Lignocellulose conversion: Convincing plants to cooperate

Goals

Define some terms from our perspective:

- **Lignocellulose**
- **Conversion**
- **Plant cooperation**

Identify and justify a research strategy

Lignocellulose = the plant's cell wall

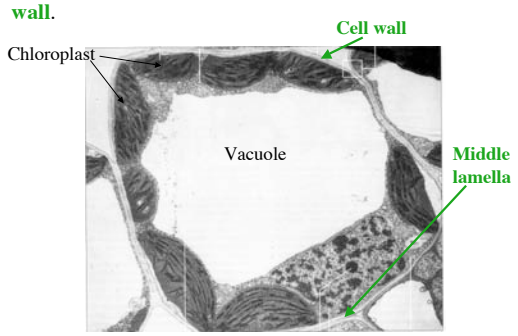
The wall is a complex fabric assembled from an assortment of threads - it is not just cellulose

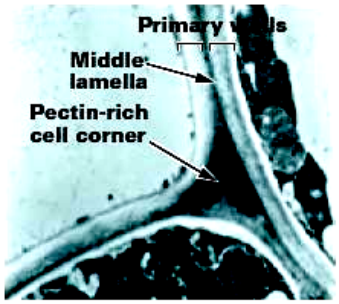
- **Polysaccharides**
- **Proteins**
- **Phenylpropanoid derivatives (lignin, simple phenolics)**
- **Ions (Ca^{2+}, Borate, Silicon)**

It is important to understand

- **The threads**
- **Specifically how they are associated with one another**
- **How each contributes to the wall's physical properties**

The fundamental "building block" of the plant body is the plant cell. The outer boundary of the plant cell is the **cell wall**.





Pectins are acidic polysaccharides that are found in cell walls, particularly where **neighboring cells are bonded to one another**. The **pectin content of grass cell walls is low** relative to that of other plant species.

Cell walls are primary (1°)

- **1° walls are extensible.**
- **They have little (or no) lignin, they may contain simple phenolic cross-links.**

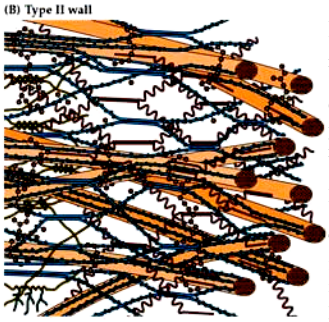
Cell walls are secondary (2°)

- **2° walls are not extensible (they are found in support tissues, water-conducting cells).**
- **They contain lignin.**
- **They are produced by cells to the inside of the 1°cell wall, just outside of the cell membrane.**

The plant cell wall

- Is an **important source of strength** (rigidity) for plant cells and, as such, supports the shape of plant cells, tissues and organs.
- It is also an important **barrier to pathogens and insects**. They generally try to breach that barrier by producing and secreting cell wall-degrading enzymes.
- However, because the barrier is made of sugars and amino acids, the wall itself is also **food for insects and pathogens**.
- Because of its composition, the wall is also a potentially important **feedstock for bioenergy production** …particularly if we can learn to operate like insects and pathogens.

Model of a cereal crop's primary cell wall



An engineering view of this wall might compare it to a reinforced concrete slab.

Cellulose & hemicellulosic polysaccharides are the rebar and wire

Pectins are the concrete

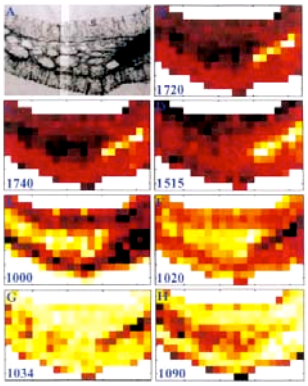
Two interacting networks fill the same space. But, this is a **fabric** and it has **porosity**.

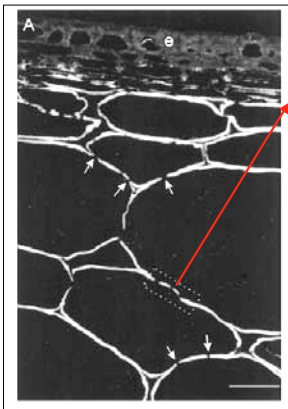
Cell wall models attempt to depict the average cell wall.

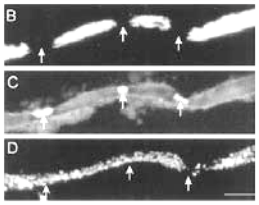
However if you "look" at a microscope section of a tissue you will see that distribution of different wall components is not uniform.

