Università degli Studi di Napoli Federico II

Dipartimento di Agraria

Corso di Dottorato di Ricerca in Scienze Agrarie ed Agroalimentari, XXX ciclo

TESI DI DOTTORATO

Exploring the potential of cell-based models in simulating tissue biophysics in plant morphogenesis: the case of woody tissues.

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a.a. 2015-2017

0.1 CONTENTS

1 GLOBAL INTRODUCTION

Tissue development and general behaviour result from extremely complicated dynamics, and classical analysis does not come very far in terms of understanding the processes underlying such dynamics. In this context, mathematical and numerical models can help to disentangle complex interactions and to analyze non-intuitive dynamics that drives tissue development and functioning (**Merks and Glazier**, 2005).

Since these are multi-scale processes, both in time and space, there is the need to develop an appropriate modelling approach.

The most promising one is hybrid modelling, that is a synthesis of the differential equation based reaction-diffusion approach at molecular and chemical continuous scales, and the Individual-Based modelling approach for simulating the mechanical and behavioural interactions of the cell ensemble constituting the tissue (**Vincenot et al.**, 2011).

A crucial actor in such interactions is the cell wall. The cell wall contributes to make plant cells remarkably different from animal ones, and has a fundamental role in controlling, among other things, the rate and the direction of elongation. As such, it is needed to consider the cell wall in order to correctly describe plant tissue growth, and more specifically to consider its mechanics (**Cosgrove**, 1997). Taking into account cell mechanics together with biochemistry and molecular biology is a daunting task to achieve using experiments alone. In fact, a multi-scale system such as the plant tissue is a good example of a context where modelling would help in advancing the knowledge of plant biology. Having a good description of the cellular mechanics could help to explore real-world problems, like wood formation in trees.

During its growth in height, a tree invests part of its photosynthates to produce wood, in order to support its structure, and to provide a means to move water through its tissues.

The process of wood formation, xylogenesis, involves proliferation and differentiation of stem cells buried inside the trunk, that will undergo substantial changes to become part of the plant vascular system. After losing its stemness, the cells undergoes a rapid diameter increase, then it starts to mature, i.e. to produce and deposit structural sugars over its cell walls, encrusting them with lignin at the same time. When the cell is completely lignified, the final step of the maturation process, programmed cell death (PCD), takes place (**Cuny and Rathgeber**, 2016).

The cell will eventually concur together with other wood cells developed along the tree radius to form a tree ring. Tree rings represent "historical register" of the plant's physiological state and are also widely used as a proxy in climate studies (**Schweingruber**, 2012).

The wood formation process shows a high degree of variability and complexity, and striking differences in size and wall thickness between tree rings develop as result. What causes this difference, and how it is caused, is still unclear: it is known that both endogen and environmental factors concur to shape it (**Hartmann et al.**, 2017).

As often happens in plant biology, the interactions between mechanical properties of the tissue and its developmental processes are usually not taken into account, mainly because of

experimental constraints, and the complexity inherent to treating plant tissue as multi-scale systems. Computational modelling has proven to be effective in exploring hypotheses related to such multi-scale systems, and at the same time, in the last ten years, some solid frameworks for modelling cell biomechanics have been proposed.

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Chapter 1

Cell-Based Models in plant developmental biology: insights into hybrid approaches.

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Published as
Hay Mele, Bruno, et al.
"Cell-based models in plant developmental biology: insights into hybrid approaches."
Frontiers in Environmental Science 3
(2015): 73.

2 CELL-BASED MODELS IN PLANT DEVELOPMENTAL BIOLOGY: INSIGHTS INTO HYBRID APPROACHES

2.1 INTRODUCTION

During the first decade of the twenty-first century, biology has been profoundly transformed. The technological advances of this period contributed to produce a tremendous amount of biological data, which in turn has made computers fundamental tools in biology research.

Nowadays, researchers have means to investigate cell biophysical, biological and kinetic properties, providing large and very detailed amount of information to the scientific community. On the other hand, knowledge on how cell processes combine and give rise to tissue and organ properties is still meagre, mainly because genetic analysis is time-consuming when the number of interacting factors is large. As time passes, and data gathers, it is becoming extremely complicated to identify the networks underlying the regulation of cell activity, while considering all the parallel interactions that underlie cellular morphogenesis (**Merks and Glazier**, 2005).

Traditional research organizes results and develops new hypotheses about the behaviour of gene networks using static schemes, an approach that is unfruitful in all but the simplest cases (**Merks and Glazier**, 2005). This suggests that reconstructing the dynamics of the genetic regulatory networks with wet research it is not sufficient. Furthermore, gene networks seem just one part of the story: insights on the role of mechanical and physical interactions are needed if one wants to truly elucidate emergent properties linked to tissues (**Dupuy et al.**, 2008). During the development of multi-cellular organisms, cells are capable of interacting with each other through a range of biological and physical mechanisms. A description of these networks of interactions is essential for understanding how it is possible for tissues and organs to co-ordinate cellular activity (**Dupuy et al.**, 2008).

Working at tissue-level many cellular dynamics have to be considered, such as cell growth, cell elongation and cell division: all these are still subjected to intense study and discussions scientific community. This is particularly felt with stem cells, whose ability to differentiate is intrinsically linked to specific biological functions in multi-cellular organisms. A widespread idea is to "interpret stem cells as non-hierarchical self-organizing dynamical systems" (**De Matteis et al.**, 2013) and "stemness" not as an explicit cellular property: stem cells would be "dynamically selected and modified in response to cell-cell and cell-environment interactions on the basis of their potential and flexibility, rather than being specialized a priori" (**De Matteis et al.**, 2013).

The difficulty of working with living tissues, together with the aforementioned multi-scale complexity, is a major limitation to describe such systems, and computer modelling appears particularly helpful to characterize the behaviour of multi-cellular systems: working with theoretical models and numerical simulations has proven to be effective in disentangling the relationship between cellular processes and tissue-level arrangement (**Jönsson and Krupinski**, 2010).

2.2 MODELLING IN PLANT DEVELOPMENTAL BIOLOGY

The first pivotal moment in the history of mathematical modelling in developmental biology was the publication of D'Arcy Thompson "On growth and form" (**Thompson**, 1942), who spawned a geometrically-oriented approach to the problem. In plant biology, this path has been followed and extended by Lindenmayer with its L-system (**Lindenmayer**, 1975), a modelling framework for representing 1D linear and branching structures (e.g. cells, leaves, or shoots) in form of a sequence of elements. The "geometrical" approach has subsequently been adapted for describing 2D structures as a graph rotation system, and coded in vv-system (**Smith**, 2006) (for more information see **Prusinkiewicz and Runions** (2012)).

A second strong contribution to the formalization of developmental processes in biology came from the works of **Hofmeister** (1863), **Sachs** (1877) and **Errera** (1886): their mechanic theories on cells are the foundation of the modern approach to cell division, even if their use bring some discrepancy between models and observations (for a solution to this, see **Besson and Dumais** (2011)).

Another decisive contribution on theoretic developmental biology has been made by Turing, with his paper on biological pattern formation (**Turing**, 1952), whose concepts have been extensively used for showing that molecular-level interactions may lead to morphogenesis and differentiation. Turing used a system of partial differential equations (PDE) in his work, a mathematical tool widely used in biological modelling, for describing substance diffusion and reaction. This approach evolved to this day in plant science as a chemical / molecular-oriented one, mainly thanks the works of **Meinhardt** (1982).

Since the diffusion of these ideas, the contributions of mathematical modelling to developmental biology have grown substantially and contributed to many interesting results (for an in-depth review see **Prusinkiewicz and Runions** (2012)), but the issues with modelling multi-cellular systems have remained.

2.2.1 History of cellular-based models in biology During the last decade, more and more developmental plant biologists turned to mathematical models to explore their hypotheses, and specialized packages appeared for facilitating model construction. Following the realization that developmental processes depend on both geometrical and molecular dynamics, a synthesis of these two approaches has been performed, and some hybrid tools appeared on the scene. This hybrid approach has a deep conceptual meaning, since joining the geometrical and the molecular point of view means working simultaneously at different temporal and spatial scales (i.e. it is a multi-scale approach). A key point in this kind of approach is to establish a certain degree of simplification of cell processes, since using complex molecular models of the cell for simulating tissue-related dynamics (**Krul et al.**, 2003) has been found very computationally demanding and too complex for gathering insights. It is possible to assimilate cells to well-mixed compartments and single cell functions can be represented in a very "simplified" way, still capturing the complexity of their spatial interactions. Considering a tissue as an ensemble of such "simplified" cells facilitates the search for the emergence of tissue organization due to the collective behaviours of the single cells; these behaviours are dependant on both cell internal processes and interactions with neighbouring cells.

Since chemical substances are signals regulating internal cell dynamics, reaction-diffusion processes, are key players in tissue modelling.

This approach is called cell-based modelling. As stated by **Palm and Merks** (2015): "The inputs to a cell-based model are the behavioural rules that cells follow. The output of a cell-based model is the tissue morphogenesis that follows indirectly from the collective behaviour of the individual cells"

Spatially explicit, cell-based paradigms can be broadly classified according to the cells being part of a grid (in-lattice models) or not (off-lattice models).

A widespread in-lattice paradigm in animal developmental biology modelling is the Cellular Potts Model (**Glazier and Graner**, 1993), or CPM, a modelling approach that considers single cells as agents trying to minimize their internal energy while growing, dividing, and interacting with chemical fields.

The CPM represent cells as a group of neighbouring pixels, distinguishing between boundary and non-boundary pixels in order to define interaction sites, and uses an energy-based approach for simulating growth, cell-cell interaction, and for maintaining cell shapes. Molecular details, i.e. substance production and diffusion, are handled by ODE and PDE (Ordinary and Partial Differential Equations) solvers coupled with geometric information and with the growth process, that also permit to consider other continuous (eventually spatially-explicit) processes. The CPM approach has been successfully applied to a vast array of biological problems: here we will cite tissue patterning (**Savill and Sherratt**, 2003; **Zeng et al.**, 2004), morphogenesis (**Zajac et al.**, 2003), tumorigenesis (**Turner and Sherratt**, 2002), and vasculogenesis (**Scianna and Preziosi**, 2012).

While some models of plant systems have been produced within the CPM paradigm, it is considered unfit for simulating plant tissue dynamics. These are strongly influenced by the presence of a cell-wall, which is responsible for maintaining cell geometry, for preventing cell motility, and it is involved in substance (e.g. auxin) transport. The CPM lacks a proper way to simulate cell wall dynamics, and the paradigm chosen for cell geometry makes cell shape and motility (absent in almost all plant cells, due to the cell wall) difficult to control.

Merks et al. (2011) proposes an appropriate solution, starting from the CPM but using for geometry an off-grid modelling approach that describes cells as polygons delimited by cell walls, considered as separate entities and shared among adjacent cells. Walls are assimilated to mechanical springs, so that growth and mechanical interactions could be computed by means of a Markovian relaxation algorithm. Additional differential equations model diffusive transport across the two cell membranes and across the cell wall separating adjacent cells. As in the original CPM, sets of ordinary differential equations in each cell describe dynamics of biochemical networks and genetic regulatory networks.

Since the hybrid approach has proven itself fruitful, other variations have been developed, changing the way cell geometry and / or topology are defined, e.g. using L-system (**Wabnik et al.**, 2013) or considering cells as polygonal (**Dupuy et al.**, 2008) or Voronoi meshes (**Mebatsion et al.**, 2006), as well as using different ways to describe cell walls and to render mechanical interactions, such

as assimilating the structure to a spring (**Shapiro et al.**, 2013) or a viscous fluid (**Dupuy et al.**, 2008), and use appropriate physical models for simulating their behaviour.

Since the diffusion of mathematical models as plant developmental biology tools, a few general purpose tools have been made, in order to allow non-programmers to enter the field.

The CPM has been integrated into the CompuCell3D software environment (**Izaguirre et al.** (2004) — `http://www.compucell3d.org`), a python-based, extensible general-purpose framework used in biology for simulating in 2D and 3D a range of biological dynamics. Compucell3D has been used mainly for studies in human tumorigenesis (**Boghaert et al.**, 2014; **Swat et al.**, 2015) and animal tissue development (**Dias et al.**, 2014), albeit other processes have been simulated (**Giverso and Preziosi**, 2014; **Popławski et al.**, 2008; **Zhang et al.**, 2011). It is not particularly suited for plant tissues, mainly because it is based on the CPM, although a model of root growth has been developed with this method (**Grieneisen et al.**, 2007).

The plant-specific approach of Merks, based on the CPM, has been implemented in the VirtualLeaf modelling framework (**Merks et al.** (2011) — `https://biomodel.project.cwi.nl/software`) for 2D tissue simulation. VirtualLeaf is written in C++ and comes with pre-programmed modules simulating many accepted results. It is possible to write new modules in order to test new hypotheses.

CellModeller (**Dupuy et al.** (2008) — `https://haselofflab.github.io/CellModeller/`) is another stand-alone environment build with a modular approach and written in python, for modelling large-scale multi-cellular systems in 2D. As in other tools cell topologies are graph rotation system (i.e. composed of nodes and edges), and inter- and intra-cellular chemical interactions are formalized as PDEs and ODEs. Mechanical interactions and growth are simulated through physical laws regulating rearrangement of the cell nodes following strain / stress of the cell wall, itself rendered as a viscous fluid. This tool has been used for modelling microbial biofilms (**Rudge et al.**, 2012), but his potential for modelling plant cells related dynamics has been often stated (**Dupuy et al.**, 2008; **Liu and Stewart**, 2015)

Some other tools have been programmed as extension for pre-existing software environment, like CellZilla (**Shapiro et al.** (2013) — `http://www.cellzilla.info/`) that extends the xlr8r (**Shapiro et al.**, 2003) Mathematica package capabilities of solving chemical reactions as sets of ODE to 2D tissues. The topology of the cell is described as a set of nodes (vertexes), with the segments joining them (edges) being the cell wall. Using xlr8r, inter- and intra-cellular chemical interactions are formulated as differential equations; growth and mechanical interactions are modelled considering the walls subject to springs whose behaviour is regulated by Hooke's law. CellZilla has been used for simulating auxin-driven development in the apical meristem of *Arabidopsis thaliana L.* (**Nikolaev et al.**, 2013)

Finally, VPlants (`https://team.inria.fr/virtualplants/`) is a set of packages belonging the OpenAlea software environment that permit to analyze, model and simulate tissue dynamics (for detail on OpenAlea see **Pradal et al.** (2008)). Its "Tissue" package permits to simulate tissues growth and division, starting from a single cell, or after reconstructing a digital tissue from a photograph. The cell is geometrically described as a polygon, and the user may choose different algorithms for growth; the division process is similarity coded on the basis of the user's needs.

VPlants has been used for simulating, among other things, vascular development in *A.thaliana* (**Muraro et al.**, 2014)

2.3 EXAMPLE OF HYBRID MODEL APPROACH

Vascular plants are characterized by a pervasive, specialized system of vessels, composed of two types of tissues: xylem, cells that transport water and mineral nutrient, and phloem cells that carry sugars and other organic molecules. The spatial position and differentiation of such vascular cells are established during early phases of tissue development. In the plant embryo, four cells buried inside the cell mass are activated as an undifferentiated tissue named procambium: they will grow and divide following specific procedures, increasing the number of cells and forming a cylinder of procambial tissue. As the embryo matures, two zones will start to act as "stock" of undifferentiated cells: one on top, called shoot apical meristem, and the other, called root apical meristem, on the lower part of the embryo. Adjacent to the two apical meristems, some procambial cells will further divide, generating precursors of vascular cells (**Jouannet et al.**, 2015). Peculiar procambium arrangement patterns will emerge during this phase, that will be propagated by the two meristems as the plant grows in height.

These arrangement patterns vary among species (**Beck et al.**, 1982) but also between the stem and the root of the same individual.

How these arrangements arise, how they are maintained, and what causes their diversity are open questions that have been puzzling plant scientists and modellers for a long time (**Jeffrey**, 1903; **Sieburth and Deyholos**, 2006; **Muraro et al.**, 2014). From an experimental standpoint, studies in plant vascular morphogenesis have followed the reductionist route, as the key players shifted from structures (**Esau**, 1960) to cells, and from those to genes (**Caño-Delgado et al.**, 2010) and proteins (**Sieburth and Deyholos**, 2006), with many regulatory elements discovered recently (**Donner et al.**, 2009), most of them in the species *A. thaliana*. At the moment there is only an approximate knowledge of the underlying dynamics (**Jouannet et al.**, 2015), derived mainly from studies on plant hormones like auxins (**Scarpella et al.**, 2006), brassinosteroids (**Vert and Chory**, 2006), and cytokines (**Mähönen et al.**, 2006); most of these focus on single aspects and there is the need for a clarification of the interaction of these aspects both spatially and temporally.

