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Tribute to an exemplary man: Yves Couder

Biology and Mechanics

Exploring the relation between apical growth, organ formation and cell wall mechanics across land plant species

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Abstract. The rapid cell growth that is associated with the formation of new lateral organs in the shoot apical meristem was linked to an increase in cell wall elasticity but not viscosity in the plant model *Arabidopsis thaliana*. To investigate the generality of this puzzling relationship, we explored in seven plant species, covering a wide diversity across land plants, the changes in mechanical properties of the cell walls that occur during organ formation. We show that, despite the considerable variation in cell wall composition among the species tested, a drop in cell wall stiffness systematically accompanied primordia formation. We also observed that meristem activity correlates with cell wall elasticity in three species. Thus it seems that cell wall elasticity and growth rate in the meristem are correlated across the land plants.

Keywords. Biomechanics, Meristem, Growth, Cell wall, Morphogenesis, AFM.

Yves Couder, with Stephane Douady's work, has provoked a significant change in the paradigm in the morphogenesis in plants. They demonstrated that a simple recursive model could generate the patterning of organ position in the meristem (phyllotaxis) with memory and inhibition fields [1–7]. This work has focused on the interest in determining the inhibitory field and its origin. The concomitant closing of the hormone transporter PIN1 as a significant element of the lateral organ initiator has enhanced the interest in the area [8]. This auxin transporter is present in a polar way at the plasma membrane of the epidermal cells permitting the accumulation of auxin at the cells, which will initiate the future lateral organs [9]. In this context, Yves Couder led the search for a mechanical sensitivity of growth and specifically for the PIN1 polarity through the cytoskeleton reorganization [10]. His efforts have permitted to demonstrate the mechanosensitivity of the microtubules [11]. In parallel, Yves Couder supported work on determining the mechanical events associated with the cell expansion that underlie the organ growth. With him, we stumbled on the change in cell wall chemistry related to cell wall elasticity changes implicated in the organ formation and the phyllotaxis patterning. How exactly mechanical changes in the cell wall relates to growing and patterning is still unclear.

In plants organogenesis and growth require the irreversible deformation of the cell wall through synthesis and remodelling of cell wall polymers [12]. Here we focus on the generation of new lateral organs. This step is achieved at the apex of the plant, where a group of cells derived from the stem cell niche are recruited into a lateral organ through a patterning process that involves the local accumulation of the hormone auxin [8, 9, 13, 14].

The formation of new organs at the meristem of the model plant *Arabidopsis thaliana* (hereafter referred to as *Arabidopsis*) was reported to depend on auxin-induced increases in cell wall elasticity [7, 15]. A similar correlation between cell wall elasticity and differential cell expansion was observed in hypocotyl, roots, leaves and flower organs [16–20]. This relation is quite puzzling as elasticity is a material property corresponding to the ability to undergo a reversible deformation in contrast to cell expansion, which reflects a biologically-driven irreversible deformation of the cell wall [12].

In an attempt to elucidate this puzzle we explored, throughout the plant kingdom, the changes in mechanical properties of the cell wall that correlate with organ formation. Cell walls display remarkable diversity, both in their chemical composition of the polymeric structure and chemical bonds [21]. Cell walls display remarkable diversity, both in their chemical composition of the polymeric structure and chemical bonds [22]. A study of cell wall composition using immunohistochemistry in bryophyte stem reviled extreme variability within the film [23]. Unfortunately, a broad survey of cell wall chemistry diversity within the whole land plants is still missing. This is undoubtedly due to the complexity of such an approach as described in [24]. The reasoning is that diversity in cell wall architecture and chemistry in land plants could reveal diversity in cell wall mechanical properties related to growth. Furthermore, most of the studies linking elastic deformation and growth were obtained on *Arabidopsis thaliana*, a model plant with a short generation time. This means extremely rapid organogenesis, which could involve growth mechanisms that are different from those of more slowly growing species as shown for instance for *Drosophila melanogaster* [25].

