresses the different process-based modules required for a systems biology approach (Fig. 6) (1) a local strain-sensing module, (2) a secondary internal signal integration module (possibly including the process of long-range propagation), (3) a growth response module (an accommodation module, yet to be formalized, has to be included whenever the response to chronic repeated load needs to be considered). Furthermore, it clearly defines and separates the scales involved (local modules vs. modules performing spatial integration). Any attempt to go into further mechanistic detail on a single module (making a "zoom sub-model") has to fit with the quantitative response of its minimal S^3m version. To illustrate, to study the mechanotransduction pathway upstream of *Pta-ZFP2* or the process of molecular accommodation of mechanosensitivity, a model of the gene regulation network underlying *Pta-ZFP2* net transcript accumulation could be produced. However, its output should still match equation (5) and Fig. 3. By the same token, any attempt to understand the growth response module (for instance by including the eco-physiological processes involved, Godin and Sinoquet 2005; Tardieu 2003) should be consistent with a logarithmic dose–response curve to an additive long-distance internal signal.

Wherever a more detailed zoom-model of a given module has been achieved, it can then be linked with the other existing modules so that the interplay of this module with the global behavior of the system can be studied. This may offer extended possibilities for testing a given zoom model and progressing more rapidly towards a proper zoom model. Moreover, it provides a template to start studying the interesting issue of long-range coupling between regulations or signaling networks in separated tissue elements (e.g., regulation of strain sensitivity in sensing tissues and of the sensitivity to secondary systemic signals underlying S_i in the responding growth zones).

3.6.3 A Tool for Genetic Dissection

Using the Sum of Strain Sensing model to analyze the response of six sympatric tree species with contrasted slendernesses and buckling risk factors, Coutand et al. (2010) found very significant interspecific differences in the mechanosensitivity of the thigmomorphogenetic control of secondary growth, with sensing threshold $S_{02strain}$ ranging from 1 to 1.8. This analysis would not have been possible without

the Sum of Strain Sensing model, as the species studied differed in diameter and bending stiffness. This study also demonstrated that S^3m was robust to genetic variation. Another plus is that only one of the S^3m model parameters was undergoing genetic variation. S^3m thus appears a valuable tool for dissecting the genetic control of a highly composite and environmentally sensitive trait: stem slenderness. More generally, integrative biomechanical models offer a clear way for increasing the genetic heritability (h^2) of the variable by removing the effects of environment and plant size/structure (Sierra de Grado et al. 2008). This may also pave the way to improved detection of robust Quantitative Traits Loci and thereby help identify controlling genes via analysis of genetic $\times$ environment variability (Tardieu 2003). Similarly, S^3m may be also used to phenotype mutants and identify unknown control genes.

3.6.4 A Tool to Identify Missing Elements and Rewarding Molecular Studies

Another interesting heuristic property of integrative models like S^3m is that they can help identify modules that are (1) more influential than others in the global thigmomorphogenetic response, and (2) insufficiently understood. This will help better target molecular studies (such as gene regulation studies) that may be rewarding in the sense that (1) the genes may trigger very significant phenotypes, and (2) their identification improves our understanding of the system response significantly. The S^3 model probably needs to be developed further before it can be made completely suitable for this application, but even in its present stage, it already clearly pinpoints the accommodation process as a major deadlock that needs to be broken to improve our understanding of wind acclimation in plants. Moreover, it points toward different tissues involved in fast and slow accommodation, and provides variables quantifying the relevant local phenotypes (the sensing threshold of the long-range secondary signal $S_{0_{\text{strains}}}$ in growing tissues for fast accommodation, and strain threshold ε_0 in slow accommodation) and methods to estimate them. Finally, the very clear shift of mechanosensitivity after three stimulations makes it a candidate for tentative modeling through a regulation network (Alvarez-Buylla et al. 2007).

3.6.5 A Platform to Ecological and Agricultural/Forestry Science?

Wind acclimation and storm hardening of crop and forest species are major challenges in agronomics and forestry research (and this may even increase with global changes; Quine and Gardiner 2002). While models are available for the mechanics of lodging, wind throw, and wind break (see de Langre 2008 and Gardiner et al. 2008 for reviews), the existing growth models disregard thigmomorphogenesis and therefore skip wind acclimation (Moulia et al. 2006). A major asset of the S^3 model is that it can be coupled with very different mechanical models. It can be coupled with mechanical models to analyze the effects of the

static and dynamic strains produced by wind-induced vibrations in plants (e.g., Gardiner et al. 2008; Rodriguez et al. 2008; Sellier et al. 2008). As the S^3 model can also handle growth responses to wind, it can be coupled with structure–function growth models (see Moulia et al. 2006; Fourcaud et al. 2008 for general discussion). There is still a lot of work to be done before mechanosensing models can be coupled with (1) wind–plant interaction and wind climate models or (2) existing growth models, but integrative mechanosensing models are surely a key breakthrough paving the way to a better understanding of the ecological and economical relevance of the thigmomorphogenetic acclimation, and to exploring the consequences of global changes in terms of stand growth and resistance to wind hazards.