2.3.1 Existing models and limitations Whilst mathematical models have proven to be a good tool in helping to understand the connections between cells and tissues (**Jönsson and Krupinski**, 2010), they do so focusing mainly on sub-cellular processes related to plant hormones, especially auxins (**Prusinkiewicz and Runions**, 2012). The first steps were laid by **Sachs** (1969), with the so-called 'canalization hypothesis', used by various molecular models exploring the vascularization processes (**Bayer et al.**, 2009; **Feugier et al.**, 2005; **Mitchison**, 1980).

The first to approach plant primary vascular structure specification and arrangement were **Muraro et al.** (2014), with a multicellular model that explores the hypothesis of a vascular patterning mechanism dependent on hormones interaction in a very specific context (the roots of *A. thaliana* in a steady-state condition). Starting from a set of experimentally determined factors (i.e. gene

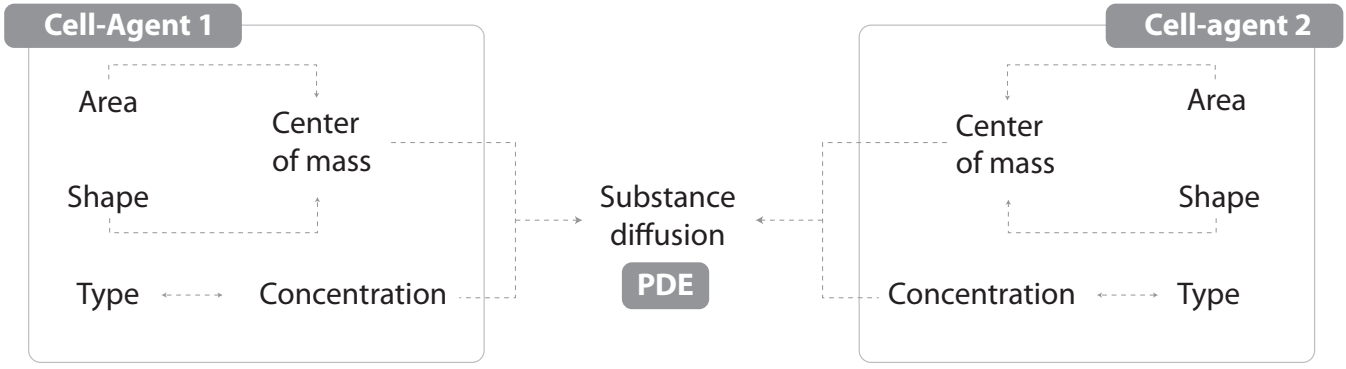

Figure 1. An individual agent is described by its geometric properties (Area, Shape and derived Centre of mass) and cell type.
Each type is characterized by a specific metabolism (ODE) with products whose concentration is dynamically linked with diffusion processes (PDE)

products and hormones), the authors build a model formalizing the interaction between these factors and propose a minimal regulatory network capable of maintaining a stable vascular pattern in arabidopsis root without predefined positional information (**Muraro et al.**, 2014). This model used VPlants from the OpenAlea environment to simulate the tissue geometry and auxin diffusion.

The theory of an auxin-dependant patterning in *A. thaliana* has been further explored with the model of **De Rybel et al.** (2014), which combined experimental evidence and modelling work to investigate the interactions between auxin and cytokinin and their involvement in early embryo development. The model, formulated using VirtualLeaf, shows how these hormones contribute to cell division and differentiation of vascular tissues providing positional cues for the establishment of a stable spatial pattern within a growing domain.

Another model, based on reaction-diffusion dynamics, has been proposed by **Cartenì et al.** (2014) for simulating the differentiation and the spatial patterning of procambium, phloem and xylem. This theoretical model was formulated as a set of activator-substrate systems (**Meinhardt**, 1982) describing the dynamics of nine diffusible morphogens whose interactions lead to the differentiation of vascular tissues and the emergence of their spatial patterns. This model, implemented in MATLAB (MathWorks Inc. — `https://www.mathworks.com/products/matlab/`) successfully recreated a broad range of vascular arrangements, working with a fixed domain and without considering individual cell dynamics.

In order to provide a procedural example of development of a hybrid model, we built a simple model of *A. thaliana* root and stem early vascular differentiation. The hybrid approach permits to take into account both the molecular and geometric perspectives (**Figure 1**), thus providing the chance to capture emergent properties linked to the interaction of these two phenomena The use of VirtualLeaf approximates spatial interactions among cells in a computationally convenient way (**Merks and Glazier**, 2005), even if it considers stochastic-based dynamics not necessarily linked with cells biological properties (**Merks et al.**, 2011).

ID	Type	Differentiation condition		Substance produced
		On S_0	ON A_P	
0	Pith	$[S_0] < \overline{S_0}$	any	none
1	Inner	$\overline{S_0} < [S_0] < \widehat{S_0}$	$[A_P] < \overline{A_P}$	S_1, A_P
2	Inner procambium	$\overline{S_0} < [S_0] < \widehat{S_0}$	$[A_P] > \overline{A_P}$	S_1, A_P
3	Outer	$[S_0] > \widehat{S_0}$	$[A_P] < \overline{A_P}$	S_2, A_P
4	Outer procambium	$[S_0] > \widehat{S_0}$	$[A_P] > \overline{A_P}$	S_2, A_P

Table 1. Differentiation and substance production rules

2.3.2 Model description As mentioned in the introduction, it is possible to work with tissue-related dynamics using a hybrid modelling approach (**Vincenot et al.**, 2011), i.e. coupling an Individual-Based Model (IBM) with a continuous PDE / ODE mathematical model. Under this paradigm, the IBM will account for internal cell rules and cell-cell interactions, whilst a set of differential equations will regulate substance physics and continuous processes.

Vincenot et al. (2011) proposed a conceptual framework comprising a set of reference cases representing combination patterns of SD and IBM sub-models, which shall serve as building blocks for hybrid models. The model described here fits into case 2b of the framework, since cells are represented as IBM individuals and an SD sub-model is embedded in each cell to compute growth and substance dynamics. Single cells are then networked depending on the adjacency of their walls, forming thereby a tissue.

Starting from **Cartenì et al.** (2014), we built a hybrid, growing-domain model of cell growth and differentiation in the roots and stems of *A. thaliana*; this model considers simplified tissue dynamics and the production of four diffusible, cross-reacting substances (S_0, S_1, S_2, and A_P) which are responsible for procambium differentiation and proliferation. In this particular case, the PDE mathematical formulation is a simplified version of the Reaction / Diffusion model presented in **Cartenì et al.** (2014).

The model is not meant to provide a one-to-one correspondence with specific gene products or plant hormones or insights into the molecular mechanisms of vascular differentiation. It was rather selected for the simplicity of formulation and for the fact that to date it is the only one that provides a general framework (although only theoretical) for the spontaneous emergence of different spatial arrangements of vascular bundles. For more detail on the model's underlying assumptions, see **Cartenì et al.** (2014).

The model domain is a transversal section of growing *A. thaliana* tissue. Each one of the cells composing the tissue is a complex agent made by the cell itself and by a polygonal cell wall, shared with neighbour cells.

The cell agent properties are the position, the type (**Table 1**), the area, and the concentration of all substances considered in the model.

The model itself considers four substances:

Module	Parameter	Description	Parameter value
IBM	$\overline{S_0}$	Threshold value for pith cell differentiation	0.02
IBM	$\widehat{S_0}$	Threshold value for internal cell differentiation	0.2
IBM	$\overline{A_P}$	Threshold value for procambium cell differentiation	0.5
PDE	σ_0	S_0 production	1
PDE	μ_0	S_0 consumption rate	0.2
PDE	D_{S_0}	S_0 diffusion coefficient	0.1
PDE	σ_S	S basic production rate	0.1
PDE	k_S	S production saturation constant	20
PDE	ρ_S	S cross-reaction coefficient	0.8
PDE	D_S	S diffusion coefficient	0.5
PDE	σ_{A_P}	A_P basic production rate	0.001
PDE	ρ_{A_P}	A_P cross-reaction coefficient	0.3
PDE	μ_{A_P}	A_P removal rate	0.02
PDE	D_{A_P}	A_P diffusion coefficient	0.001

Table 2. List of parameters values

- S_0, produced by the cell walls contacting the medium / outside. It represents the signal involved in the creation of a radial gradient that determines the establishment of pith, inner, and outer layers of cells.
- S_1 and S_2, produced respectively by the inner or the outer cells. These represent signal substrates regulating the production of the activator A_P.
- A_P, produced by the reaction between S_1 and S_2. It represents the procambium activator that triggers the differentiation of procambial cells.

All substances diffuse between cells but not outwards the tissue, and all four are considered homogeneous in the individual cell space.

In the model, cells grow at a constant rate (controlled by the parameter k_a, see **Table 2**), dividing if their size reach twice the original size. A theoretical substance (S_0), is then produced in the outermost layer of cells, contributing to the creation of an inside / outside gradient; and the other substances are produced according to cell type (**Table 1**). Each chemical species inside the cell diffuse through the tissue and react with each other according to the following equations:

$$\frac{\partial S_0}{\partial t} = \sigma_0 - \mu_0 S_0 + D_{S_0}\nabla^2 S_0 \tag{1}$$

$$\frac{\partial S_1}{\partial t} = \overline{\sigma_1}\left(1 - \frac{S_1}{1 + k_S A_P}\right) - \rho_S A_P^2 S_1 S_2 + D_S \nabla^2 S_1 \tag{2}$$

$$\frac{\partial S_2}{\partial t} = \overline{\sigma_2}\left(1 - \frac{S_2}{1 + k_S A_P}\right) - \rho_S A_P^2 S_1 S_2 + D_S \nabla^2 S_2 \tag{3}$$

$$\frac{\partial A_P}{\partial t} = \sigma_{A_P} + \rho_{A_P} A_P^2 S_1 S_2 - \mu_{A_P} A_P + D_{A_P}\nabla^2 A_P \tag{4}$$

with:

$$\overline{\sigma_1} = \begin{cases} \sigma_S & \text{if cell is not type 0 and is (type 1 or type 2)} \\ 0 & \text{else} \end{cases} \tag{5}$$

$$\overline{\sigma_2} = \begin{cases} \sigma_S & \text{if cell is not type 0 and is (type 3 or type 4)} \\ 0 & \text{else} \end{cases} \tag{6}$$

Algorithmically, at the beginning of each simulation time step the concentration of the substances inside each cell is checked and, if any of the threshold values in **Table 1** are met, the cell type attribute is updated; then the cell grows taking into account mechanical constraints due to cell-cell interactions, and the area and shape attributes are updated. After growth, the cell size is checked for evaluating if to divide or not. The model then computes the reaction / diffusion module, based on its type (**Table 1**), and all substances first diffuse and then react among each other following the aforementioned system of Partial Differential Equations, taking into account geometry and topology. Then, the model enters the next time step.

In order to further explore the hybrid-model capabilities of VirtualLeaf, another simple assumption has been implemented for the description of cell growth and division. In this case, cells still grow at a fixed rate, but the division threshold is modelled as inversely proportional to the S_0 concentration. Cell size increases with the distance from the outermost layer of cells.

Parameter definitions and values for both the variants of the model are the same; they are presented in **Table 2**.

2.3.3 Model output and discussion **Figure 3** (at the end of the chapter) shows the output of two model simulations run for 1785 (**3a**) and 11505 (**3b**) time steps. A set of simulations was performed to test the capabilities of the model to reproduce the provascular patterns observed in *A. thaliana* plants (**Figure 2**, at the end of the chapter). All parameter values for the two simulations were the same. From these results, it is possible to observe how procambium arrangement is an emergent property dependent both on the reaction / diffusion dynamics that lead to the formation of Turing patterns (**Turing**, 1952) and on the tissue size, that influence the aforesaid dynamics and contribute to the differences between the stem (bigger domain) and the root (smaller domain) patterns. This model thus confirm, in a growing domain, the results presented by **Cartenì et al.** (2014). that the shift between a protostelic arrangement (**3a**) and a eustelic arrangement (**3b**) could be attributed only to the size of the domain in which the molecular dynamics occur. Moreover, both simulated sections show roughly the same cell number and the same total area of the reference photomicrographs (**Figure 2**c_2, **2**b_2, at the end of the chapter).

However, in the real system, cells are heterogeneous in dimensions and the difference between the model and the real system can be reduced if a further assumption for growth and division dynamics is added to the model.

The output from the third model simulation (**Figure 4**) shows how a simple rule can affect the system state producing a difference in terms of size among the cell rows, linked to the concentration

of S_0 inside the cells. Cells leaving meristematic zones often enlarge to hundreds of times their original size and the final size depends on the position they occupy in the radial arrangement. Cell expansion usually results from the combination of two processes: the increase in cell ploidy by endoreplication (DNA replication with no mitosis), and the complex process of cell expansion, which is driven by internal turgor pressure and restricted by the ability of cell walls to extend (**Perrot-Rechenmann**, 2010). These processes are under the control of several stimuli that can be spatially heterogeneous. In the presented simulation, this was achieved inserting the inverse of the concentration of a certain substance (S_0) as a term in the algorithm that triggers cell division. The condition may simulate the dynamics of a substance that inhibits cell growth, whose biological counterpart may be a sugar or a plant hormone (e.g. **Evans et al.** (1994)).

The proposed solution effectively reproduced the radial pattern of cell sizes observed in arabidopsis stems (**Figure 2**, at the end of the chapter). This result is an example of how a simple individual rule makes a complex collective behaviour emerge and illustrate the potential of VirtualLeaf for exploring spatial-explicit tissue dynamics.

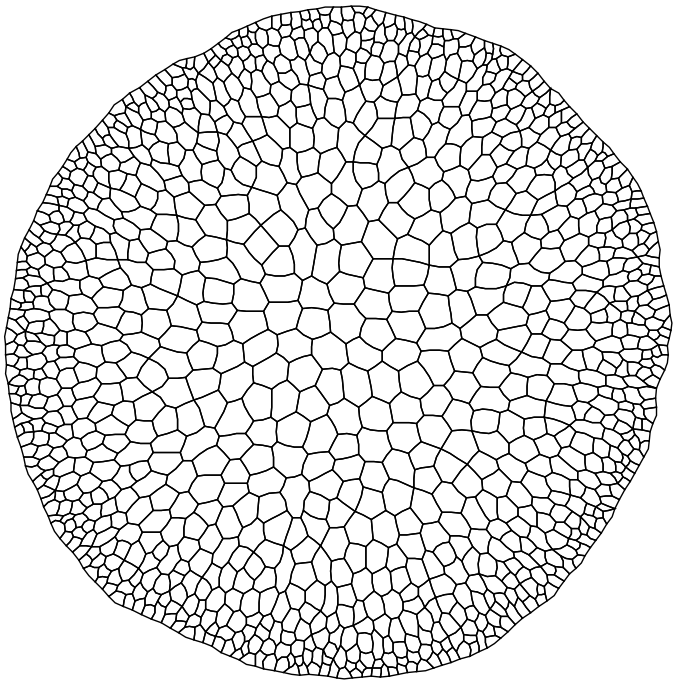

Figure 4. Output from alternative growth model.

The spatial position of plant vascular cells is established during early phases of development, when some cells are activated as procambium, and it is maintained along the plant life-span. Procambium arrangement patterns vary among different species but also between the stem and the root of the same individual: how they arise, how they are maintained, and what causes their diversity are open questions. Modelling the dynamics of the system in a manner appropriate to answer these questions is considered a complex challenge, since the system analyzed is multi-scale and characterized by a vast number of processes. IBMs and PDEs are unsuitable for this task, mainly because taken separately, these approaches are not able to efficiently describe multi-scale systems: the former cannot account for continuous dynamics and the latter cannot work with discrete elements.

In our case, a purely IBM approach would not efficiently model the diffusion of substances among cells (a continuous process) while a PDE model would fail to represent the properties of the single cells, mainly because it considers the tissue as a "whole" characterized by "average" properties (for the meaning of "whole" in this context, see (**Vincenot et al.**, 2011)). A hybrid modelling approach, instead, may be suitable because it permits to analyze multi-scale systems like tissues without oversimplifying them. Beside preventing conceptual approximations, this approach may become

necessary in order to inspect properties emerging from the interaction of processes that cannot be modelled with the same paradigm, as those linking individual properties with diffusion dynamics. In order to test this hypothesis we built a model using a hybrid approach, i.e. individual-based rules for modelling networks and interactions, and differential equations to model growth and diffusion.

2.4 CONCLUSIONS

This paper presented a brief review of cellular-based modelling in plant developmental biology, pointing out its strength and showing available tools. The present state is that models addressing the dynamics of root and stem vascular differentiation are few, and almost all of them work with static-domains, albeit the modelling tools proposed are more than capable to handle growing domains.

