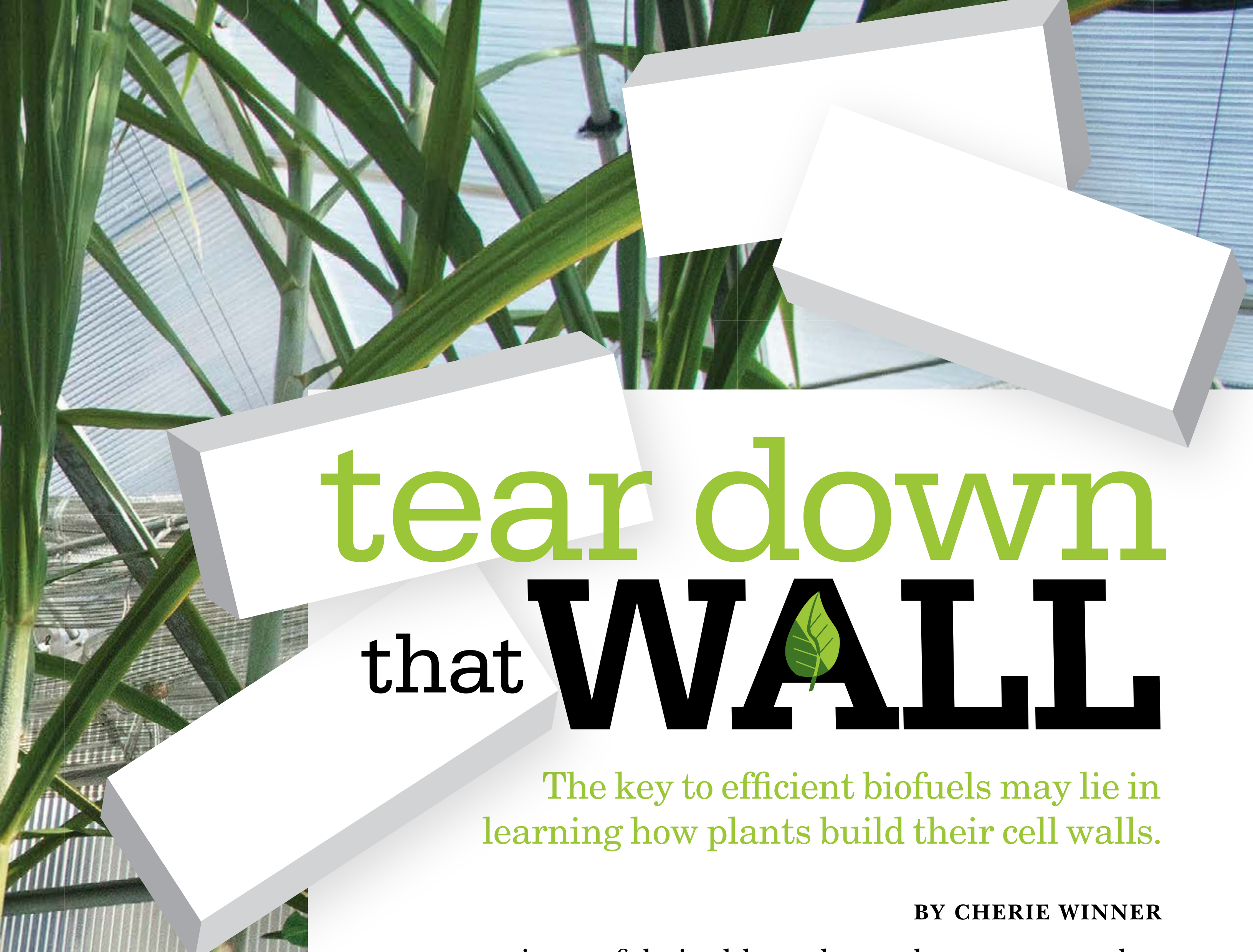


# tear down that WALL

## The key to efficient biofuels may lie in learning how plants build their cell walls.

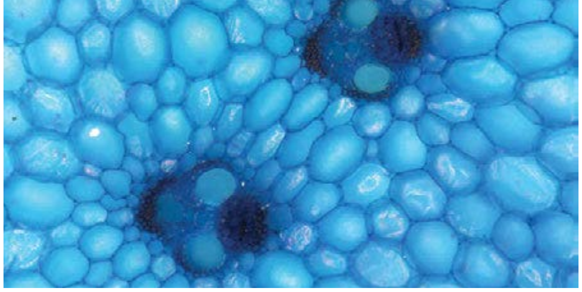
BY CHERIE WINNER

By virtue of their chloroplasts, plants are superb harvesters of solar energy. They use it to build leaves, flowers, fruits, stems, and roots. We harvest a small percentage of that energy in the form of food and a smaller amount in the form of wood for heating.

But the vast majority of plant biomass goes unused by us. In every agricultural region on Earth, the structural parts of the plants we grow—things like cornstalks, sugar canes, beanstalks, and wheat stems—are discarded because we haven't figured out a way to convert them into fuel.