This indicates:
- **Different cell types**
- **Different cell maturities**

Fourier transform infrared (FTIR) **spectroscopic** wall analysis (Maureen McCann, Purdue)



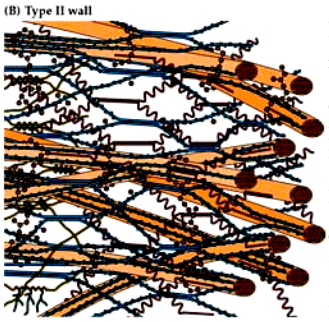




Sections through a tomato fruit

These views show polysaccharide distribution through the thickness of the wall. Different polymers are identified by reaction with specific antibodies.

Model of a cereal crop's primary cell wall



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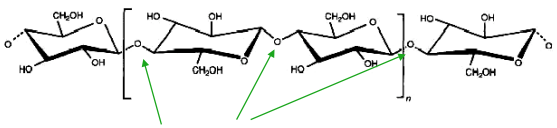
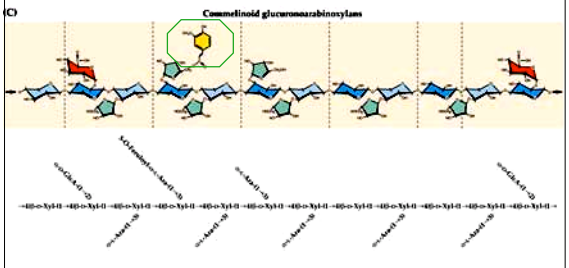
Cellulose:
- The **primary strength-conferring polysaccharide**
- β-1,4-linked **glucan**
- Each glucan will have a **high degree of polymerization** (DP).
- Its structure allows alignment and cross-linking of **many glucans** via H-bonds, forming a **multimolecular microfibril**.
- The microfibril's "crystallinity" **excludes water**.



In: Biochemistry and Molecular Biology of Plants (2000)
(Eds. Buchanan, Gruissem and Jones

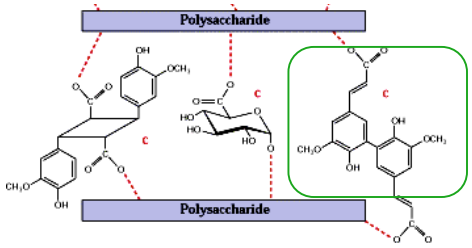
The primary structural hemicelluloses in grass walls are polymers called 1,4-β-xylans. Their backbones are built from the 5-carbon sugar Xylose.

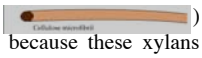
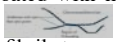


Xylan structure can vary - 1° *vs.* 2° walls

Many microbial and plant enzymes that can completely deconstruct various xylans are known.

The xylans of grasses are often cross-linked to one another via cross-links involving phenolic acids.



In cell walls, cellulose microfibrils () are coated with hemicelluloses and, because these xylans () are long polymers, they can cross-link microfibrils to one another.

This:

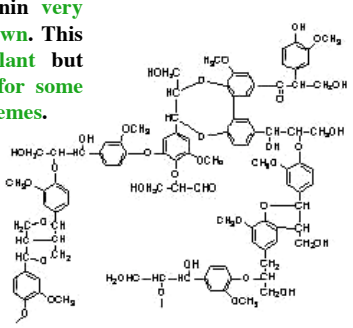
- **Provides strength** ("the rebar and wire")
- **"Hides" the cellulose** from celluloytic enzymes
- **Defines**, in part, **the wall's porosity.** That is, large molecules do not easily diffuse within the wall. The cell wall space is not a "well stirred beaker".

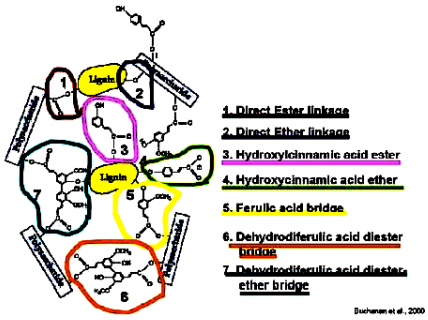
While **lignin is a component of secondary cell walls**, it must also be considered in many schemes for replacing petroleum products with renewable feedstock sources.

Models suggest that tree plantations can **provide particularly dense (per acre) yields of useful plant biomass**.