4 Conclusions: The Challenges of Integrative Mechanobiology

This review has shown that a picture is emerging in the field of integrative mechanobiology. This emergence is based on intensive, long-standing interdisciplinary collaborations harnessing solid mechanics, fluid mechanics, nonlinear physics of dynamic systems, biomechanics, and mechanobiology. Integrative modeling and continued confrontation with experiments is central to this movement. These models provide tools for (1) specifying the consequences on measurable variables of a set of hypotheses in interaction with plant structure and (2) designing and interpreting experiments to assess these hypotheses. This approach, employing systems biology as an extension of the experimental method, contrasts with (and complements) alternative setups in which experimentalists intensively collect data while bioinformaticians set up models and data mining programs. In the case of plant biomechanics and mechanobiology, the integrative models also rely (1) on a clear identification of what is purely physical (the ISM model in our case) and what is mechanobiological (the S^3m model), and (2) on an explicit coupling of the two (sub-)models. This makes it possible to integrate the constraints on plant phenotype related to the physics of plant structure into the coupled model. The mechanical model can then be adjusted whenever necessary, such as to cope with a different experimental setup or to natural conditions. In each submodel, the effects of the topology and geometry of the plant structure (or of the studied biological system, cell, meristem, etc.) have to be taken into account, while a clear modular design and explicit setting of organizational levels has to be implemented. However, models can only generate useful insight if they are kept simple enough. Multiplying the number of elements and degrees of freedom makes models very difficult to analyze and experimentally assess. Hence, a concomitant effort to simplify the models (as done in physics through dimensional analysis; see de Langre 2008; Rodriguez et al. 2008) and address them to specific questions for which their outputs and hypotheses can be experimentally falsified is extremely valuable.

This approach thus requires setting a common interdisciplinary culture (remember the stress vs. strain issue) and common projects.

This type of interdisciplinary research program is being applied to the integrative mechanobiology of (1) the interaction between plants and their mechanical environment (wind and gravity) and (2) the growth-induced autostresses in the apical meristem (that is not reviewed here; see chapter "Plants as Mechano-Osmotic Transducers" and Traas and Moneger 2010 for recent reviews). A first step in this program is to set a minimal model of mechanobiological integration over the considered structure, coupled with a structural mechanics model (Coutand and Moulia 2000; Hamant et al. 2008). Both models have to be validated on their output (e.g., Moulia and Fournier 1997; Coutand and Moulia 2000). But mechanistic models also have to be validated in terms of their basic mechanistic modules (e.g., Coutand et al. 2009) and their capacity to dissect natural genetic variation (e.g., Sierra de Grado et al. 2008; Almeras and Fournier 2009; Coutand et al. 2010). In the case of the thigmomorphogenetic responses to external bending loads, the Sum of Strain Sensing model, S^3m, has fulfilled these requirements.

However, evolution does not comply with minimal models. There are several reasons for this, such as the lack of pure optimality principles (apart from those governed by physics) (see Gould and Lewontin 1979; Moulia and Fournier-Djimbi 1997; Niklas 1998 for a discussion on a topic going far beyond the scope of this review). Selection for mutational robustness, for example, can induce a great deal of redundancy (Alvarez-Buylla et al. 2007).

The minimal model can, however, be used as a template to integrate more detailed and mechanistic zoom modules, and as a guide for pinpointing relevant and rewarding extensions.

We have illustrated this program in the example of the S^3m model. However, the field of integrative mechanobiology is still in its infancy, and we have a long way to go before achieving a satisfactory understanding of the processes involved. Hence, we shall end this review by highlighting some of the unresolved questions and challenges that, from our point of view, are key hurdles to the mechanobiology of how plants acclimate to their mechanical (wind and gravity) environment.

4.1 Wind Loading and Accommodation

The first challenge is to take into account the repetitive nature of wind loads. This entails integrating the vibrational mechanics of wind–plant interactions (Py et al. 2006; Sellier and Fourcaud 2009). Mechanical models of vibrational dynamics are already available but have not yet been re-engineering for coupling with mechanosensing models. Specific mechanosensitive responses to strain rate or strain frequency should also be assessed. Indeed, frequency dependence has been observed in other living systems (e.g., Ehrlich and Lanyon 2002), and there are clues to indicate a plant-led regulation of its modal frequencies (Rodriguez et al. 2008).