The paper also presents an example on how to analyze tissue-related dynamics using a hybrid modelling approach. The presented modelling process showed that this approach represents an efficient way to simulate tissue-level dynamics because it provides an intuitive manner to aggregate individual based dynamics with continuous processes like diffusion. A model built with this technique permits to observe emergent processes linked to the interaction of these discrete / continuous elements. A simple tissue growth model with a process-based condition of cell division shows how VirtualLeaf is capable to link spatial individual properties and process-based internal processes.

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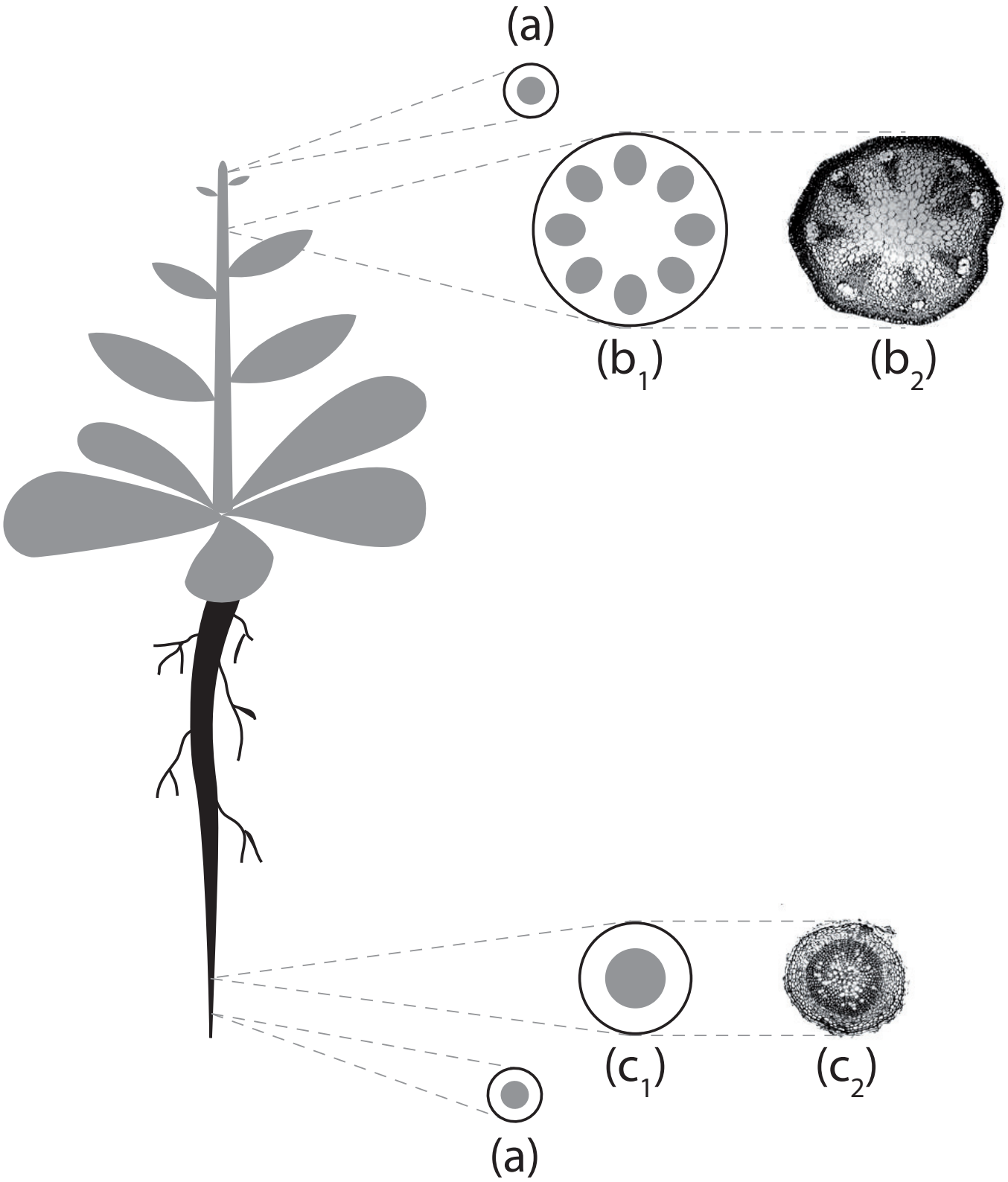

Figure 2. Schematization of *Arabidopsis thaliana L.* vascular structures in stem and primary root: (a) stem/root tip; (b_1) stem section scheme; (b_2) stem section micrograph; (c_1) root section scheme; (b_2) root section micrograph

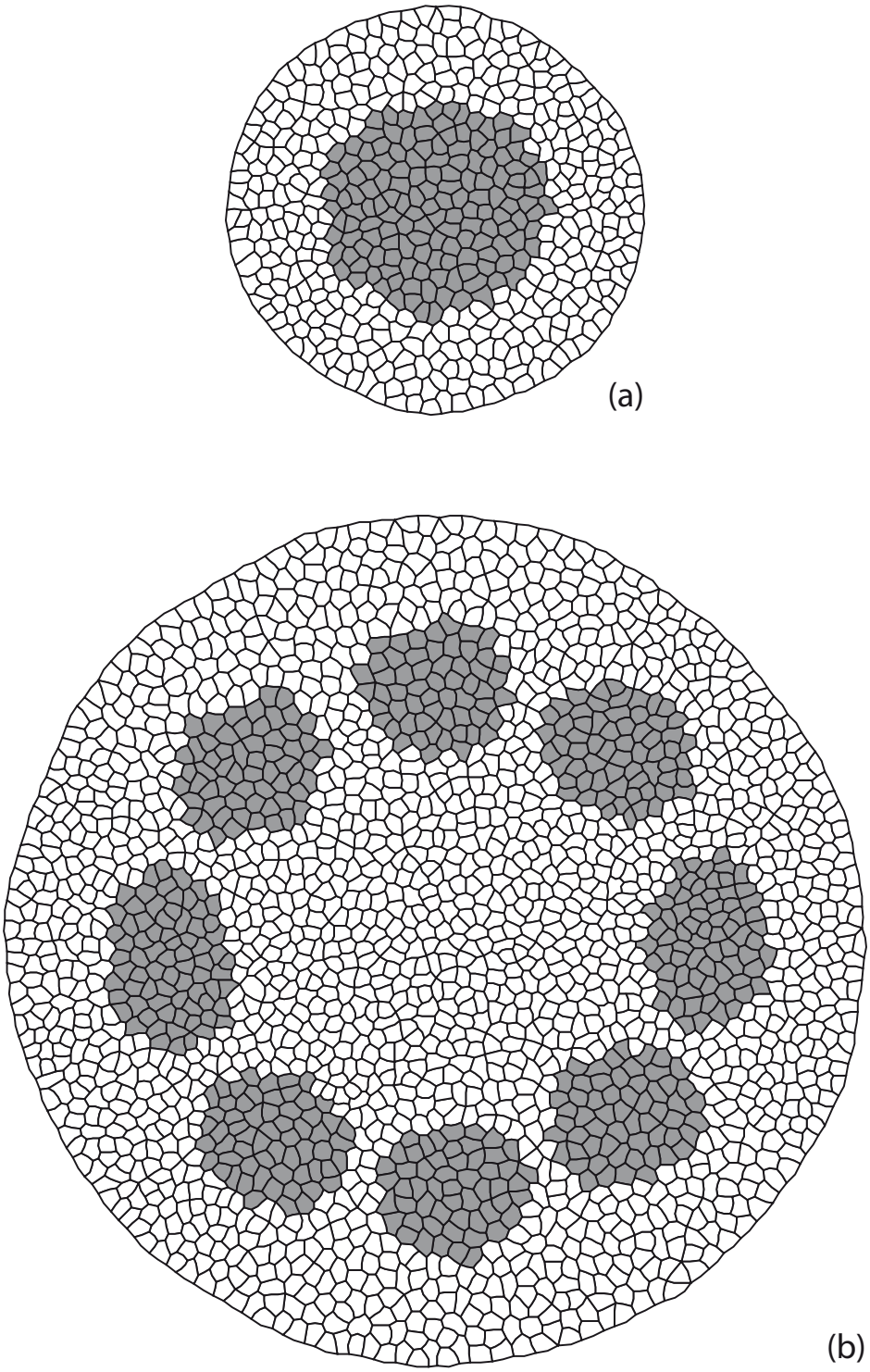

Figure 3. Model output for root (a) and stem (b) sections. Gray is procambium, white is non-procambial tissue.

Chapter 2

Simulating the effects of cell wall properties on plant tissue morphogenesis

3 SIMULATING THE EFFECTS OF CELL WALL PROPERTIES ON PLANT TISSUES MORPHOGENESIS

3.1 INTRODUCTION

In the last few years, scientists have collected a wealth of evidence regarding the actors involved in morphogenesis. Genes have been discovered, and molecular pathways have been built, with the intent to unravel the "under the cover" dynamics that makes possible for life to develop its forms. Even if many of the key players have been discovered or hypothesised, there still a conspicuous gap in our knowledge of how molecular information interacts with mechanical processes during morphogenesis, and how mechanical processes feedback on the biochemistry and molecular genetics of the cell. At present, relevance has been given to the study of the biomechanics of morphogenesis, and substantial evidence has been found of physical constraint over development (**Mirabet et al.**, 2011). Due to the complex, multi-scale dynamics involved, mathematical formulations and computational modelling have been found useful tools to investigate these processes (**Prusinkiewicz and Rolland-Lagan**, 2006).

The developmental biology of plants stands apart from that of animal life forms for two key reasons: the indefinite nature of plant development (due to the meristems) and the existence of the cell wall, which constrains the cell and almost always prevent cell migration. In fact, the cell wall can be thought as a physical barrier surrounding the cell, acting as a prominent controller of the cell size and shape with its ability to counteract the force of turgor pressure. Over the past decades, researchers worked out to understand how the cell wall is made, how it is formed, and how its properties contribute to the cell (and by extension to tissue) behaviour. The interest is not only outside of scientific curiosity, but also because to the importance that the cell wall constituents have for society (think of timber, paper, and more recently cellulosic biofuels, see Loque 2015). A good number of reviews have been written to organise the wealth of knowledge around this topic (**Cosgrove**, 2005; **Saxena and Brown Jr**, 2005; **Somerville**, 2006; **Joshi and Mansfield**, 2007; **mohnen**, 2008; **Guerriero et al.**, 2010; **Scheller and Ulvskov**, 2010; **Fujita et al.**, 2011; **Li and Gu**, 2012; **Wolf et al.**, 2012; **Li et al.**, 2014; **Bashline et al.**, 2014)

A cell wall is a complex object, mainly composed of cellulose and polysaccharide polymers such as hemicellulose and pectin, with also a few proteins. Through evolution, the cell wall has been selected to be both rigid enough to contribute to maintain the plant shape against gravity and to be plastic (referring to the ability to react to change, and also to its material properties (**Cosgrove**, 2016)) enough to make the plant cell shape optimal to the developmental and to the physiological situation it is in. Thus, understanding how the cellular wall is made it is fundamental to find out how cells control growth through cell wall synthesis and cell wall remodelling (remodelling being the breaking and re-insertion of elements of the pectin/hemicellulose matrix (**Sassi and Traas**, 2015)). Various hypotheses, all partially supported by experimental evidence, have been proposed to describe cell wall structure and how synthesis and remodelling work. Nowadays, the consensus is toward a cell wall represented as a complex viscoelastic entity with a coupled loosening dynamics, that goes toward a loosening/ yielding/ extension cycle during cell growth, and also thickens and lignifies in wood cells (for a thorough review see **Cosgrove** (2016))

Non-lignified cells area always under high internal pressure (i.e. turgor pressure) to maintain their shape. The only thing preventing them from bursting is the presence of the cell walls, that act as a "rigid exoskeleton", counteracting the pressing force, but also confines the cell to its actual shape and size. Thus, for the cell to grow, the cell wall must irreversibly yield (cfr. **Ali and Traas** (2016)), so that internal pressure lowers below turgor levels, causing a water inflow until the turgor level is re-established and counteracted by wall tension. Such "turgor-driven" cell expansion is based on the pioneering work of Sachs (reviewed in, e.g. **Hamant and Traas** (2009)) who discovered that plant cell expansion could only take place as long as there is a pressing force inside cells. The first attempt to model the turgor-driven hypothesis was made by **Lockhart** (1965), that described in mathematical terms the viscoplastic behaviour of cell walls under turgor pressure: in a non-growing isolated cell, the internal pressure is counterbalanced by the tension in the cell wall. If this pressure further increases and reaches a certain threshold, the load-bearing parts of the cell wall yield and the rate of growth of the cell is proportional to the excess of turgor pressure. The formulation also has the merit of stating that biophysics and biomechanic mediates the biological and biochemical control of plant cell expansion. The description has been largely used since its formulation, even if its assumptions (wall as a linear viscoelastic element, without plastic components) have been challenged by recent experimental discoveries (see **Cosgrove** (2016)). Nonetheless, the formulation is robust and simple and is widely used in modelling plant cell dynamics, together with a description of the water intake, for defining the rate of plant cell expansion.

As stated in **Hay Mele et al.** (2015), plants are built by complex and multi-scale tissues, where biochemistry, molecular biology, and biomechanics interact with each other. Their multiscale complexity plant tissues very interesting from a modelling standpoint, and also represents an excellent example of a context where modelling would help in advancing the knowledge of plant biology. As of today, many modelling paradigms have been proposed as an addition to the biologist's toolbox, and some of them can explicitly describe cell wall dynamics. Among them, the cell-based approach seems to be the most promising, as they tradeoff unnecessary cell complexity with a clean and compact way to describe cell dynamics, while taking into account processes at the tissue (like diffusion) and cell (like individual proliferation and differentiation) scales.

Here we developed a cell-based model to reproduce the development of a two-dimensional plant radial section, to investigate the role of mechanical heterogeneity of the plant tissue on the emergence of shape. More specifically, this work aim is to study the effects of cell wall thickening on the morphogenesis of plants. Our hypothesis is that wall thickness provide an additional way for the tissue to control some of its properties, like anisotropy, growth rate and division timing. To achieve this goal, we programmed cell wall thickness inside a cell-based modelling framework, tested the behaviour of a single cell, and then proceeded to analyse the behaviour of tissues composed of cells whose thickness is different.

3.2 MODEL DESCRIPTION

3.2.1 Simulator framework VirtualLeaf is a hybrid modelling framework built to describe plant tissues as multi-scale systems using an energy-based approach for modelling mechanical dynamics and differential equations for modelling biochemical dynamics (**Merks et al.**, 2011). This modelling

approach has been found extremely valuable for exploring new hypotheses associated to plant tissue dynamics where tissue shape and mechanical forces are considered key players and possibly interact with other dynamics (e.g. substance reaction and diffusion) to generate complex behaviours.

In VirtualLeaf, tissues are represented by a dynamic mesh of nodes, and cells are represented by non-overlapping polygons located on the same mesh. For every cell, it is possible to calculate an "internal energy" (H) that represents the balance between turgor pressure and wall tension:

$$H = \lambda_A(a_i - A)^2 + \lambda_L(l_j - L)^2 \tag{7}$$

where λ_A represents the cell resistance to shape changes, λ_l represents the wall resistance to length changes, i and j are the indexes corresponding to each cell and polygon edge, a_i is the actual cell area, l_j is the actual wall length, A and L are target area and target length, respectively. The target values (A and L) represent the values that area and length would assume in the absence of stress. It is noteworthy that in the original framework, both lambdas are general properties of the tissue (i.e. the mechanical properties of the tissue are the same at every point) and hence are constant parameters.

During tissue growth, every single node is randomly displaced, and the internal energy is calculated before and after the displacement, for all the cells associated with the displaced node. The energy difference ΔH is then used to calculate the probability (p) of transitioning from the old note position to the new one. The common approach is to calculate:

$$p = \begin{cases} 1, & \text{if } \Delta H < 0 \\ \exp\left(-\frac{\Delta H}{T}\right), & \text{if} \Delta H \geq 0 \end{cases} \tag{8}$$

where T is a measure of the "randomness" of the system (i.e. a proxy of the frequency of cells random movements). VirtualLeaf enables a slightly different formulation that makes use of another parameter S (**Savill and Hogeweg**, 1997):

$$p = \begin{cases} 1, & \text{if } \Delta H < -\sum_i^{\text{cells}} S_i \\ \exp\left(-\dfrac{\Delta H + \sum_i^{\text{cells}} S_i}{T}\right), & \text{if} \Delta H \geq -\sum_i^{\text{cells}} S_i \end{cases} \tag{9}$$

This formulation has a clear conceptual meaning: the higher the S parameter, the higher the energy needed to expand the cell. After the successful displacement of all nodes (i.e. the cell is now in a lower energy state than before), the energy difference between the old and new tissue configuration (ΔH_{Tot}), is compared against a mechanical energy threshold (ΔH^*_{Tot}): if the transition is successful ($\Delta H_{Tot} < \Delta H^*_{Tot}$), diffusion and subcellular dynamics take place. If the transition is not successful ($\Delta H_{Tot} \geq \Delta H^*_{Tot}$), the cell is not considered to have reached a mechanical equilibrium state, and the node displacement routine starts again.