The fact that the **poplar genome is now sequenced** suggests that many **useful modifications could be introduced to woody (i.e., lignin-rich) plants to make them of even greater potential value**.

Lignin structure is complex and this makes lignin very resistant to breakdown. This is good for the plant but provides problems for some biotechnological schemes.





Only a few microbes can digest lignin, because it **excludes water** and **contains a variety of different linkages**.

An important target of recent public and private initiatives is to make the production of fuel ethanol economically sustainable.

The **conversion** of lignocellulosic biomass to ethanol requires **the integration of many harvest, "postharvest", and physical, chemical and biochemical process engineering steps**.

A flow diagram for field-to-fuel utilization of the ligno-cellulose in crop residues or dedicated biomass energy crops would look something like this:

Plant cultivation & management

↓

Harvest & postharvest management

↓

Pretreatment

↓

Cellulose digestion

↓

Fermentation

One important goal of **pretreatment** is to make the cellulose of biomass more accessible to cellulases. Pretreatment should:

- open up the organization of the cell wall so **that enzymes can reach cellulose…**and
- open up the cellulose microfibril so that **cellulases can digest cellulosic 1,4-β-glucans more rapidly and completely**.

Our impression is that **lessening the costs** (energy, environmental protection/clean-up, physical plant) for **biomass pretreatments** is an important goal. Another goal would be to **use, rather than lose**, the sugars in non-cellulosic polymers.

What shape would **plant participation in the conversion** process have**?**

How we would be **go about convincing the plant** to help us out**?**

We feel that the **keys to answering these questions** can be found in an understanding of the **ways plant cells make, assemble and disassemble their cell walls**.

REVIEW

Toward a Systems Approach to Understanding Plant Cell Walls

Chris Somerville,[1,2]* Stefan Bauer,[1] Ginger Brininstool,[1] Michelle Facette,[1,2] Thorsten Hamann,[1] Jennifer Milne,[1] Erin Osborne,[1] Alex Paredez,[1,2] Staffan Persson,[1] Ted Raab,[1] Sonja Vorwerk,[1] Heather Youngs[1,2]

One of the defining features of plants is a body plan based on the physical properties of cell walls. Structural analyses of the polysaccharide components, combined with high-resolution imaging, have provided the basis for much of the current understanding of cell walls. The application of genetic methods has begun to provide new insights into how walls are made, how they are controlled, and how they function. However, progress in integrating biophysical, developmental, and genetic information into a useful model will require a system-based approach.

24 DECEMBER 2004 VOL 306 SCIENCE P. 2206-

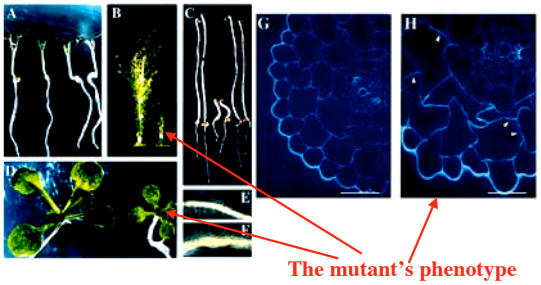
The **cell wall** of a grain or biomass crop could be **genetically manipulated** so that a more "**biomass conversion compliant**" wall is made.

In this age of molecular biology, researchers have **identified** many of the **genes that encode cell wall proteins and enzymes** that are **important in the synthesis of cell wall polysaccharides and lignin**.

Thus, if a particular wall component causes difficulty in the conversion process, we could engineer a modification that leads to **changes in the amount or structure of that component made by cells**.

However, we must remember that **before a plant is a feedstock, it has to be a photosynthesizing, growing and developing plant**.

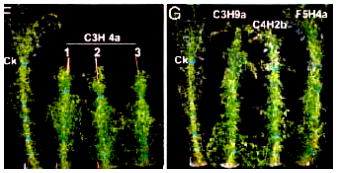
Thus, we view this very feasible strategy to be one that will **yield benefits only in the longer term**.



The *KOR* gene encodes a protein important in cellulose synthesis. If the gene is mutated then the plants are dwarf and drastically modified in many ways that we can see.

While longer term, these efforts are likely to be valuable. Potential applications are already at hand through manipulations of the **lignin biosynthesis pathway** so that the plants with modified lignin are:

- Easier for **livestock to digest**
- More **compliant for wood pulp manufacture** (paper production)



These transgenic alfalfa plants have **altered lignin structure**. Some are shorter, but others are **full size and more digestible**.