Here, as in our previous work on the *Arabidopsis* meristem, we used a Atomic Force Microscope (AFM) to locally compress cell walls in the meristem of 7 different plant species covering the diversity across land plants [15], and map in the tissue elasticity in the meristem. We observed that organ formation was always associated with an increase in elasticity of the cell wall.

1. Results

1.1. *Lateral organ initiation is associated with a reduction in cell wall stiffness in a wide variety of land plants*

In *Arabidopsis*, organ initiation and outgrowth was correlated with a reduction in cell wall stiffness as quantified by the apparent Young's modulus Ea [15]. To investigate whether this was also the case in other species, we mapped the Ea onto the apical meristems of 7 species: For the Bryophytes, we chose *Polytrichum commune*. Note that this moss does not have a true meristem, but instead a single apical cell that acts as a shoot meristemoid. From the Gymnosperms we chose *Pinus nigra* (pine), from the *Ginkgophyta, Ginkgo biloba* (ginkgo) and from the Angiosperms, the monocot *Zea mays* (maize) and the dicots *Quercus robur* (oak), *Solanum lycopersicum* cv. "MoneyMaker" (tomato) and *Arabidopsis*.

As shown in Figure 1, in all meristems studied, emerged organs showed a roughly 4-fold reduction in apparent stiffness compared to meristematic regions. In some species, (oak, maize, gingko, pine) decreases in elasticity could also be seen at sites of incipient, or future, organs. Thus, like in *Arabidopsis*, organ emergence was associated with a decrease in cell wall stiffness.

The measured stiffness varied between species within a range of ~100–400 kPa, with the stiffest and softest meristem being from oak and *Arabidopsis* respectively. These differences probably