To test the effects of the interaction between different cell types (i.e. cells with different elastic properties of the wall) on the final shape of a developing plant tissue, the second term of Equation 1 has been modified. λ_L is now a function of subcellular dynamics, thus making cell wall elastic properties cell-specific and heterogeneous within the tissue:

$$H = \lambda_A(a_i - A)^2 + \lambda_{L_i}(l_j - L)^2 \tag{10}$$

3.2.2 Individual-based rules Every individual cell, defined by the nodes' mesh, is subject to two basic processes, growth (i.e. cell expansion) and division (i.e. generation of two daughter cells). Growth is performed by the displacement of the nodes which tend to arrange in a way such that the actual area of the cell (a) is closest to the target area (A) to minimise the internal energy (H), as described in the previous section. Cell division is described by a simple conditional rule: when a cell reaches twice its initial area, it splits up along the shorter axis. This assumption is consistent with the knowledge that plant cells usually divide perpendicularly to the direction of higher stress, that coincides with the smaller moment of inertia (i.e. the shorter cell axis).

Moreover, two different cell types are defined in our simulations: "thickening" and "non-thickening" cells. In addition to the properties described above, the first type of cells also undergoes wall thickening which increases the stiffness of its walls, slowing down its expansion. During the simulation, cells do not differentiate, and daughter cells inherit the type of the cells that generated them.

3.2.3 Subcellular dynamics Within the modelling framework, each cell has two properties that change in time and are described by Ordinary Differential Equations (ODEs). First, the target area (A) of each cell (both cell types) is assumed to follow a logistic curve:

$$\frac{dA}{dt} = g_A \left(1 - \frac{A}{A^*}\right) A \tag{11}$$

where g_A is the maximum growth rate, and A^* is the maximum target area that a cell can achieve.

Moreover, the subcellular dynamics of wall thickening also takes place while cells of the second type ("thickening") grow. The assumption is that the cell wall thickness (W) increases according to a maximum growth rate gw and then slows down while converging towards a maximum thickness W_*. These assumptions are summarised in the following ODE:

$$\frac{dW}{dt} = g_w \left(1 - \frac{W}{W^*}\right) W \tag{12}$$

The value of cell wall thickness (W) is used to calculate the halting parameter S, which is assumed to increase during cell growth proportionally to cell wall thickness as follows:

$$S = k_S W \tag{13}$$

Parameter	Description	Value	Cell Type	Experiment
g_A	rate of TargetArea increase	0.001	All	All
A^*	TargetArea limit	8000	All	All
g_W	thickening rate	- 0.1 1 0.1	Non-thickening Non-thickening Thickening	Exp 1_a Exp 1_b Exp 2
W^*	maximum thickness	- 100	Non-thickening Non-thickening	Exp $1_a, 2$ Exp 1_b
W_0	initial thickness	0 to 100 0	Non-thickening Non-thickening	Exp 1_a Exp $1_b, 2$
k_S	thickness / halting parameter conversion factor	0.1	All	All

Table 1. Parameter table

where k_S is a conversion constant. The value of S for each cell is then used to calculate the probability of transition from the old nodes' configuration to the new one as previously described.

3.2.4 Simulations setup First, we wanted to test the effect of wall thickness on the growth dynamics of a single cell. The first set of eleven simulations was initialised with a single "non-thickening" cell, each one with a fixed wall thickness value (w) ranging from 0 to 10 arbitrary units. Then, another set of eleven simulations was initialised with a single "thickening" cell, each with a fixed maximum wall thickness value (w_*) ranging from 0 to 10 arbitrary units.

We also wanted to test the effect of five different spatial arrangements of the two cell types ("non-thickening" and "thickening") on the final shape of a plant tissue. The starting tissue was set up with a total of 177 cells representing a cross-section of a developing plant tissue (e.g. stem or root). Five different initial configurations have been designed, differing in the number and the position of cells of a specific type (Figure 3, second column). All simulation parameters are reported in **Table 1**.

3.3 RESULTS AND DISCUSSION

3.3.1 Single Cell In a single cell, expansion is achieved through four subsequent events: cell wall relaxation, intracellular pressure drops, cell water intake, and cell wall yielding. In the simulation environment, wall relaxation is characterised by monitoring the increase in the number of nodes, pressure drop and water intake are described by monitoring area and target area dynamics, and wall yield is described by monitoring perimeter dynamics. The target area was established as a proxy for pressure stress, and the distance between the equilibrium wall length and the real wall length was established as a proxy of wall strain.

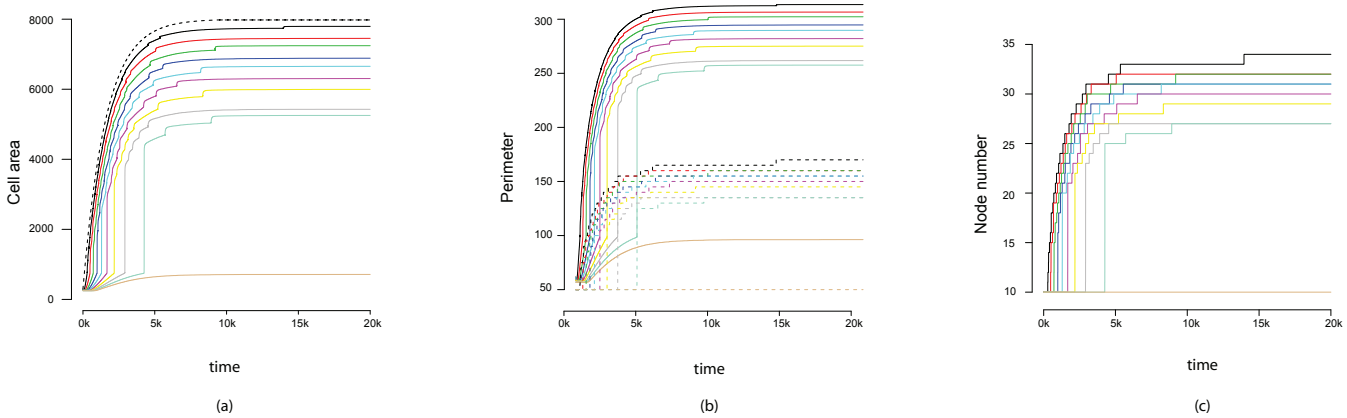

Figure 1. Cell area and wall dynamics for a single "non-thickening" cell.A. Cell area; B. cell perimeter; and C. number of nodes.The colour of the curves represents different values of wall thickness (w): 0 (black), 1 (red), 2 (green), 3 (blue), 4 (cyan), 5 (magenta), 6 (yellow), 7 (gray), 8 (aquamarine), 9 (burlywood), 10 (darkgoldenrod). Values of all simulation parameters are reported in **Table 1**.

We analysed the behaviour of a single plant cell without ("non-thickening" cell type, Fig. 1) and with ("thickening" cell type, Fig. 2) dynamic variation of the cell wall thickness, as described in the "Subcellular dynamics" section.

Figure 1 shows growth dynamics for single cells with fixed wall thickness (in different colours). Growth dynamics for a single cell with fixed wall thickness (Fig. 1, different thickness coded as different colours), shows that thickness influence both the equilibrium area and the expansion trajectory of the tissue, with the equilibrium area decreasing as the thickness increase (Fig. 1a). It is possible to distinguish two behaviour classes: "constrained growth" (for thickness in the range [1–6] and the maximum thickness value), where the area dynamics are always proportional to the target area, and "burst growth" (for the remaining thickness values). In this case, the cell goes through a first phase of "constrained growth" before experiencing a moment of instant growth and then settling around its equilibrium value. Perimeter (Fig. 1b) and wall relaxation (fig. 2b) closely follow the area dynamics, maintaining the "constrained" and "burst" behaviour separation. The figure also shows how the instant growth event is accompanied by simultaneous relaxation of the wall. It is also possible to appreciate how the mechanical stress associated with the target area increase significantly strains the cell walls (Fig1b), and how wall relaxation seems to be restrained proportionally to wall thickness (Fig1c). Figures 1b and 1c also show that for the maximum thickness value the yielding curve does not have the same shape as the other and no relaxation occurs (Fig 1b and 1c, darkgoldenrod line).

In the framework used here, the weight of the wall tension in the Hamiltonian (Eq. 7) is proportional to wall thickness. Thus, thicker cells can deviate more from the target area than soft cells (i.e. withstand greater internal pressure) while still being at mechanical equilibrium. On the other hand, if the turgor pressure rises to a value high enough, it will overcome the wall tension, and the system will rapidly (in our case instantaneously) converge toward the target area.

In all the constrained growth cases, the system will minimise the area delta (i.e. the difference between actual and target area) while maintaining an equilibrium area proportional to the wall

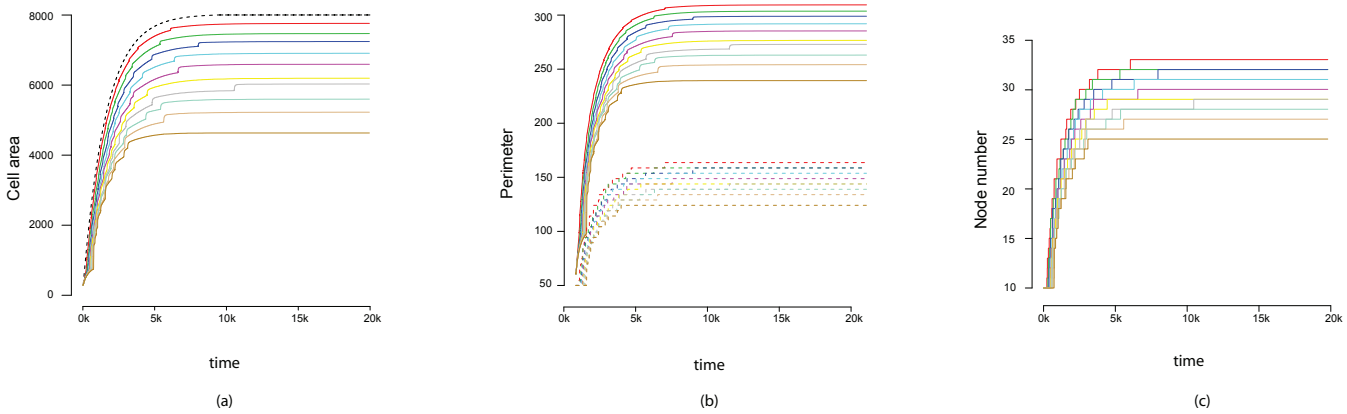

Figure 2. Cell area and wall dynamics for a single "thickening" cell. A. Cell area; B. cell perimeter; and C. number of nodes. The colour of the curves represents different values of maximum thickness (w*): 0 (black), 1 (red), 2 (green), 3 (blue), 4 (cyan), 5 (magenta), 6 (yellow), 7 (gray), 8 (aquamarine), 9 (burlywood), 10 (darkgoldenrod). Values of all simulation parameters are reported in **Table 1**.

thickness, because of its effect as a weight parameter in the Hamiltonian. In the burst growth cases, the system will start with a higher weight put on the length constraint, but when the turgor pressure (i.e. the internal pressure) rises above a threshold level, the area effect becomes stronger than the length one, and the tissue experiences instantaneous growth.

For the maximum thickness value used in the experiment, deviation from the target length is always costlier than a deviation from the target area (since the former is weighted ten times more), so that the total system always minimises the length delta, heavily dampening tissue growth.

In the case of the "thickening" cell type, the cell wall thickness increases in time (see Eq. 1b) up to a maximum value (w_*). Simulation outputs (Fig.2) shows that the "bursting" behaviour is no more evident, being relegated to the early phase of the growth dynamic. The disappearance of the "bursting behaviour" is due to the coupling of the thickening dynamics with the pressure dynamics: since they grow together, the pressure never accumulates up to the "bursting" threshold.

We can conclude that in our system thickness constrains wall yielding and reduce wall stress, thus slowing down growth and absorbing energy destined for cell expansion. Both effects cause alterations in expansion trajectories, with the latter also being responsible for the value of equilibrium area.

3.3.2 Tissue description To survey the effects of cell wall thickness on inter-cellular mechanical interactions in a multi-cellular context, we arranged five different initial tissue configurations and simulated cell expansion and proliferation for each one of them. The five tissues have the same geometry (Fig. 3, first column), but differ in the localisation and number of thickening (brown) vs non-thickening (green) cells (Fig. 3). The arrangements are labelled "soft" (no thickening cells), "border" (thickening cells localized only at the tissue border), "inner ring" (thickening cells forming an internal ring in the tissue), "inner core" (thickening cells localized everywhere but the border) and "peripheral mass" (thickening cells forming a single mass localized on the tissue border).

3.3.3 Effect of cells with different mechanical properties on morphogenesis Figure 3 shows simulation results after 200 timesteps for each of the five initial arrangements. From the simulation

outputs, it is possible to observe that all the arrangements differ in size, with "border" and "inner core" producing the smallest tissue. "Inner ring" shows an intermediate tissue size, and "peripheral mass" shows no difference with the "soft" tissue. Tissue shape is strongly affected only in the "peripheral mass" case, whereas all the other cases show isotropic growth.

These results suggest that thickness heterogeneity impact on tissue size, and thus on its growth rate. As observed in the case of the single-cell experiment, the growth rate of "thick" cells is lower than its "soft" counterpart. The lower growth rate does efficiently slow down cell proliferation, that is linked to reaching a size threshold. We can define this as "cell-autonomous slowdown", and we can also define a "cell non-autonomous slowdown", where a "soft" cell growth is constricted by mechanical interactions with "thick" neighbours.

An example of "cell-autonomous slowdown" is the "inner core" case (Fig. 3, fourth tissue from the top). This tissue is composed mainly by "thick" cells, so it is expected to grow less when compared to a "soft" tissue (Fig. 3, first tissue from the top) where cells are free to proliferate for the same amount of time.

The "border" configuration shows an example of "cell non-autonomous slowdown": in it, "thick" border cells heavily constraint the growth (thus the proliferation) of the soft internal cells. It is worth noting that in this model, cell-cell mechanical interactions can also work the opposite way and force cell expansion. In this case, the cell whose expansion is forced will eventually divide itself to be not ripped out.

This forcing means that all tissues where soft cells represent the majority will eventually expand and continue to do so. It also implies that layers of thick cells around thin cells will, in some condition (like those of the "inner ring" arrangement), act as "delays" that periodically inhibits growth. The latter is explained as follows: the dynamic starts with thick cells preventing soft cell expansion by "cell non-autonomous slowdown", with the system remaining stable (i.e. non-growing) while soft cells' target area increase. At some point, like the case of "burst growth" in single cells, the system will reach a critical energy level, that will force the thick ring expansion. The expansion of the thick ring will lower the internal energy level, returning the tissue in a stable state. In the "inner ring" configuration dynamics this causes the outer ring of cells to behave differently than the internal soft core. In fact, while the former will proliferate gradually, and always at the same rate, the latter will be subject to intense (i.e. strong but short) proliferation events.

The extent on which heterogeneous mechanical properties (in our case due to different wall thickness inside the tissue) impact on morphogenesis could be appreciated in the "peripheral mass" example. In this configuration, a small boundary mass of thick cells causes the tissue to lose its circular shape, ending with a shape that resembles the heart-shaped phase of *Arabidopsis thaliana* embryo development (**Wendrich and Weijers**, 2013). The "peripheral mass" tissue also follows the same dynamics, and produce a qualitatively similar result, of wound healing after callus formation. It is known that after a fire, cells around the wound de-differentiate and form a callus, to protect the wound itself. Then, during secondary growth, the callus will be engulfed inside the trunk (**De Micco et al.**, 2013).