Besides this, a further major challenge is to understand the broad long-term accommodation of mechanosensitivity. This requires an explicit two-way coupling

of the mechanosensing model and the mechanical model – a task that has yet to be undertaken. A biomechanical and mechanobiological model of cell (and tissue element) mechanosensing, and its possible regulation through gene regulation networks, is clearly lacking. Once developed, this model could be integrated into a plant model such as S^3m to handle crosstalk between distant tissue elements. This is a huge task, but one that may benefit from a similar program in the mechanobiology of meristems (Green 1999; Traas and Moneger 2010).

Finally, our understanding of the transmission and reception of secondary systemic signals remains far too coarse (Brenner et al. 2006). In particular, the mechanisms underlying the setting of the threshold for a growth response to the long-range signals $S_{01\mathrm{strain}}$ and $S_{02\mathrm{strain}}$ need to be studied, as changes in this parameter shape the thigmomorphogenetic response. This makes it necessary to understand and model the reception of mechanosensitive secondary signals, and the genetic regulation and accommodation potentially involved. This would in turn help define proper dimensionless variables for the internal signal and the Sum of Strain. Quantitative analysis of gene regulation in growing tissues does, however, remain a delicate task, but a proper formalism (set through interdisciplinary biomechanical research) has just been set (Merret et al. 2010).

4.2 *In Search of a Function: Plant Mechanical Design and Biomechanical Strategies*

Besides improving our understanding of mechanosensing, the adaptive value of this process should be addressed. To what extent does the thigmomorphogenetic response really acclimatize plants to wind, whatever the climate? Does thigmomorphogenesis control the risk of buckling? Is strain sensing involved in acclimation to water stresses? To tackle these questions, we need to compare the mechanosensitive responses of the plants and their behavior to environmental constraints such as height growth, water losses, and storms. To achieve a sufficient level of generality requires comparison (and possibly the coupling) between biomechanical models of risks (wind damage, buckling) and the mechanosensitive growth model. This is actually the most appropriate way to ascribe a real function to thigmomorphogenetic processes and to bring mechanosensitive models into the study of dynamic structure–function models (Godin and Sinoquet 2005).

By the same token, the combination of mechanical acclimation with the capacity to recover from mechanical hazards (Moulia and Fournier 2009) should be taken into account. Plant mechanical design has to be considered through a complete biomechanical strategy for ecological resilience to mechanical constraints (Fournier et al. 2006). This sets clear connections between integrative mechanobiology and biomechanical functional ecology. This issue is of major interest for assessing the potential resilience of plants (and of ecosystems) to the possible increase in storms.

The first successes of integrative mechanobiology have thus opened up a large set of questions for interdisciplinary research with a continuum of scientific challenges ranging from cell biology and gene regulation to functional ecology and more applied issues such as crop and forest management for better resilience to global changes in the mechanical environment. This is a lively, active, and attractive research community. We really believe the next version of a review like this, say in 5–10 years (?), would be very different from this one, and we really look forward to the kind of developments on the horizon!

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Hydraulics of Vascular Water Transport

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Abstract The science of plant water transport is equal parts of physics and biology. Plants have evolved a complex wick system that harnesses the cohesive hydrogen bond energy of liquid water and suppresses the heterogeneous nucleation of cavitation. Trade-offs between making the wick safe against cavitation and implosion, yet efficient in moving water, result in the process being limiting to plant performance. Cavitation limits the range of negative pressures that can be harnessed to move water, and the hydraulic conductance of the wick limits the flow rate that can be moved at a given negative pressure gradient. Both limits constrain CO_2 uptake via the water-for-carbon trade-off at the stomatal interface. Research in the area concerns the mechanisms of cavitation, its reversal by embolism repair, consequences for plant ecology and evolution, and the coupling of water transport to plant productivity. Very little is known of the molecular biology underlying xylem physiology.

1 Introduction

Few transport processes generate more controversy and misunderstanding than the long-distance movement of water in the xylem of plants. The transport mechanism is very simple and very remarkable – remarkable enough to be contentious. In its essentials, it is a physical process. Yet the physics impose major constraints on the biology of plants. This chapter explains water transport and identifies issues where research is active or needed. It is written to be accessible for the nonspecialist.

Of all biological transport systems, plant xylem transports the most fluid over the longest distances (Vogel 1994). A tree with a trunk the size of a man's waist transports on the order of 100 l of water on a clear day (Enquist et al. 2000). Summing over a watershed, plant water use matches the runoff in rivers, and constitutes just under half the annual precipitation (Schlesinger 1997). This conspicuous consumption makes water a major limiting resource for plant life. What is

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