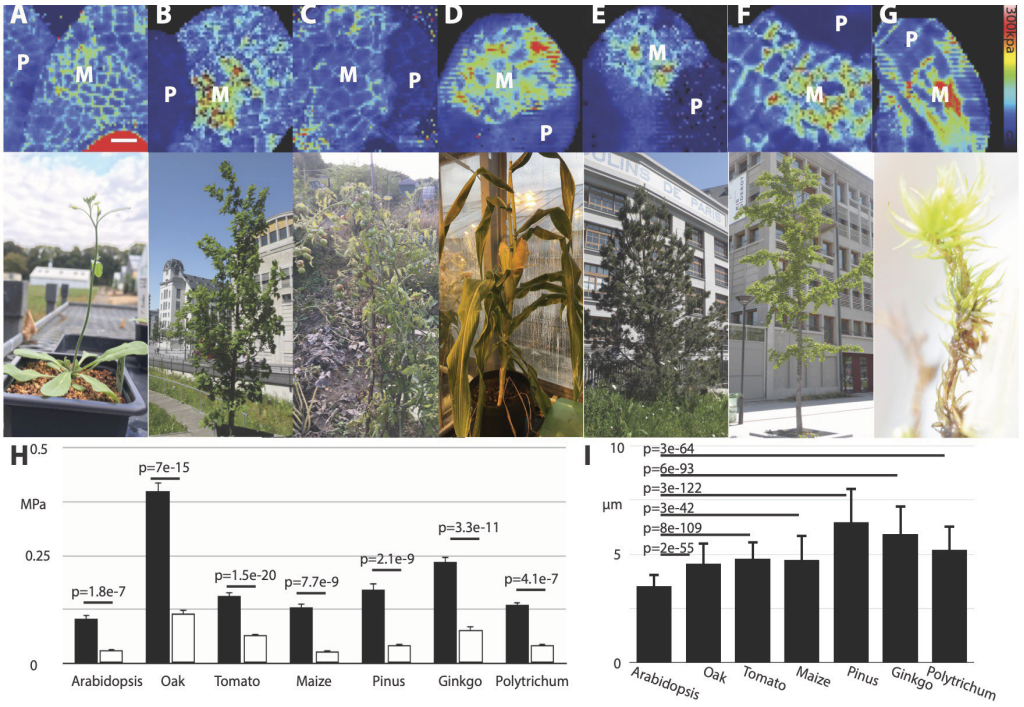


Figure 1. Elasticity of periclinal cell walls of meristem and new lateral organs across the plant kingdom. Representative apparent Young's (Ea) modulus map on the meristematic region of (A) *Arabidopsis thaliana* ($n = 8$) (B) *Quercus robur* ($n = 27$) (C) Tomato or *Solanum lycopersicum* ($n = 24$) (D) *Zea mays* ($n = 8$) (E) *Pinus nigra* ($n = 12$) (F) *Ginkgo biloba* ($n = 30$) (G) *Polytrichum* ($n = 13$). (H) Average and standard deviation from meristem to meristem of Ea observed in the meristematic dome (black) and in young lateral organs (white). (I) Average and standard deviation for cell with in meristematic cells. M meristem P primordia. Bar 10 µm. *P*-values were obtained with equal variance two-sample student.

reflect variations in cell wall thickness and/or composition, since cell size and meristem shape were remarkably similar between oak and Arabidopsis and meristem stiffness was remarkably similar among *Arabidopsis*, maize, and *Polytrichum* despite large variations in cell size and meristem shape (Figure 1I). Overall, the variability in cell wall elasticity among species was remarkably small relative to the within meristem variability for the same plant and the variability between cell walls within the same meristem (Figures 1 and 2).

1.2. *Changes in overall stiffness correlate partially with meristem activity*

Apical meristems can, depending on the environment, undergo transitions between a dormant stage and an active growth stage. In the first part of this study, we focused only on rapidly growing meristems taken in the spring or in plants grown under long day conditions. In order to evaluate the effect of cellular growth rates on the observed cell wall stiffness, we studied dormant meristems of pine and oak (Figure 2). We also compared the rapidly growing meristem of Arabidopsis to slow growing ones by changing the illumination period. We observed a two-fold increase in the overall stiffness associated with the reduced growth rate in *Arabidopsis*, oak and pine meristems. Despite this increase, the difference in stiffness between central zone and primordia was conserved regardless the growth rate.