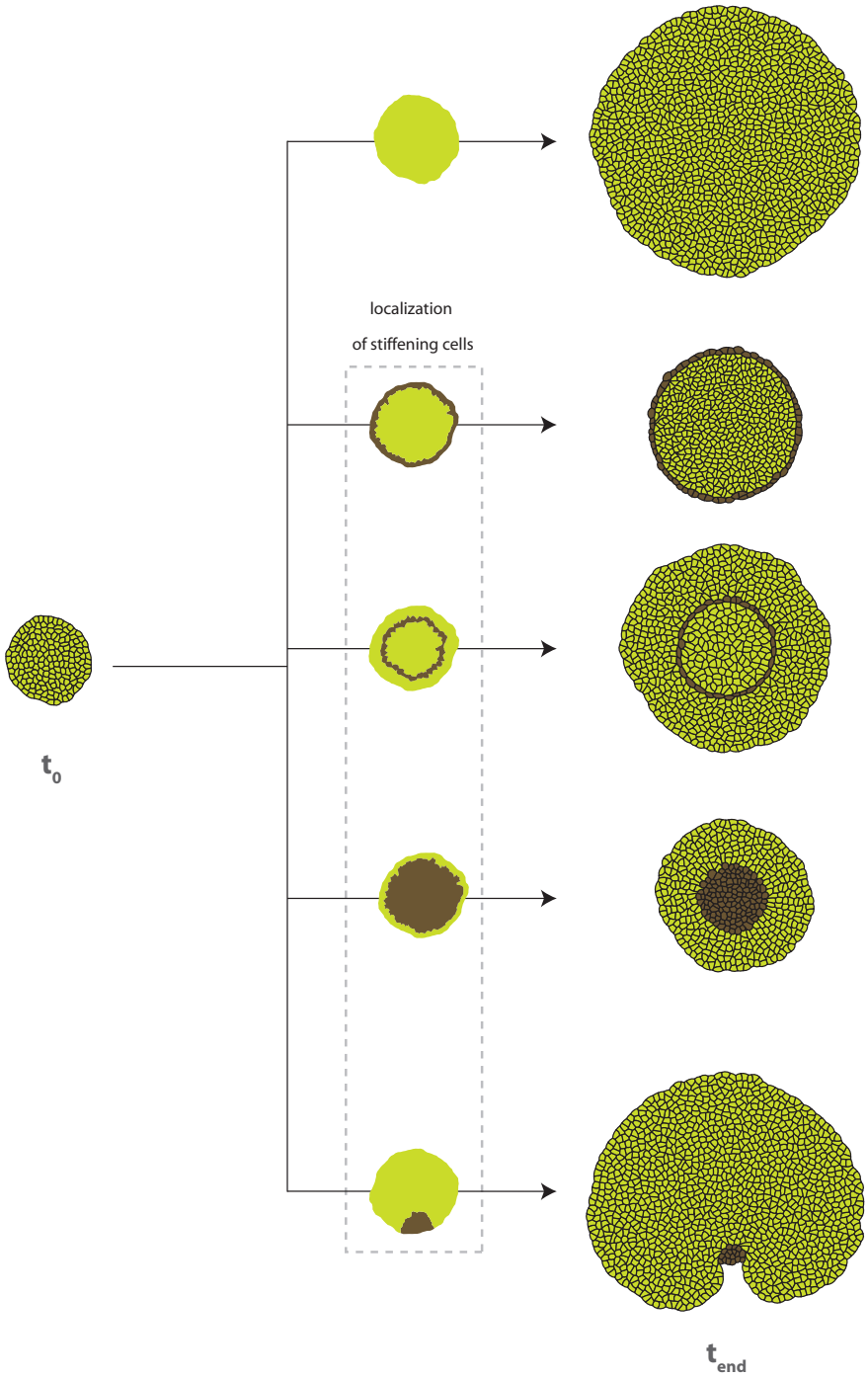

Figure 3. Effect of heterogeneous mechanical properties on tissue morphogenesis. Five different arrangements of the "non-thickening" (green) and "thickening" (brown) cell types are presented. From the top: absence of stiffening cells ("soft"); "border" localization of "thickening" cells; "inner ring" localization; "inner core" localization; and "peripheral mass" localization. Values of all simulation parameters are reported in **Table 1**.

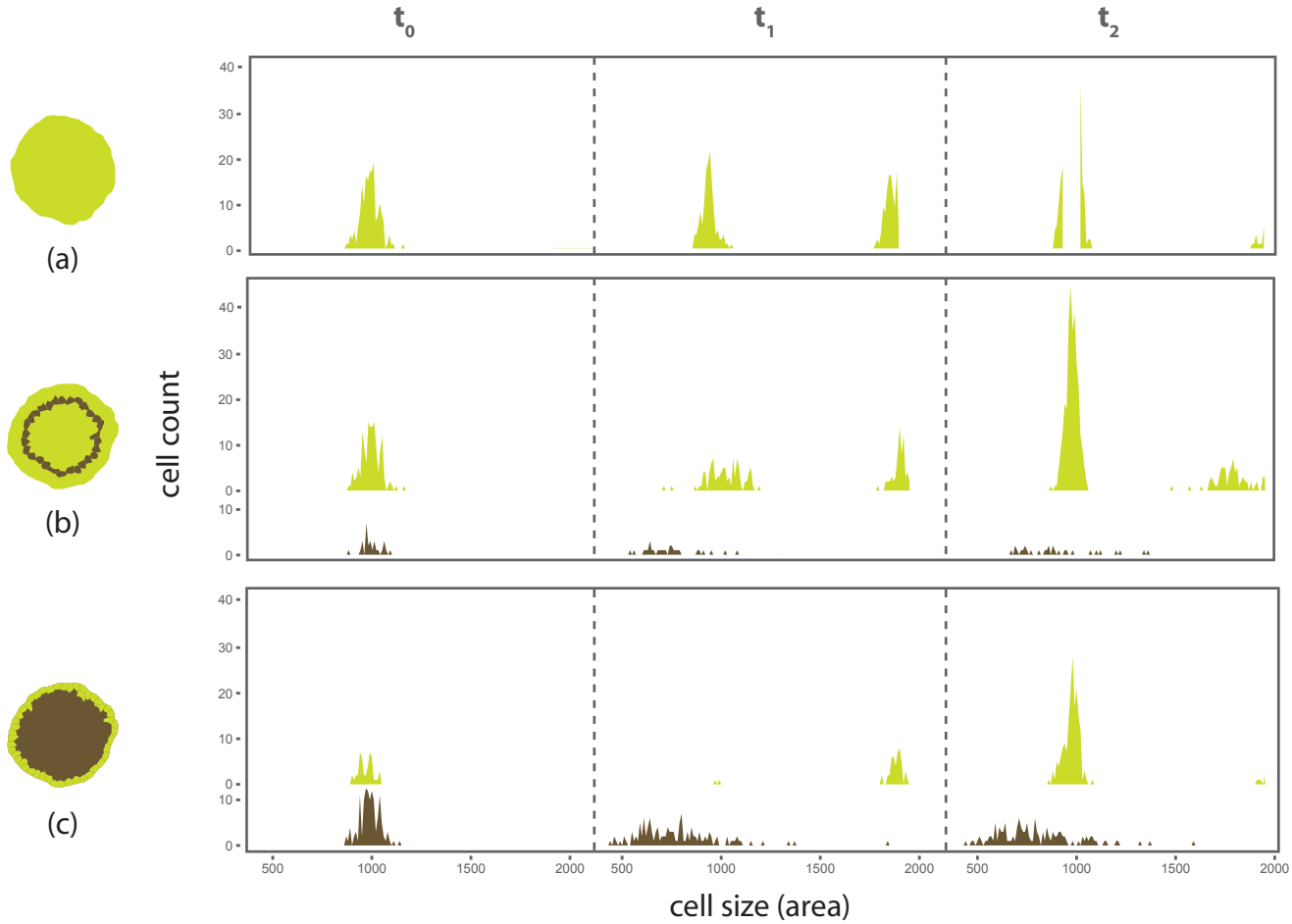

Figure 4. Distribution of sizes for "non-thickening" (green) and "thickening" (brown) cells in three different spatial arrangements. For each configuration (a. "soft"; b. "inner ring" and c. "inner core"), cell sizes' distribution is calculated at the beginning (t_0), in the middle (t_1) and at the end (t_2) of the simulation. Values of all simulation parameters are reported in **Table 1**.

3.3.4 Distribution of cell sizes for the different tissues Figure 4 shows changes in cell size distribution for three of the cases presented in the previous section ("soft", "inner ring" and "inner core" arrangements). Every box represents the cell size distribution of the tissue shape shown on the left at the beginning (t_0) middle (t_1) and end (t_2) of the simulation. Except for the first arrangement (no "thickening" cells), the global cell population is sub-setted in the "non-thickening" cell population (green, top) and the "thickening" cell population (brown, bottom).

In all arrangements, cells of both populations start with a normal distribution (t_0).

- In the "soft" tissue (Fig. 4a), the simulation goes through a bimodal phase and ends with three distinct modes, with the rightmost one (i.e. the biggest value) being the least numerous.
- In the "inner ring" arrangement (Fig. 4b), soft cells' areas spontaneously organise in a bimodal pattern during the simulation, crowding around modes almost at the same value of the "soft" tissue configuration, with the only difference that sizes distribution is broader around the first

mode. The bimodal distribution is maintained until the end of the simulation, but the dispersion around the mode is inverted for the two sub-populations. The distribution of the areas in the "thickening" cells ring spreads over the initial mode during the simulation, while slightly moving toward the left.

- In the "Inner core" pattern (Fig. 4c), the "thickening" cells show the same behaviour of the inner ring arrangement, while the distribution of the soft cells remains unimodal through the duration of the simulation. THe "inner core" arrangement is the only case examined where cells sizes spread around a single area class (cfr. Fig 4a and 4b).

During growth, every cell can potentially travel along the area classes, eventually "jumping" from the last class (theoretical maximum size, i.e. the size that triggers division) to the first class (initial size of a daughter cell). This jump explains the general tendency of soft cells' area to organise into a bimodal distribution, reflecting the separation between "old" cells (i.e. big cells, that are almost on the point of dividing) and "young" cells (i.e. cells that just underwent mitosis). The "intermediate" mode appearing at the end of the simulation (t_2) for the homogeneous "soft tissue" can be interpreted remembering that the cell's area is dependent on mechanical interactions with other cells, and that boundary cells are less constrained (hence grows faster) than cells located inside the tissue.

Figure 6 shows the comparison of simulation outputs with real tissues.

3.3.5 Lineages of cell proliferation Figure 5 shows the proliferation trajectory dynamics of "non-thickening" cells in three of the simulated arrangements. Proliferation trajectories are displayed as the segments joining cells sharing the same ancestor. For every row, the final tissue (as in Fig. 3, last column) and the proliferation trajectories are shown.

- In the first case (Fig. 5a, "soft" tissue), the proliferation trajectories are mostly isotropic, and almost all cells divide the same amount of times, producing a 'Z'-shaped pattern.
- In the second case (Fig. 5b, "inner ring" tissue) the core of inner cells is not proliferating at all (o trajectories are present), while in the outer ring the boundary cells tend to maintain the 'Z'-shaped proliferation pattern, while most of the inside cells tend to proliferate in straight directions.
- In the last case (Fig 5c, "inner core" tissue), there is again a strong homogeneity in proliferation trajectories. In fact, almost all the trajectories are straight line departing from the centre, except a boundary cell that displays the 'Z'-shaped trajectory.

As stated in the model description, cells always divide along their shortest axis. This behaviour means that the proliferation trajectory is mainly dependent on the direction of cell expansion. Cell expansion, in turn, depends on both autonomous and non-autonomous cell mechanical interactions. Autonomous cell stress contributes to isotropic cell expansion in all the cells, regardless of their thickness or the thickness of their neighbours. Conversely, in soft cells partially surrounded by thick cells, non-autonomous cell stress could constrain expansion to be anisotropic. This effect is because cell expansion on the cell walls shared with the thicker cells (i.e. wall elongation) would force the

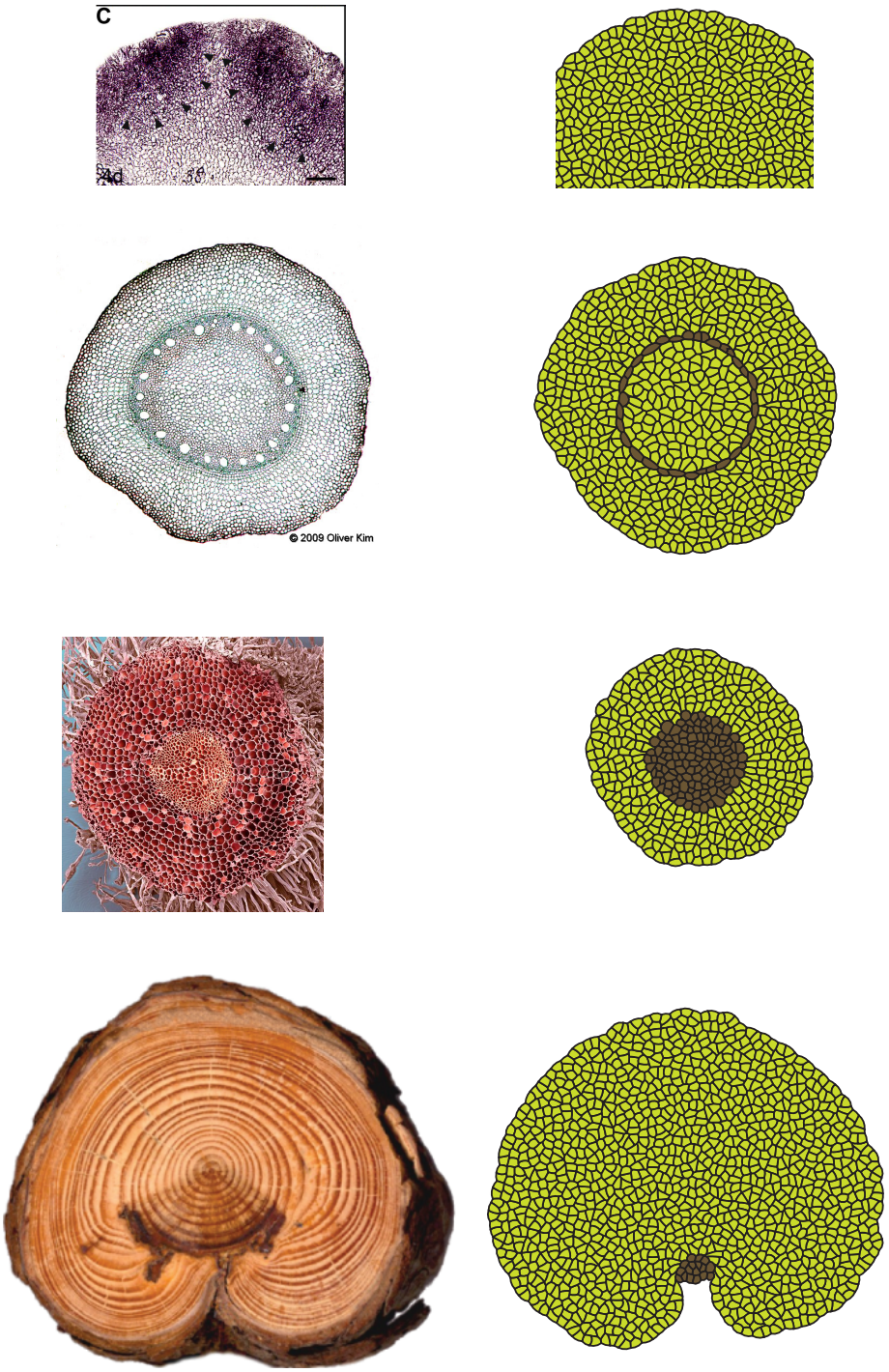

Figure 5. Comparison of simulation outputs with real tissues.

thicker cell in an unfavourable energy state. Since cell growth is a statistical process that is triggered by the cell internal energy moving along favourable states, cell expansion does not happen in the direction of cell walls shared with thicker cells. It is also worth to note that boundary cells are

subject to a lesser degree of non-autonomous stress than other cells. These two dynamics explains the different behaviour showed in Fig. 3. In the "soft tissue" case, the homogeneity in mechanical properties and the non-existent cell thickness make possible isotropic cell expansion, thus producing a disordered proliferation trajectory. In the other two cases, the mechanical heterogeneity contributes to sustaining anisotropic growth toward the tissue boundaries making proliferation proceeds along a straight direction.