From: Reddy et al. (2005) PNAS

The other approach to manipulating cell wall participation in bioconversion is to **enhance the propensity to disassemble cell walls.**

All plants actively modify their cell walls, at specific times, as they grow and develop.

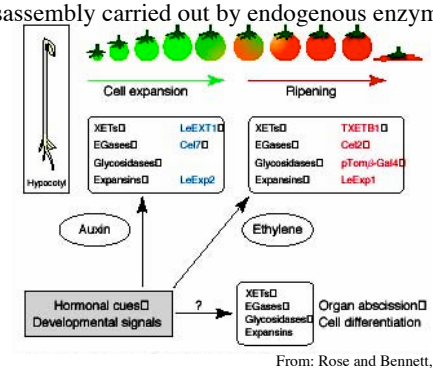
Can we **enhance and manage** this innate capacity for wall breakdown so as to make biomass and crop plants that **assist in their own bioconversion**?

Pathogens must break down the cell walls of their plant hosts, if plant cells are to grow they must selectively digest cell wall polymers so that cells can expand, if fruits are to ripen, they must digest their cell walls so that the fruits soften etc.

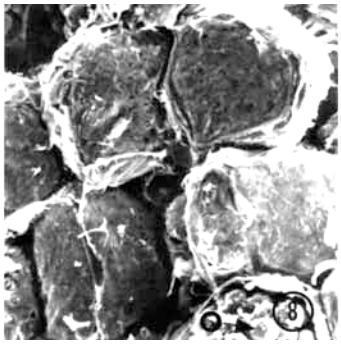
The messages from these observations are that:

- In nature **there is a great diversity of cell wall digesting enzymes**
- Plants and microbes can **synthesize cell wall digesting enzymes and export them into the cell wall space**

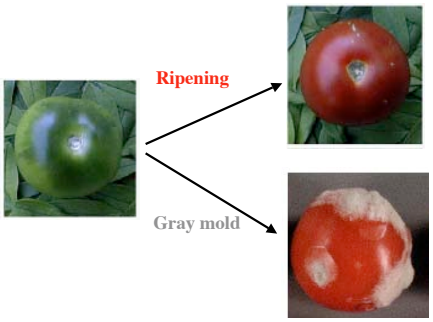
Cell growth and **fruit ripening** involve wall disassembly carried out by endogenous enzymes.



From: Rose and Bennett, 1998



When an avocado ripens **even the cellulose appears to be broken down**. (Platt-Aloia et al. 1980. *Bot. Gazette* **141**: 366-373.)



With either tomato fruit "fate", wall disassembly is accomplished with the secretion of wall-digesting enzymes into the cell wall space.

Those very skilled individuals who worked to drop the Seattle Super Dome directly into the space where it had been sitting for several years studied its structure intensively before they decided where to place their explosive charges.

➢**We know a great deal about the structure that we want to deconstruct.**

➢**Can we identify the correct "enzymatic charges" needed to deconstruct cell walls?**

➢**Can we manage the timing of our internal explosion?**

In Davis, on campus and off, we have access to many plant and microbial enzymes (both the proteins and the genes that encode them) useful in stripping the "shielding" polysaccharides away from cellulose and converting those polysaccharides to their component sugar building blocks.

➢**Novozymes, Inc: Joel Cherry and colleagues**
➢**Plant Sciences:**
Alan Bennett, Ann Powell et al. - Assorted plant wall-modifying proteins
Alison Berry - *Acidothermus celluloyticus* xylanases and cellulase
My program (Greve, Labavitch et al.) -enzymes from plant pathogens

We have the capacity to use genetic engineering to "add" the expression of these genes to a wide assortment of plant species.

Furthermore:

➢We have programs that are devising **schemes to cause these genes to be expressed at a very high level** in plants (McDonald, Falk & Dandekar)

➢We have access to **promoters** (nucleic acid elements analogous to dimmer switches) **to control the expression of genes at specific times and places in the plant**, allowing us to set off the wall's deconstruction in an appropriate way.

Our group:

➢Bennett, Dubcovsky, Powell, Greve and Labavitch (Plant Sciences)

➢Jean VanderGheynst (Bio and Ag. Enginering)

➢Joel Cherry (Novozymes)

Plans to begin addressing both sides of the equation for "controlled cell wall modification", that I have described, focusing on wheat.

Any success we achieve at the plant modification level, will have to be integrated into the overall scheme for bioconversion of plant biomass that will be evolving at UC Davis in the next decade or more.