Evaluating cell wall structure Tobias Baskin University of Massachusetts

The form of plant organs such as leaves, roots, and flowers, reflects an equilibrium between an osmotic force drawing water in and a tension borne by the cell wall that resists this influx. This opposition holds plants erect against gravity and yet lets them bend rather than break in wind or rain. This equilibrium is broken by growth, which here means an irreversible volume increase. Growth occurs when the tension within the cell wall weakens, thus allowing water uptake. The osmotic force is isotropic; however, expansion is usually anisotropic, implicating the mechanical properties of the cell wall as governing the pattern of growth. Beyond this implication, relationships between cell wall structure and rates of expansion are largely conjectural.

The scanning electron microscope (SEM) is a powerful method for revealing the ultrastructure of the plant cell wall (figure, last page). Whereas the transmission electron microscope (TEM) requires heavy metals to interact with the sample, an interaction that is weak and prone to spurious precipitation, the SEM images the wall more or less directly (a heavy metal is needed but in this case in the form of a coherent coat).

SEM images show the high degree of structural anisotropy present in the wall. In general, the cell wall is a two-phase composite material. This is usually described as stiff cellulose fibers embedded in an amorphous matrix. The fibers are the cellulose microfibrils and the matrix is the pectin and hemicellulose. But the matrix (as seen in the figure) appears to be more ordered than amorphous. The diameter of the prominently aligned fibers is far larger than even the most generous estimates of that of a cellulose microfibril. Although several microfibrils might coalesce, diameter appears to vary continuously along any given fiber. This is inconsistent with coalescence of additional cellulose microfibrils insofar as the dimensions of the unitary microfibril are expected to be constant (so coalescence should lead to rather abrupt increases in diameter). This suggests that part of the matrix sheathes the fibers. For simplicity, in the following, I will refer to these prominent and well aligned structures as “macro-fibrils”. Likewise, short structures run roughly perpendicular to the fibers, consistent with a cross-linker but inconsistent with an “amorphous” matrix.

Furthermore, a paramount question is how the plant controls the anisotropy of expansion. In cell walls such as those shown in figure 1, maximal strain rate is perpendicular to the net alignment of fibers. It is assumed that deformation parallel to the fiber axis is hindered compared to deformation perpendicular. However, various treatments or genotypes are known that reduce expansion anisotropy but have little or no effect on the overall alignment of fibers, at least as judged by eye in SEM images. Insofar as these treatments change the deformation status of the cell wall (either as cause or consequence) it is reasonable to expect structural alteration. One might expect the spacing between fibers, their persistence length or angular dispersion to be affected. Similarly the characteristics of the sheathe and the cross links might change too.

The ultimate aim is to understand how expansion rates in length and in width are set. Chemical modifications and structural deployment are presumably both involved. This project is to be able to assess cell wall structure on a more quantitative level.

Practical matters.

I have two plant genotypes (one ‘wild type’, one mutant) that differ in growth anisotropy. The null hypothesis is that there is no difference in cell wall structure between them. I have a collection of ~300 images, ~150 from each genotype (four plants per genotype, ~four sections per plant, ~10 images per section). The images are at the same magnification. Any differences in instrument settings are minor. However, some images contain

irregularities such as a blob or fold in one part, or are slightly out of focus. More importantly, the imaged cells have different types of texture. That is, the images do not all look alike. Some have more prominent cross links, while the cross links in others are fewer and smaller. Also varying is the amount of microfibril undulation.

Questions:

Macro-fibrils

What is their average diameter, orientation, undulation, spacing, and persistence length?

How do the above parameters vary in an image?

Crosslinks

What is their average diameter, density, orientation?

How do the above parameters vary?