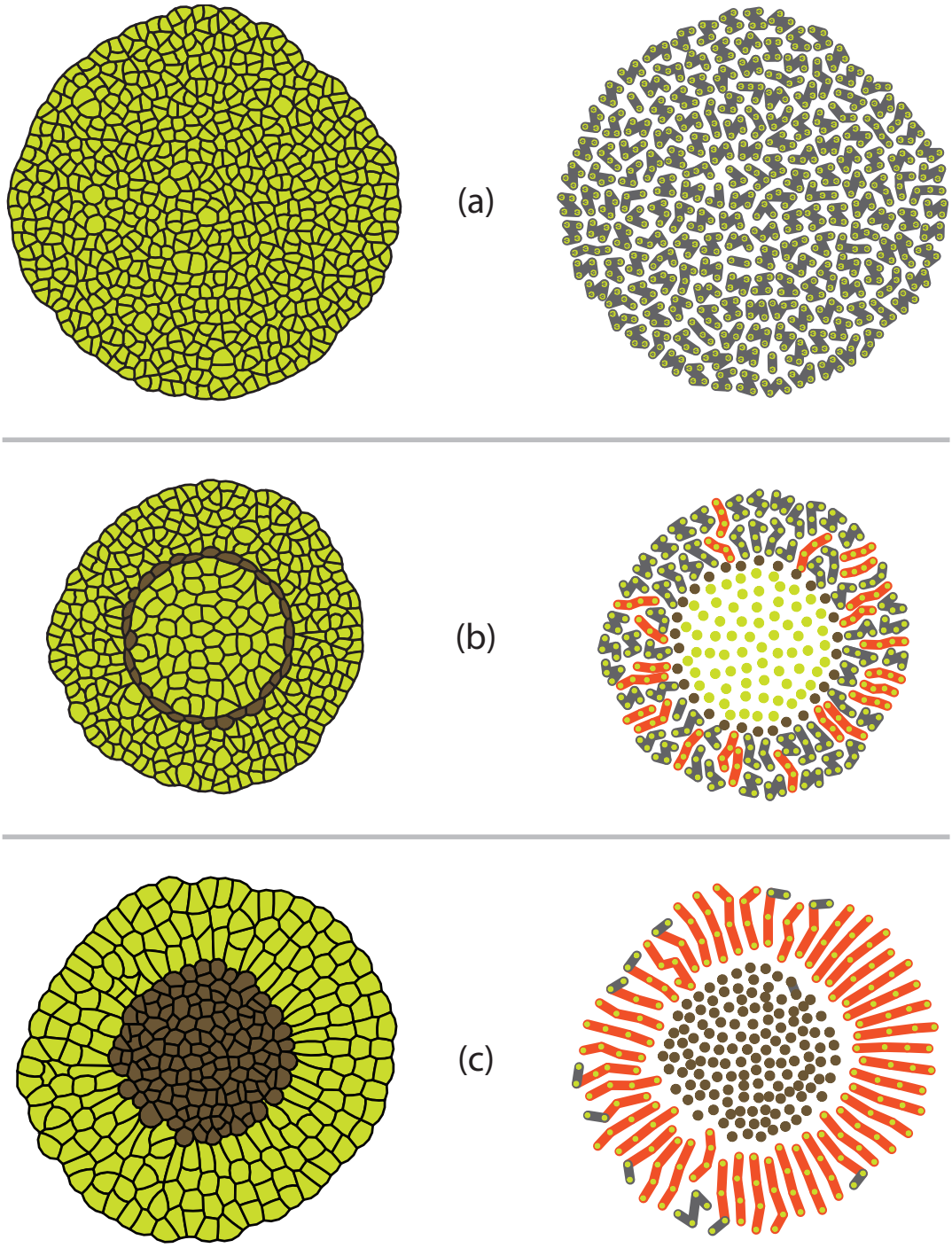

Figure 6. Lineages of cell proliferation during the simulation a. “soft”, b. “inner ring” and c. “inner core” configurations. On the left is shown the simulation output while on the right the corresponding proliferation trajectories are shown. Lineages are defined as the composites of segments that join cells produced by the same mitosis event. Lineages following radial direction are highlighted in red. Values of all simulation parameters are reported in **Table 1**.

3.4 CONCLUSIONS

In plants, the cell wall is a key actor, mediating many of the organism's interactions with the environment. It also contributes to the force balance fundamental for almost all the mechanical processes associated with plant growth and plant shape maintenance. It is thus fair to hypothesise that regulating the cell wall mechanical attributes would provide an additional way for the tissue to control some of its properties, like anisotropy, growth rate and division timing. To test this hypothesis, we first expressed the contribute of one of such attributes (i.e. wall thickness) to the internal energy of the cell, using an Hamiltonian-based modelling framework. Our expression was tested with a single cell model, and we proved that it does not contradict the fundamental biomechanical assumption at the basis of cell growth. Such kind of frameworks relies on a phenomenological description, and they do not permit to investigate the sub-cellular mechanism that produces wall thickening. On the other hand, Hamiltonian-based approaches give us a suitable way to model these dynamics and their effects on plant tissue mechanics. The presented simulation framework, although entirely theoretical, showed a very promising potential to investigate the appearance of several patterns of tissue arrangements in plants. Being able to observe tissue's shape evolution while keeping a cell-based description of its processes renders the framework useful in linking different fields of plant biology and in testing hypotheses on plant development. After the single-cell test, we used the framework to simulate the development of a generic two-dimensional plant tissue where two cell types were arranged in different configurations. The simulations' output showed that cell arrangement impacts on tissues properties such as cell growth rate, proliferation trajectory, and tissue growth anisotropy. In conclusion, this work presents a suitable framework for exploring thickness-related dynamics in plant tissues and provides evidence that the tissue behaviour could be regulated at cell level by the process of cell wall thickening.

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Chapter 3

Modelling plant tissues with geometrical dynamics: the case of xylogenesis

4 MODELLING PLANT TISSUES WITH GEOMETRICAL DYNAMICS: THE CASE OF XYLOGENESIS

4.1 INTRODUCTION

Wood is a plant structural tissue associated with water transport and structural support. Trees produce wood when they increase in diameter, during the secondary growth. The mechanism of girth increase involves proliferation and differentiation of specific groups of cells arranged in two rings, buried inside the stem at different distances from the centre and called cambia. The cambium associated with wood development is called vascular cambium, and its proliferation produces "cambial derivatives" along the radius in both directions. In temperate trees, vascular cambial activity has been observed to be periodical, with the cambium usually active between spring and autumn (**Prislan et al.**, 2013).

Cambial derivatives produced toward the inner side receive a signal that inhibits their proliferation and triggers the differentiation into wood cells, but no definitive evidence about the nature of the signal has been produced (**Hacke et al.**, 2016). During the early phase of differentiation, a rapid anisotropic increase in cell's diameter is observed, that could be driven either by auxin dynamics (**Shibaoka**, 1994) or by mechanical stress (**Landrein and Hamant**, 2013). Subsequent to the distension phase, the cell enters the maturation phase: it starts to produce and deposit a secondary wall and begin the production of lignin that encrusts the cell wall. After complete wall thickening, the xylem precursor undergoes through programmed cell death and become a mature xylem cell (**Wilson**, 1984).

Cohorts of cells simultaneously go through these phases, forming "zones" along the tree radius and ultimately generating annual tree-rings. The fact that these zones are spatially consistent suggests the existence of a signal that enforces zonation, clearly hinting at the ideas first introduced by **Wolpert** (1969) and the hypothesis of the existence of a morphogen gradient (**Bhalerao and Bennett**, 2003). The morphogen has not been identified, and while many point at auxin as the best candidate (**Bhalerao and Bennett**, 2003), some evidence is supporting small peptides for this role (**Etchells et al.**, 2013).

The wood formation process is complex and variable, with fivefold differences in size and wall thickness among cells produced during a growth season (**Koerner**, 2015). It has been observed that xylem cells formed during the first part of the growth season (i.e. spring) have a wider lumen and thinner walls than cells formed during the late growth season (i.e. summer). The transition between these two forms is a remarkable developmental process (**Deslauriers et al.**, 2014; **Schweingruber**, 2012), with deep technological and ecological implications: wood is a significant sink of CO_2 in plants (**Vaganov et al.**, 2006), its anatomy is fundamental for the plant functioning (earlywood contributes to water transport and latewood to structural integrity), and timber is a primary economic resource for some countries (**Tojo and Hirasawa**, 2013; **Funada and Kajita**, 2014). Notwithstanding experimental evidence, it is not entirely clear how and which environmental factors influence xylogenesis (**Vaganov**, 1990; **Vaganov et al.**, 2006; **Cuny et al.**, 2013), for it is hard to disentangle the complex network of physiological and developmental processes involved in wood formation (**Cuny and Rathgeber**, 2016) without losing systemic information. Moreover, the mechanical feedback of the cell wall structure over both its developmental processes and the

developmental processes of the cell's neighbours are rarely and scarcely considered (this is a common trend in plant morphogenesis, albeit partially reversing thanks to the use of computational models, see for example **Grieneisen et al.** (2007) and **Traas and Monéger** (2010)). Wood cell anatomy and mechanical properties are still subject to substantial measurement constraints (**Cuny and Rathgeber**, 2016), and no cohesive view of cell wall structure and dynamics have been found yet by the scientific community (**Cosgrove**, 2016).

Even if models of cambial activity have been published in the past, most of them focus on the ecophysiological dynamics associated with the tree during xylogenesis (**Wilkinson et al.**, 2015; **Vaganov et al.**, 2006; **Deleuze and Houllier**, 1998), while neglecting the biophysical dimension (i.e. cell growth and division). No model takes into account the mechanical feedbacks between cell structure and cell biochemistry, in spite of the strong feedbacks existing between mechanical forces, cell wall properties and substance diffusion (for an example of such feedbacks, see **Laskowski et al.** (2008)).

Hartmann et al. (2017) produced model-based evidence that both cambial differentiation into xylem and zonation in wood-forming tissues could be controlled by a morphogenetic gradient. The authors acknowledge that the same gradient cannot be accounted for size differences in wood since the morphogen field fails to regulate final cell size. Furthermore, the model cannot maintain the homogeneity of cellular structure, unlike real wood, that shows a strikingly stable structure (**Balducci et al.**, 2016).

It is clear from this introduction that a plant system undergoing secondary growth is a complex, multi-scale system, where biomechanic is tightly coupled to differentiation, spatial dynamics are key players, and where signal transport interacts nonlinearly with growth. Thus, plant tissues are systems that would be usefully explored by a multicellular, quantitative and dynamic model. Here we implement explicit wall dynamics in our VirtualLeaf extended framework (Chapter 2) and we will use it to investigate the effect of cell-cell mechanical interactions over xylogenesis in an adult conifer's stem. More specifically, we will answer the following questions:

- How the interaction between growth, cell wall deposition and lignification contribute to modulate the lumen size and the wall thickness of mature wood elements?
- Do seasonality-induced water and structural sugar fluctuations associated with growth and wall deposition produce tree-ring structures?

Here we hypothesize that tree rings formation is driven by the effects of thickening over cell expansion and that the early- to latewood transition is mediated by water and structural sugar availability, both strongly influenced by environmental fluctuations.

4.2 MODEL DESCRIPTION

Based on theoretical considerations over the nature of the system of interest, expressed in the introduction and in **Hay Mele et al.** (2015), we choose our enhanced VirtualLeaf framework (Chapter 2) to build a hybrid growing-domain model of xylogenesis in the stem of a woody plant.

In VirtualLeaf, every cell of the tissue is a sophisticated agent formed by the cell itself (base cell") and by a polygonal cell wall, shared between the cell and its neighbours. Separating the base cell from the wall permits to give both the same level of detail and also to define cell-wall interactions efficiently. For each "base cell", geometrical properties (all calculated using the cell wall's vertex coordinates) mechanical properties and concentration of every substance considered are assigned and dynamically updated over the simulation time. All the continuous dynamics (intracellular substances dynamics and diffusion) are described using a set of differential equations (ODE submodel), while an Individual-Based model (IBM submodel) controls cell differentiation and division. The IBM defines which Ordinary Differential Equations (ODEs) are active in the cell, and regulates the diffusive term in the PDEs. Furthermore, both the continuous and IBM submodels contribute to the Hamiltonian that controls cell spatial properties (such as area, perimeter and shape) that, in turn, influence intracellular properties.

Formulating the model in this way means that we have an actual hybrid model able to work on multiple time and space scales while coupling biochemical and mechanical dynamics.

4.2.1 Model definition The model domain is a specific ara in a transversal section of an adult conifer's stem undergoing secondary growth, namely the zone starting with the vascular cambium cells facing inward and proceeding toward the stem centre. The domain boundary coincides with the boundary between cambial cell producing xylem precursors and cambial cell producing phloem precursors. Thus, we have three cell types: cambial cells, xylem precursors (i.e. cells in the distension and maturation phase) and mature tracheids (i.e. dead xylem cells).

Following the current knowledge of xylogenesis dynamics (**Rathgeber et al.**, 2016), the model considers proliferation in cambial cells, and distension, thickening by deposit of structural sugars, lignification and PCD in xylem precursor.

The model also considers the diffusion of a morphogen M (**Hartmann et al.**, 2017), that is homogeneous in the individual cell space, enters the cambium cells from the outside of the model domain and move by diffusion.

M represents the substance involved in the creation of the radial gradient that determines which cells will lose the cambial status (i.e. the ability to divide) to become xylem precursors (**Hartmann et al.**, 2017). All the dynamics are intended to be generalised, phenomenological versions of the actual dynamics occurring during xylogenesis (**Rathgeber et al.**, 2016). As such, our model is not meant to be a molecular-level explanation of the process, but rather an example of how the synergy among mechanical and biochemical processes contribute to tissue dynamics (in this case xylogenesis). It is also intended as a general chemo-mechanical framework to support the hypothesis of difference between early- and late-wood as motivated by mechanical interactions.

The modelling system codes the transition between individual-based and continuous dynamics as seamless, going back and forth among them. Nonetheless, we choose to describe these dynamics separately in the text, to facilitate reading.

4.2.2 Geometric formulation and Hamiltonian Since cell wall deposition and lignification are tightly linked to the spatial attributes of the cell (i.e. its perimeter and its lumen area) we gave our

framework the ability to display cell wall, and also to explicitly calculate its absolute and percent area. As a side result, the system is now able to calculate the area of the cell lumen. Thus, the new system is able to use cell geometry to calculate how much the cell wall is thickening in time and to feed back this information to the Hamiltonian-based cell growth algorithm.

4.2.3 Drawing wall thickness and building the cell's lumen We can describe wall thickening in a single cell as the shrinking of its associated polygon toward its centre of mass by a factor ρ, that is moving every cell's node i from the position O to the position N according to the formula:

$$N_i = \rho(O_i - C_i)C_i \tag{14}$$

where

$$\rho = 1 - \frac{W_i}{\bar{r}} \tag{15}$$

With W_i thickness of the cell wall to which i belongs, and $\bar{r}$ averaged distance of the cell's nodes from the centre. The new polygon represents the cell's lumen.

Based on vertexes coordinates, we can calculate cell's area (using the Gauss's area formula) and perimeter in real time: this makes the new framework able to describe lumen's contraction dynamics. The formulation also makes possible to calculate the wall area as the difference between cell and lumen areas.

4.2.4 Hamiltonian Since the Montecarlo-based geometric framework has been widely explained elsewhere (**Hay Mele et al.** (2015), Chapter 2) we will mention here only the changes needed to formulate our model. The first difference is that i is defined per-wall-element, as the average between values of thickness among all the owners of the wall element. The second change is that the cell stiffness is proportional to the cell wall thickness by a factor k_s. All the geometric information are calculated after the acceptance of the Monte Carlo step (for information about the Monte Carlo dynamics in VirtualLeaf, see **Hay Mele et al.** (2015); **Merks et al.** (2011)).

4.2.5 Individual-based rules The model considers three cell types: cambial cell, xylem precursor, and mature tracheids. For every type, a specific set of action is performed (**Figure 1**):

1. If the cell is a cambial cell, the model first checks if the cell is still detecting the M signal: if the signal is undetectable ($[M]$ lower than a certain threshold $[M]^*$), the cell differentiates to xylem precursor. Otherwise, the cell enters the yielding, extension and water intake phase (**Cosgrove**, 2005), i.e. cell's target area increases of a fixed amount k_g, proportional to water intake (i.e. in limiting conditions k_g is lower). If the cell is twice the original size, it will divide (**Fiorani et al.**, 2000) perpendicularly to the tensile stress (**Louveaux et al.**, 2016), here assumed to be along its long axis. This is because it is fair to consider the cell shape as the results of mechanical forces. Since cell's elongation is in the direction of the stress, it is reasonable to conclude that the cell's major axis is where the tensile stress is happening in cambium cells. After division, M quantity is halved in each of the two daughter cells.