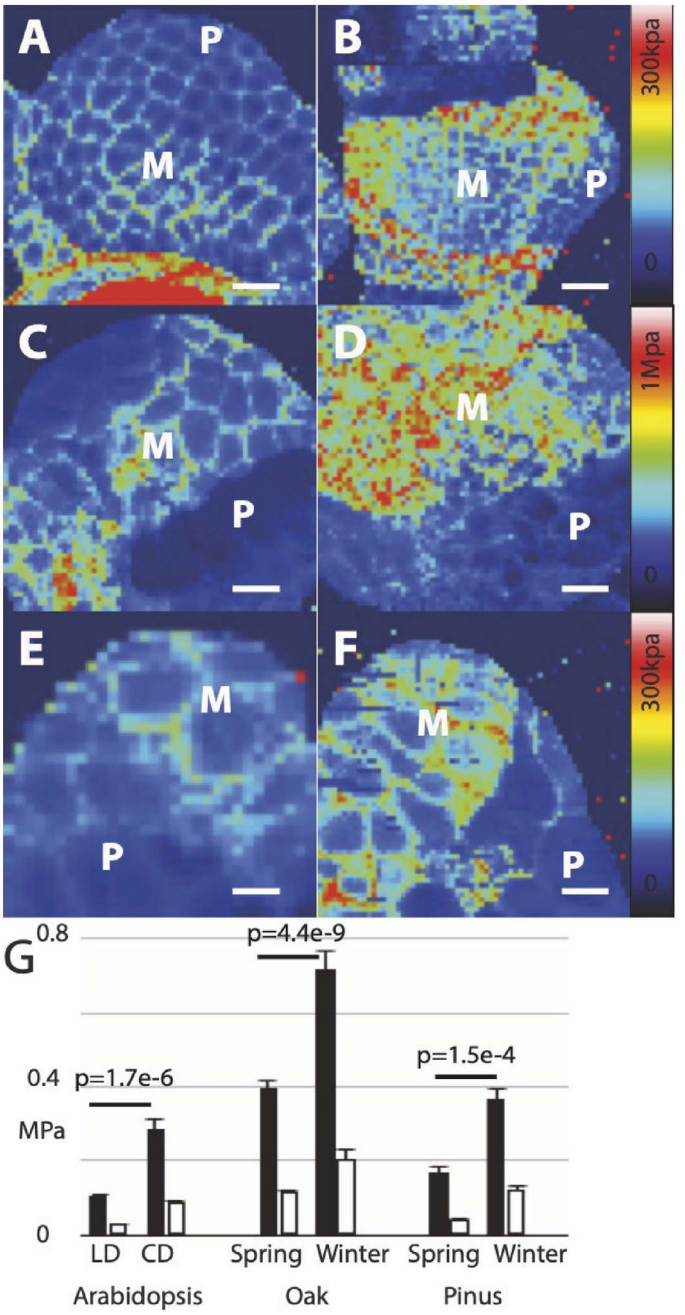


Figure 2. Effect of dormancy or short day length on meristem and stiffness of primordia. Representative apparent Young's (Ea) modulus map on the meristematic region of *Arabidopsis thaliana* (A) grown in long days conditions (n = 8), (B) in short day conditions (n = 9), of *Quercus robur* (C) in spring (n = 27) (D) in winter (n = 9), *Pinus nigra* (E) in spring (n = 12) (F) in winter (n = 10). (G) Average and standard deviation from meristem to meristem of Ea observed in the meristematic region (black) or in young lateral organs (white). LD: Long day, CD: Short day. M meristem P primordia. Bar = 10 µm. P-values were obtained with equal variance two-sample Student test.

2. Discussion

The change in cell wall elasticity associated with organ formation, initially observed in *Arabidopsis*, also seems to be conserved in a moss, a ginkgo, a gymnosperm and dicot and monocot angiosperm species. This conserved mechanical feature could be linked to the differential growth underlying organ formation and the function of the stem cells. In most of the plants we could observe, like in Arabidopsis, a change in stiffness *before* the organ outgrowth, supporting a causality between the change in cell wall mechanics/chemistry and the growth rate increase.

The similarity in cell wall mechanics observed across the seven species is striking. We and others previously showed that in *Arabidopsis* the cell wall stiffness is controlled by the methyl-esterification state of the pectin. The results in this study suggest that changes in other cell wall polymers might underlie the observed changes in the stiffness in species that do not accumulate large amounts of pectin as in maize [26]. Alternatively it could sugest that the extrapolation of cell wall composition observed in differentiated cells to the composition of meristematic cell wall is inacurate. Quantitative cell wall composition measurement in situ using technics such as FTIR, Raman or immunochistochemistry associated to dSTORM would be extremely informative in the future [22].

The correlation between meristem dormancy and cell wall rigidity in oak and pine reinforces the link between changes in cell wall elasticity and growth. Stiffening of the cell wall could be one of the growth limiting mechanisms controlling dormancy. Recent work on sunflower seed dormancy supports this potential link (Hayat Bouteau, personal communication). The next step will be to document precisely when the changes in the stiffness occur and to identify the underlying biochemical processes. Yet other factors besides cell wall stiffness could be critical for the control of cell growth. The changes in cell wall elasticity (a reversible phenomenon) could be only an epiphenomenon linked to more important changes in the cell wall conformation of polymer following chemical changes [27]. This could explain way changes in cell wall elasticity correlates to growth. Yet the fact that similar effects are observed in all the meristems studied here suggest that regardless of direct or indirect is the effect of elasticity on growth the link could be conserved in all land plants.