Concepts

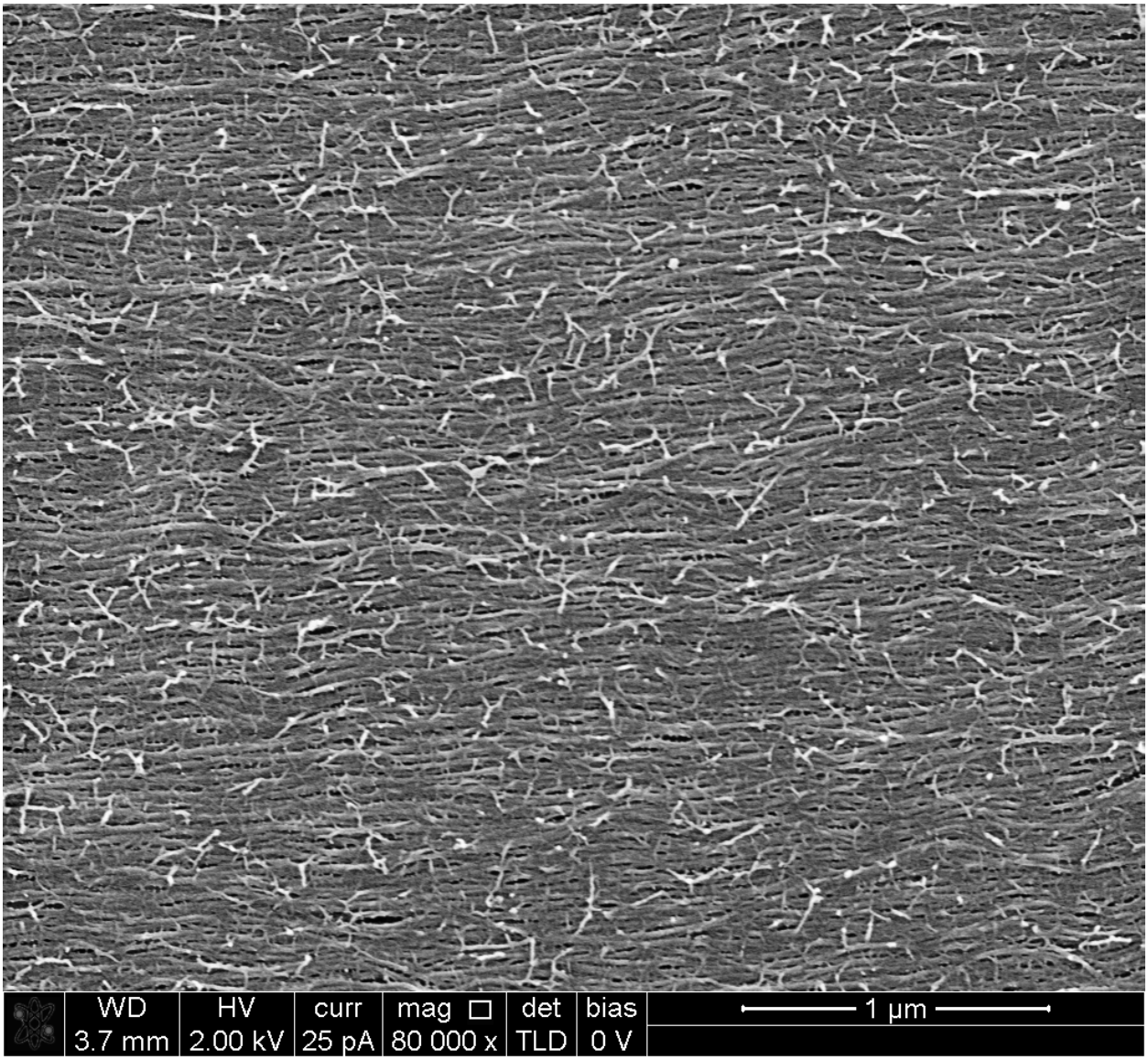
Can a better description be found for the cell wall than fibers + amorphous matrix?

Can cell walls that appear to be from different cell types be distinguished quantitatively?

Can the cell walls from the wild type and mutant be distinguished based on the images?

Can a mechanical model for the role of cellulose and matrix in controlling expansion anisotropy be elaborated?

Note. The above list is meant to be illustrative of the kind of information I would like to obtain. It is by no means complete or exact. Nor do I require all of them to be answered.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