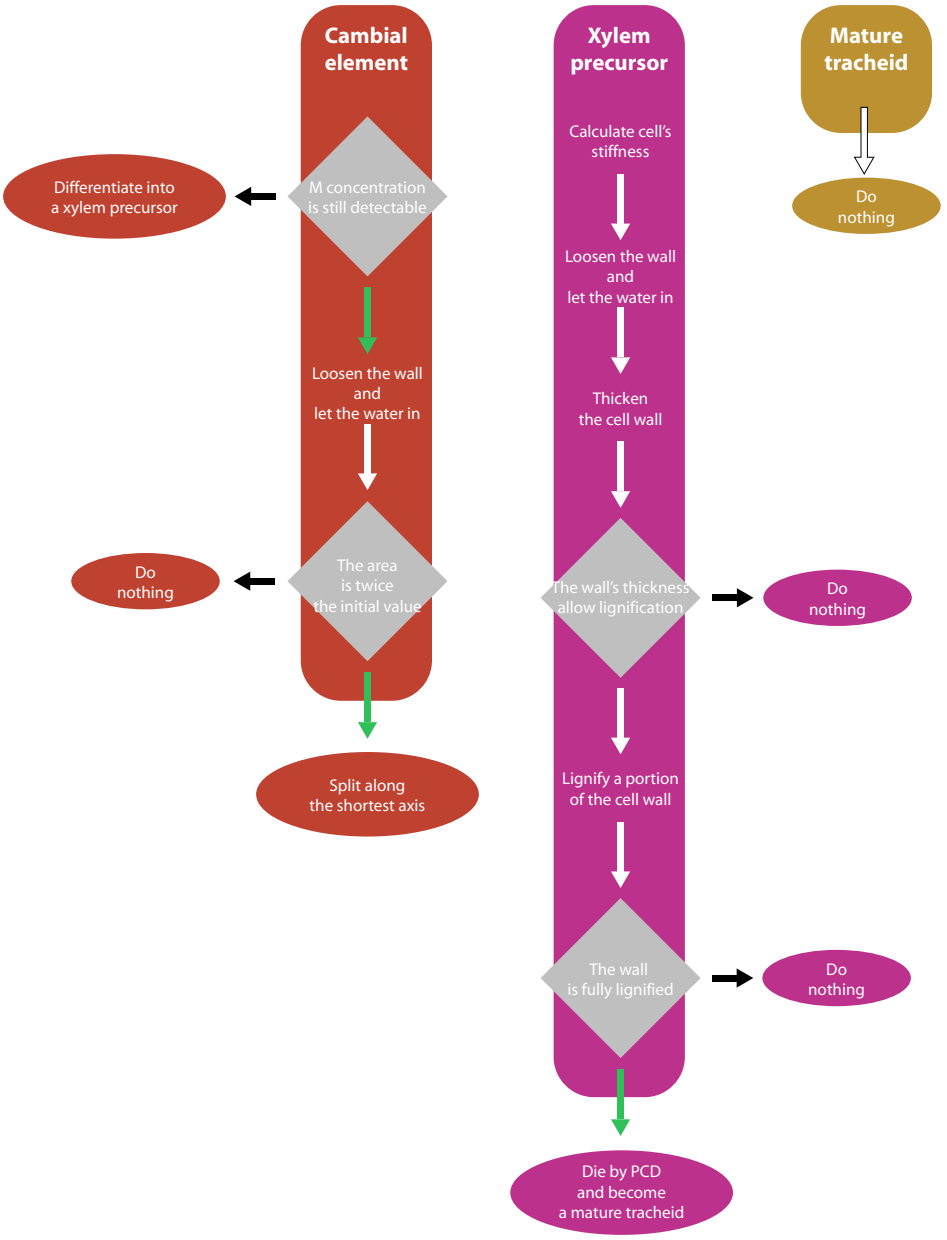

Figure 1. The flow of actions regulating cell behaviour, described per cell. Diamonds stands for control conditions, with green arrows for true and black arrow for false. "Do nothing" signals the program to stop simulating and move to another cell. For details of the single processes, see the main text.

2. If the cell is a xylem precursor, the cell stiffness is updated. Then, the cell enters its enlargement phase (cell's target area increases in the same way as a cambial cell), and start to deposit the wall. Then lignin dynamics take place, and if the grade of lignification is severe (i.e. that is, lignin has encrusted k_l percent of the wall area) the cell dies (simulating programmed cell death after complete lignification) and become wood (**Fukuda**, 1997). Both cell wall deposition and lignification are continuous dynamics, described in the next section.

3. If the cell is a mature tracheid, it does nothing.

4.2.6 Continuous dynamics If the cell is a boundary cell, then M quantity is reset to the value M_0 (the assumption here is that M is continuously entering the cell from the outside, i.e. from the phloem). Else, M is degraded proportionally to its concentration. Thus, we assume that M diffuses through the apoplast (simplified here by the cell walls) obeying a diffusion-decay transport mechanism (**Grieneisen et al.**, 2012), where diffusion is limited proportionally to wall thickness. As such, the dynamic changes in M concentration associated with a single cell i are described mathematically as:

$$\frac{\partial [M]_i}{\partial t} = \left(\sum_j^{\text{walls}} l_j \omega_j D([M]_i - [M]_{Cj}) \right) - k_d [M]_i \tag{16}$$

Where l_j is the length of wall J, D is the diffusion coefficient for M, the footer Cj represent the cell sharing wall j with the current cell, k_d stands for decay rate and ω_j represents the logistic effect of thickening over substance diffusion in wall j as:

$$\omega_j = \max \left(0, 1 - \frac{0.5(w_1 - w_2)}{w_n} \right) \tag{17}$$

with w_1, w_2 represent the thickness over the two sides of the wall, and w_n representing the thickness at which a wall is rendered impermeable to M.

Wall thickening in xylem precursors is measured in units of weight over units of length, and described as:

$$\frac{dW}{dt} = a_s u_t \frac{A_l}{A_c} \tag{18}$$

Here we assume that the extent of thickening is proportional to the amount of structural sugars (a_s), it is a fraction of the thickening happening in optimal conditions (u_t). We also assume that cell thickening is limited by the amount of lumen space available (hence the ratio between lumen's area A_l and cell's area A_c).

Wall lignification in xylem precursors is measured in area units, and described as:

$$\frac{dL}{dt} = \mathcal{K} u_l \left(1 - \frac{L}{k_l A_w} \right) \tag{19}$$

where

$$\mathcal{K} = \begin{cases} 1, & \text{if } W \geq k_f \\ 0, & \text{otherwise} \end{cases} \tag{20}$$

Here we assume that lignin production starts when there is enough free space in the secondary wall (i.e. condition $\mathcal{K}$, stating that lignin production will start when the thickness reaches the value k_f). We also assume that the amount of lignin produced is a fraction of the production in optimal conditions u_l (measured in unit of area over unit of time). Since lignin is deposited in the space between structural sugars microfibrils and this space adds up to k_l percent of the total wall area (A_w,

Parameter	Description	Value
k_s	Conversion factor between thickness and stiffness	1
M^*	M minimum level for maintaining cambial properties	0.001
k_g	Quantity of target area increase per unit of time	2/1
k_l	threshold % of lignin in the cell associated with cell death	0.2
M_0	Steady quantity of M in boundary cells	1
D	Diffusion coefficient for M	0.04
k_d	Decay rate of M	0.2
w_n	Thickness at which the wall is rendered impermeable to M	10
u_s	Quantity of structural sugars produced per unit of length in unit of time	5000/200
u_l	Quantity of lignin produced in unit of time	0.5
k_p	% of the cell wall area available to lignification	0.3
k_f	Minimum thickness required for lignification	1

Table 1. Parameter table

calculated geometrically as the difference between the cell area and the lumen area), we consider lignification as a logistic process, modulated by the amount of available space. The value of the parameters for all the simulation described in the chapter are shown in **Table 1**.

4.3 MODEL OUTPUT AND DISCUSSION

Figure 2a represents the starting tissue, produced running the model on a single cell. This starting tissue represents the aforementioned inner core of the stem section at the beginning of the spring: cambial cells are in red, pith is white. The shape of the cambial cell is variable (Fig 2a, inset +) and cells sometimes form ordered "rows" (Fig 2a, inset ++).

We simulate growth during spring setting $u_w = 5000$ and $k_g = 2$, to account for higher sugar availability and non-limiting water: after 835 time steps (**Figure 2b**) the cambial ring (in red) has produced a ring of mature tracheids (in brown) with thin walls, a wide lumen and an elongated shape. Cells proliferation during the interval (a) to (b) displays a centrifugal, straight direction (inset +), and the tracheid produced have a shape more regular than the cambial cells. The output produced during the interval (a) to (b) demonstrates that the new Hamiltonian, the algorithm for the lumen perimeter and area calculation, and the graphical method for wall thickness all work correctly and coherently with the hypotheses stated here and elsewhere (Chapter 2). Moreover, accordingly with our previous observation on the effect of thickening on tissue properties, cell thickening contributes to direct the proliferation along pseudo-rows (e.g. Fig.2b, insert +). Using this output as a new input, we simulated late spring, setting $u_w = 200$ and $k_g = 1$, to account for low structural sugar availability and limiting water: after 835 time steps (Fig.2c) the cambial zone has grown a new ring of mature tracheids, with thick walls, a narrow lumen, and a compressed shape. Cells still proliferate along the radius (inset +), and "thick" tracheids show neither concavities nor convexities in their shapes compared to "thin" tracheids (inset ++). Also, "thick" tracheids appear

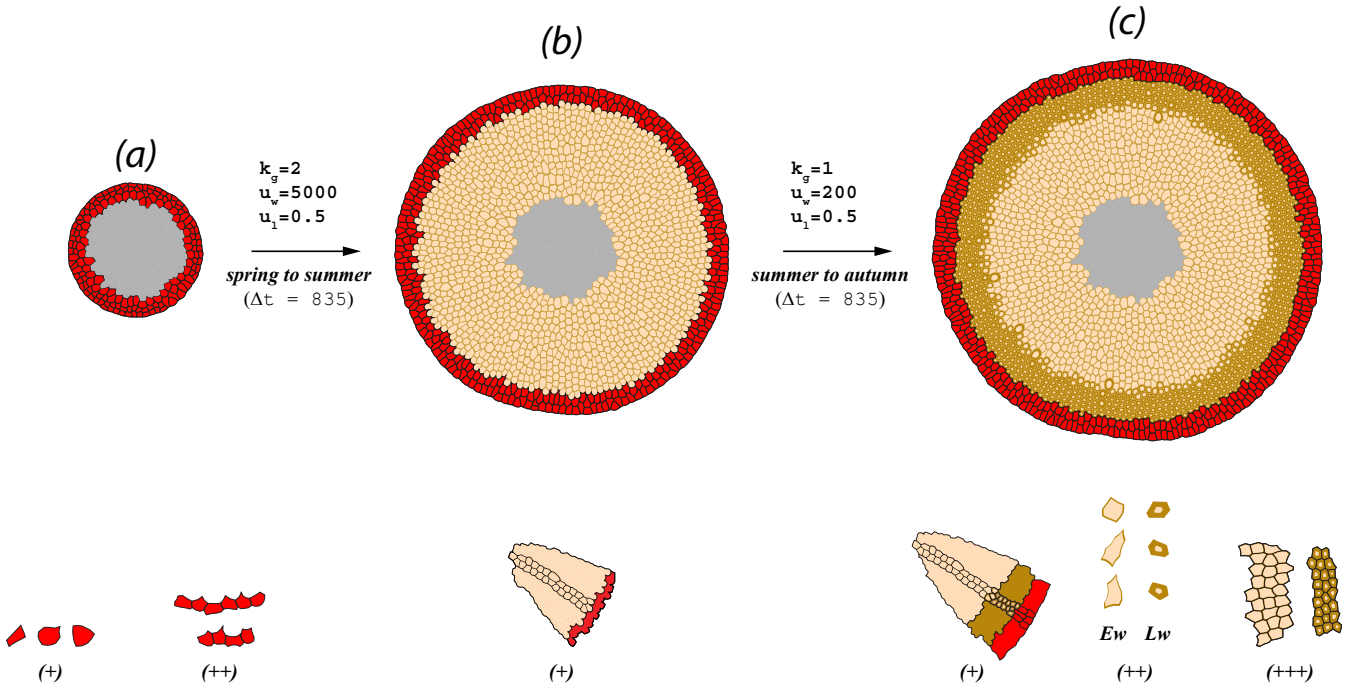

Figure 2. Panel of simulation outputs describing the time evolution of tissue (a) during two phases of diameter growth. The cell type is colour-coded: red for cambium, brown for wood, and grey for pith. Below each output are displayed the most significant properties of the cells/tissue associated with it: phase a, inset (+) shows cambial cell shape plasticity, inset (++) shows spontaneous radial rows of cells; phase b, inset (+) shows the emergence of directionality in wood cells production; phase c, inset (+) shows the persistence of the directionality after simulated seasonal shifts, inset (++) shows the difference in shape, size, and wall thickness among cells produced in the first phase and cell produced in the second phase, inset (+++) shows differences in the packaging of cells between first phase and second phase cell populations.

to be more densely packed than "thin" tracheids (inset +++). The output produced during the time interval (b) to (c) demonstrates the effect of structural sugar availability on cell's shape and wall thickness. Comparing (b) to (c) permits to appreciate the "firmness" of the wood during the growth process; the pseudo-rows tend to maintain their alignment (Fig 3c, inset +). Also, "thick" cells show neither concavity nor convexity in its walls' structure compared to "thin" (Fig 3c inset ++) and "thick" cells are also packed tighter (Fig 3c, inset +++). As side results, it is interesting to note the emergence of penta- and hexagonal shapes in almost all the "thick" cells. These two phenomena (non-bending walls and almost regular shapes) suggest that the effect of thickness on the cell shape extends beyond the cell's area. The final output of the simulation showd in FIgure The simulation outputs suggest that a model considering a morphogen field-based differentiation, cell-based wall dynamics, and that also explicitly considers the feedback of thickening over cell dynamics could account for the regularity in tree-ring formation. Even if auxin seems prominently involved in all the processes mentioned above, contemporary views on its role in vascular development are often very complex and not considered on a systemic scale, making not clear to what extent auxin transport is a determinant of the process itself (for example, vessel diameter (**Hacke et al.**, 2016)).

It is worth remembering that since cell wall expansion and cell wall thickening are fundamentally different processes, they are expected to be influenced by different factors: turgor-pressure dependent expansion (**Cosgrove**, 1997) relies on water availability (**Hsiao**, 1973), while carbohydrate availability-dependent thickening (**Demura and Ye**, 2010) relies on CO_2 and light levels (**Körner**, 2003). Furthermore, part of the complex array of lignification processes is susceptible to temperature (**Donaldson**, 2001; **Simard et al.**, 2013).

Finally, it has been hypothesised that environmental processes influence the rates of physiological processes rather than their duration (**Cuny and Rathgeber**, 2016).

Measuring mean cell area and cell wall thickness (respectively coloured blue and brown in **Figure 3**) along the radius of the tissue c of **Figure 2** for mature tracheids confirms the qualitative results. Different trends for the two attributes are shown, that together contribute to the distinction between "thin" zones (light brown background in **Figure 3**) and "thick" zones (in dark brown in the figure). The measure also highlights the existence of a transition zone (white background in **Figure 3**), produced during the first moments of the transition between tissue (b) and tissue (c). Finally, cell area seems to fluctuate more than wall thickness along the radius.

These results show that cell wall thickness has a limiting effect on cell expansion, and also support the hypothesis of lignification as a controller of the wall thickness at maturity. This means that the shift between an early and latewood cell could be determined solely by the quantity of structural sugar available during the wood growth period. This is interesting because, as stated before, the main hypotheses about xylogenesis rely on environmental factors and hormone dynamics to explain how and why it produces one type of wood cell rather than the other one.

It is fair to assume that cell wall thickening and lignification impact on mechanical dynamics, thus interacting with cell expansion and influencing tissue growth. Even if wood developmental processes are clarified at the molecular level (**Heo et al.**, 2017), and notwithstanding early evidence (**Prendin et al.**, 2017), no model accounting for mechanical interaction has been proposed yet.