Yves Couder, when confronted with the puzzling correlation between changes in elasticity and growth, would always refer to one of his exhibit: a piece of wood in which the fibers will arrange in lines around a branch akin to flow lines of fluid around the obstacle. Turbulence lines and long-distance interaction between them were remarkably similar to the ones observed in fluid mechanics. For him, it was a clear demonstration that regardless of all the repetitive correlation between elasticity of the cell wall observed with AFM, understanding how a plant grows will require much more effort. In this sense, this work is not satisfactory as it only reinforces a known correlation between growth and changes in cell wall elasticity. Future work will need to figure out what precisely this correlation means in terms of growth, a direct effect, or an epiphenomenon.

3. Materials and methods

3.1. *Plant material and growth conditions*

Arabidopsis WS, tomato cv “MoneyMaker” and maize “Goldenmais Bantam” plants were grown in soil in long day conditions (16 hours light) at 25 °C. All other species used in this work were sampled from the campus of the University of Paris Diderot or Jardins Grand Moulins Abbè Pierre, Paris, France or, for *Polytrichum*, in the “Fausses Reposes” Forest in Chaville 92370 France.

3.2. *AFM measurements*

The AFM data were collected following the same protocol as previously described [15]. Apices were immobilized on glass slides and surrounded by stiff agarose after dissection from plants or cuttings. Measurement of the wall properties alone was ensured by suppression of turgor pressure by immersion of the apices in a hypertonic solution for a minimum of 30 minutes before measurement (0.55 M mannitol). We have previously demonstrated that this causes plasmolysis in meristems of *Arabidopsis* [15]. We assumed it is sufficient to plasmolyse all the meristems. The observation of the cell contour in the rigidity map confirms this hypothesis. In addition, this concentration was sufficient to plasmolyse even the highly turgid cells of onion epidermis. The following numbers of apices were analyzed: *Polytrichum* (n = 13), *Pinus nigra* (spring n = 12, winter n = 10), *Gingko biloba* (n = 30), *Zea mays* (n = 8), *Quercus robur* (spring n = 27, winter n = 9), Tomato (long days n = 24), *Arabidopsis* (long days n = 8, Short days n = 9). The following cantilevers were used: "Nano World" (NanoWorld AG Headquarters, Neuchâtel, Switzerland) TL-NCH-20 tips with a spring constant of 10–130 N/m (those used were estimated to be 1.5 N/m) with Sphere Tips of a 2500 nm radius. All force spectroscopy experiments were performed as previously described; briefly, rigidity of samples was determined as follows: an AFM cantilever loaded with a spherical tip was used to indent the sample over a 100×100 μm square area, within the area 64×64 measurements were made resulting in 4096 force-indentation experiments, each force-indentation experiment was treated with a Hertzian indentation model to extrapolate the apparent Young's modulus, each pixel in a stiffness map represents Young's modulus from one force-indentation point. For topographical reconstructions, the height of each point was determined by the point-of-contact from the force-indentation curve; each contact point is from the same curve used to determine Ea. Stiffness data were projected onto topographical maps using a MatLab routine (available upon request).

Cell width was evaluated from the AFM rigidity map on the cells presenting a strong contrast in rigidity between the periclinal and anticlinal wall. The number of cells measured was at least 130.

3.3. *Apparent Young's modulus calculations*

As in our previous work, the Apparent Young's modulus in this study was calculated using the JPK Data Processing software (ver. spm-4.0.23, JPK Instruments AG, Germany) [15, 18], which allows for a more standardized analysis and estimation of Young's modulus using a standard Hertzian contact model. Only the retraction curve was used in our analysis. The best fit was obtained using a Hertzian model with 2.5 μm as tip radii, for a cantilever loaded with a 5 μm spherical beads. A Poisson ratio of 0.5 was assumed for the material. For "mean of means" graphs standard propagation of error calculations were applied. Cantilever elastic constant was measured using Athef Asnasios reference cantilever [28].

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