Detailed individual measures of area and thickness (**Figure 4**) along the radius of the same tissue (c in **Figure 2**) permits to fully appreciate the fluctuation of these attributes between individual cells, and also the emergence of variability among both wall thickness and cell area, that ultimately produced a sub-population of outliers along the radius, with higher area and wall thickness at maturity. During the evolution of the simulated system, a narrow transition zone appears: this is due to the continuous nature of the thickening process, and its dependence on the lumen area. In fact, when the season (i.e. the parameters k_g and u_w) change, immature xylem precursors with broad lumens will rapidly thicken their walls, generating transition wood (cfr. Fig 3). This phenomenon is characteristic of some tree species, like Larix (Fig. 6)

The simulation then continued for 5900 time steps (**Figure 5**) simulating other three wood growth cycles, each one composed of two model runs: one with the "spring" parameters and the other with the "summer" parameters. It is worth to note that cells appearing during the later growth cycles show a decrease in the number of sides: this suggests that the rectangular shape of conifer's wood cells is modulated both by cell-cell mechanical interaction and autonomous cell processes (i.e. cell wall thickening). THe long-run simulation permits to appreciate some later dynamics that deviates from the real behaviour of wood. For example, during earlywood formation some cell rows "fork" due

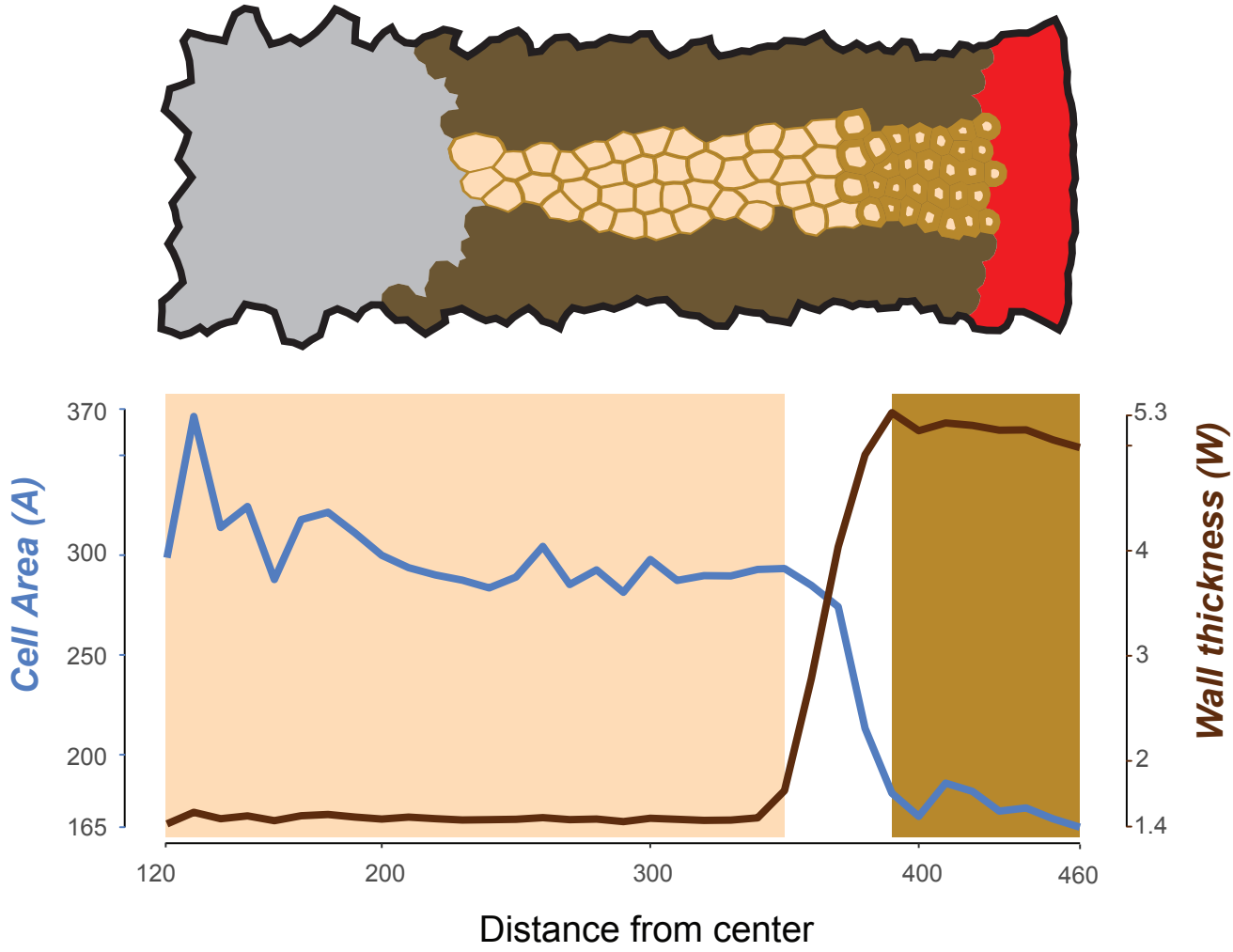

Figure 3. Detail of cells produced along the radius (a), and quantification (b) of cell area (blue line) and wall thickness (brown line). Area and thickness units are expressed in arbitrary units. The plot displays the two attributes averaged over increments of ten length units along the radius. The background of (b) separate cells produced during the first phase of growth (light brown) from cells produced during the second period of growth (dark brown). No background (white) has been used to mark the zone of transition between the two cell types.

to anticlinal division events. This type of division is generally associated to girth increase in plant stems, and is not usually performed by cambial cells undertaking differentiation into xylem. In our simulation, anticlinal divisions of cambial cells tend to break the cell "cords" that emerges during tissue development. Further modelling experiments should be performed in order to evaluate what could prevent this behaviour. THe simulation makes also possible to appreciate another interesting difference between early and latewood, namely that the former tend to orient their sides radially and their vertexes tangentially, while the latter behave the opposite way.

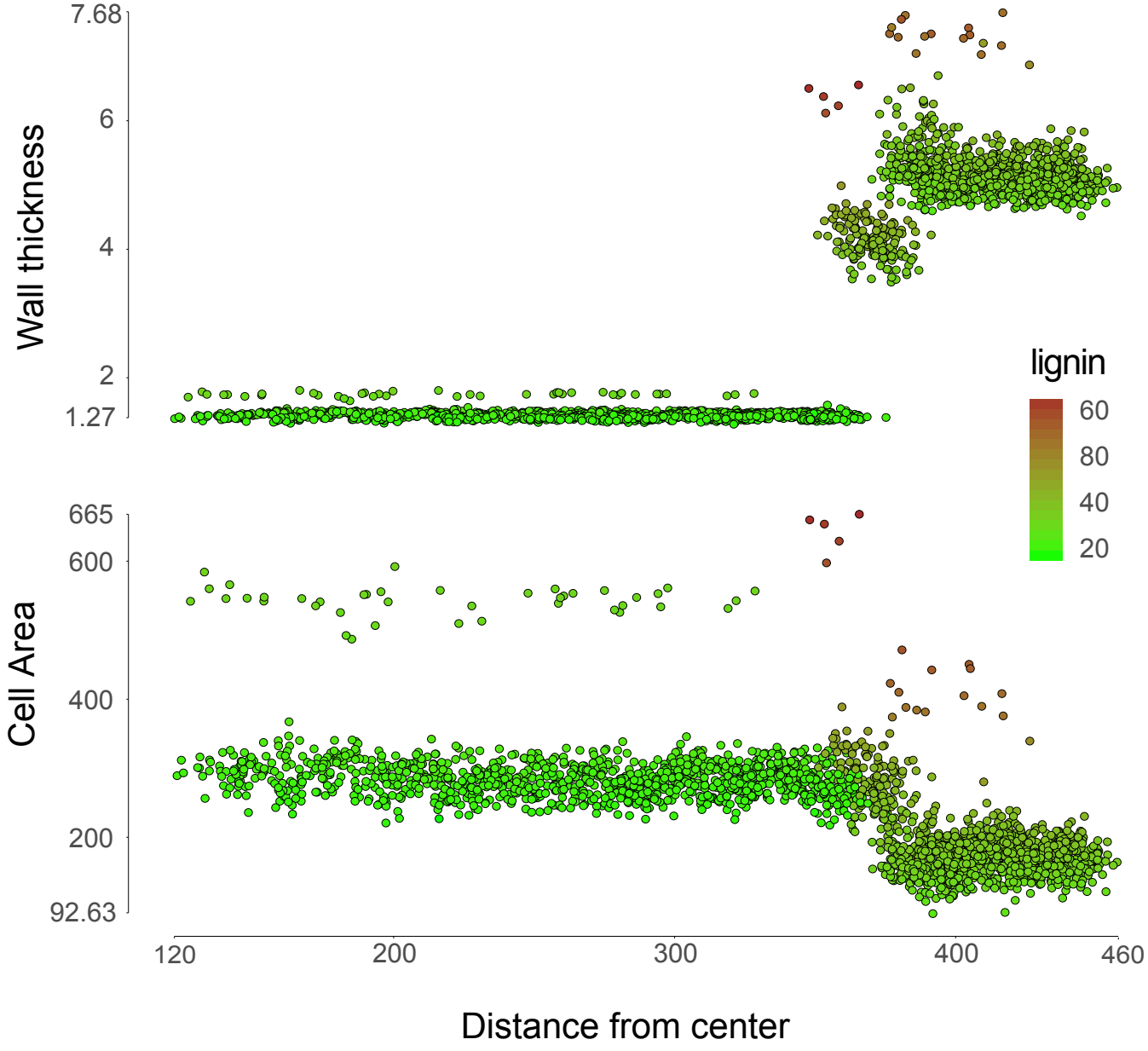

Figure 4. Area and thickness units are expressed in arbitrary units. Wall thickness and cell area values of single cells, ordered by distance from the centre of the tissue. The amount of lignin per cell has been quantified as colour.

4.4 CONCLUSIONS

Modelling xylogenesis in a manner appropriate to answer biological questions may be considered a complex challenge, due to the lack of experimental evidence and the multi-scale nature of the phenomena, that is also heavily dependent on mechanical interactions (whose study is also lacking). Trying with a single modelling paradigm, also without considering mechanical dynamics, would produce scarce results, for well-known limitations (**Hay Mele et al.**, 2015). In this specific case, a

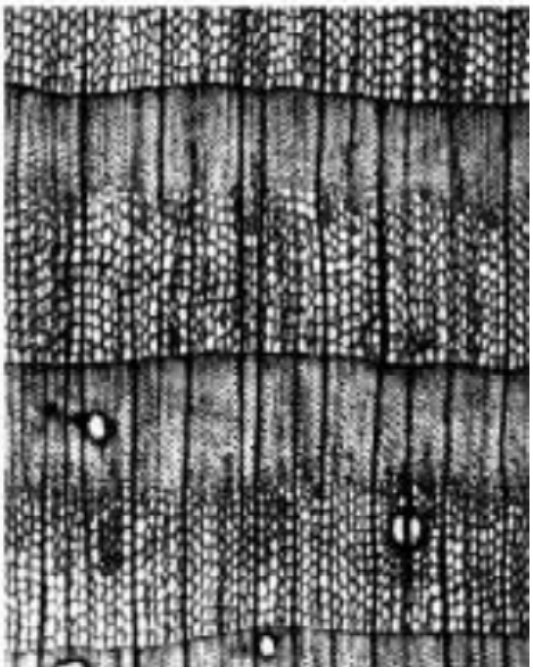

Figure 5. Photomicrography opf *Larix Cajanderi* Mayr growth rings evidencing abrupt early to latewood transition. Picture taken from (**Blokhina et al.**, 2017)

generic hybrid model approach that would not take into account geometric and topological dynamics would also fail, because of the strong dependence of cell wall dynamics on the geometry of the cell.

Our enhanced VirtualLeaf framework has proven to be a valid tool to test our initial hypotheses related to wood formation, because it permits to consider both biological and mechanical dynamics, and also their interplay. This is another proof of how this approach is necessary to inspect dynamics emerging from the interplay of processes that are not happening at the same scale (like diffusion and cell division), are described by completely different rules (like mechanical and chemical processes), or both.

Xylogenesis is a complex, multi-scale dynamics, of relevant interest to many scientific disciplines and they also have a substantialsubstantial impact on the economics of some countries whose timber is a precious resource for construction and consumption. Plant biology still struggles in identifying the processes that define the switch toward one form of the wood cell rather than the other, and while it is certain that the climate change will influence wood formation in trees, it is hard to predict such changes with confidence.

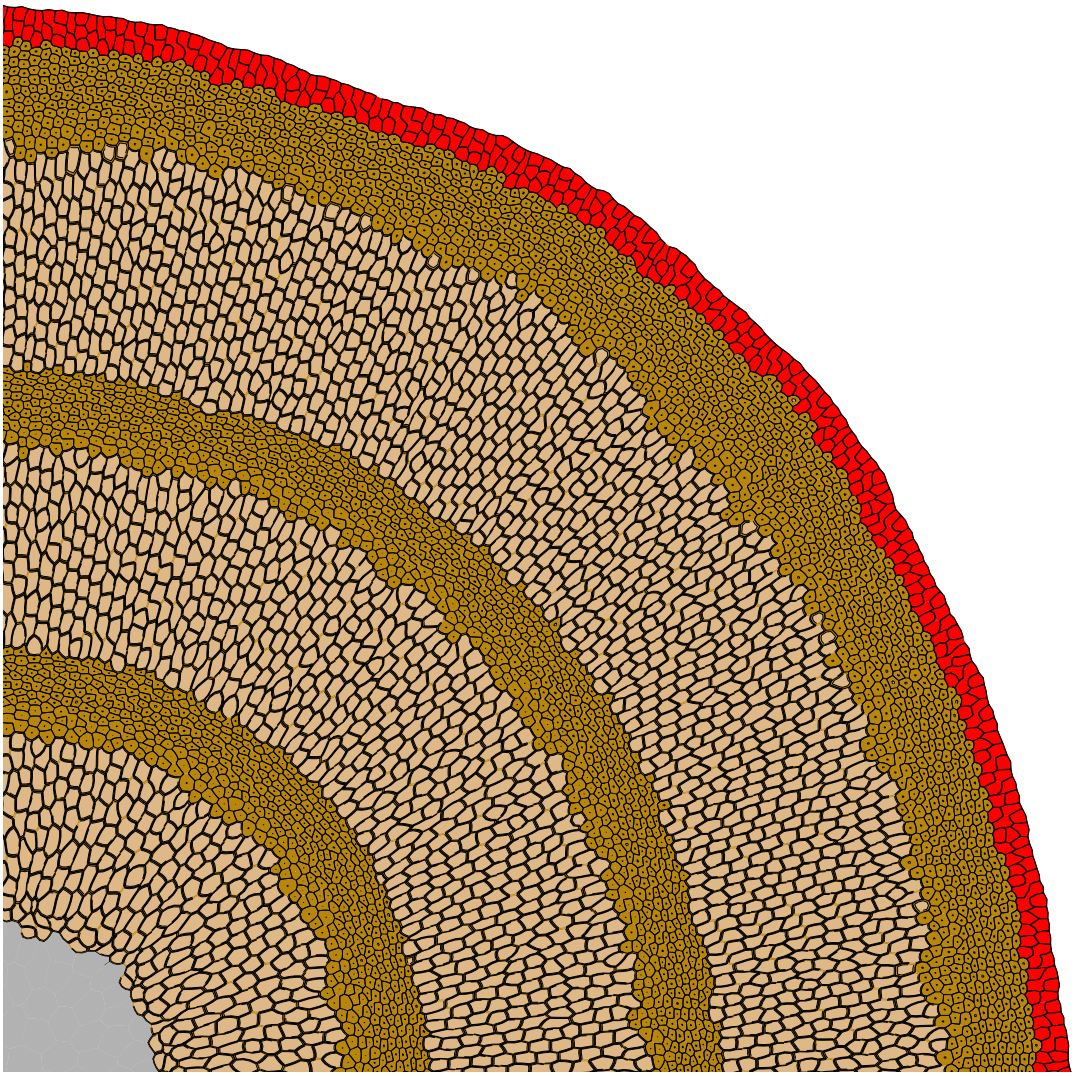

Figure 6. Long-term simulation output at the end of the third growth cycle.

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5 GLOBAL CONCLUSIONS

This thesis aimed to advance the use of hybrid cell-based modelling in plant biology and to further expand the knowledge on the interaction between mechanical processes and morphogenesis, albeit on a small subset of processes (i.e. cell wall thickening and xylogenesis).

The thesis starts with a review of the fundamental concepts in plant tissue modelling, evidencing how complicated is to render the multiscale structure of tissue dynamics. In the text is evidenced how mechanical properties (like cell wall thickness) are seldom considered in plant tissue modelling, and how more than often simulation consider static (opposed to growing) tissues.

The work then moves to analyze the current modelling philosophies and their reference software, and proceeds to acknowledge the cell-based paradigm as the most promising for modelling plant tissues. To present a typical cell-based model's structure, and to display its advantages, a simple model describing vascular differentiation in *Arabidopsis thaliana L.* is presented. The model manages to prove how intuitive is to simulate dynamics associated with a growing tissue while taking into account cell's shape and mechanical properties.

The thesis then continues with the description of the VirtualLeaf modelling framework and its Hamiltonian-based formulation of cell geometry. To explore how tissue mechanics and morphogenesis interact with each other, the author has coupled the λ_{length} term of the VirtualLeaf Hamiltonian to cell wall thickness. Then, the framework has been tested with a single cell, and after the evaluation of the simulation outputs as bio physically corrects, the system has been used for simulating theoretical tissues where cells with thick walls coexisted within populations of cells with thin walls. We found that the position of "thick" cells may affect both single cells' properties and the behaviour of the whole tissue, giving way for the tissue to fine-control its behaviour.

The framework is further extended in the last part of the thesis with the capability to display cell wall thickness and to calculate the lumens' perimeter and area. The extension made possible to explore tissues where thickening dynamics have a fundamental role, and we chose to study hypotheses about xylogenesis because it is a topic of interest, and mechanical interactions are critical players for the whole duration of the process. Our system produced outputs remarkably similar to real wood and is was able to describe the early- to latewood transition using a mix of continuous dynamics, individual-based rules, and mechanical formulations.

The closing thought is that often experimental approaches are limited because they can only take "pictures" of the phenomenon, so that biologists must rely on intuition and do not have a solid environment where to test their ideas: modelling could provide that, transforming the "pictures" into a "movie".