Plant scientist **Daniel Cosgrove**, who has devoted decades to studying the cell walls that make plant matter resistant to chemical conversion, thinks it doesn't have to be that way.

Given the scale of our need for energy and the size of that untapped, renewable resource, he says, "plant cell walls are the obvious target."



Freshly-cut piece of sugar cane, stained with toluidine blue to make the cells easier to see. Each cell is surrounded by a thin, fibrous cell wall. The two large, dark areas are vascular bundles that transport water and sugars. Photo by Edward Wagner.

## UNLOCKING PLANT ENERGY

We are expert at using the structural parts of plants to make things—wood for buildings and furniture, flax and cotton for clothing—but when it comes to using them for energy, we haven't progressed much beyond the Neanderthal stage: We burn them.

"I have a wood pellet stove at home, and with the cold spell we've got right now, we're cranking through a lot of those wood pellets to keep warm," says Cosgrove, holder of the Eberly Chair in biology. "The problem is, unless you want to go to an old steam engine or something of that fashion, it's hard to harvest the energy in wood pellets to power an automobile."

What we need, he says, is a liquid fuel, preferably one that's carbon-neutral. The closest we've come is ethanol, made mainly through fermentation of corn, which we then mix with petroleum gasoline. But corn ethanol is not a long-term solution. It diverts prime agricultural land away from food production, decreases ecosystem diversity, and, when production and transportation are considered, it is not carbon-neutral.

It also misses the point. We make ethanol from the starch inside corn kernels. The millions of tons of corn stalks are wasted. So is all the energy stored in chemical bonds in their cell walls, because we don't know how to break down the walls and release it. Biofuel crops such as poplar and switchgrass are problematic for the same reason.

"The problem is, we don't understand cell wall structure well enough to approach its conversion scientifically," says Cosgrove.

The U.S. Department of Energy (DOE) agrees with him. In 2009, the agency funded three Energy Frontier Research Centers geared toward finding a good way to turn wood and fibrous plant material into liquid fuel. Two of the programs focus on trying to break down cell walls. The third, the Center for Lignocellulose Structure and Function headquartered at Penn State, looks at the problem from the opposite perspective: how cell walls are made in the first place. The idea is that understanding how cell walls are made will make it easier for us to take them apart.

"We have a unique angle and a unique group of investigators, mostly from Penn State but also from five other institutions," says Cosgrove, the Center's director. "We're doing a variety of trans-disciplinary work that involves physicists and computational modelers and biologists and geneticists. We're interested in the fundamental problems of how cell walls are put together, because it's not just biochemistry that determines cell wall properties."

## HOW YOUR GARDEN GROWS

The mystery of plant structure starts with how plants grow. In specific zones at the tips of the stems, shoots, and buds, new cells are added through proliferation: The cells take in nutrients, approximately double in size, and then divide to form two daughter cells, which themselves grow and divide. When the structure or organism reaches full size, both cell expansion and cell division stop. That kind of growth is much like what happens in animals.

But other parts of a plant grow, sometimes massively, without cell division. The stem gets longer and thicker because the cells in it elongate and expand. This kind of growth occurs in all plants, from petunias to redwoods—and it is essential for them to attain large size. If plant cells didn't get larger, if they stayed the same size they were in the seedling, the landscape would look much different.

"Someone has calculated that if the tallest tree in the world, a redwood tree, grew the way your liver cells grow, it would be about hip-high, waist-high. About three feet high," says Cosgrove.

## LET IT FLOW

The puzzling thing is how, exactly, plant cells expand so much. Each cell is enclosed by a flexible cell membrane and, just outside that, by a cell wall, a box-like structure that is as strong and tough as it sounds.

Most major constituents of the cell wall have been known for almost 200 years. Perhaps the most familiar is cellulose, the indigestible-but-good-for-colon-health polymer we know as "dietary fiber." In its most basic form, cellulose is a long chain of glucose (sugar) molecules. Cell walls contain dense layers of cellulose microfibrils made of two dozen of these chains adhering to each other.

But our understanding of how all the parts are made and how they interact with each other is still developing. During the past four years, Center scientists have learned a lot about the Cellulose Synthesis Complex, or CSC, that produces cellulose chains and microfibrils. Hundreds of CSCs are embedded in the cell membrane that surrounds each growing cell.

This "nanomachine," as Cosgrove calls the CSC, is a big, donut-shaped structure comprising at least 18 enzymes, each of which builds cellulose chains. The newly-made chains pass through the "donut hole" in the middle of the CSC to the outside of the cell. There, they line up parallel with other new chains to form a microfibril. Then the microfibrils become embedded in and cross-linked to a matrix of other molecules.

After one entire sheath around the cell is completed, a new one begins to form just inside the first. Over time, many layers develop, the innermost one always being the newest. In woody plants, there's a second phase of development in which the walls lignify, or become woody. At that point, the cells don't get any bigger, but their walls get thicker and harder.

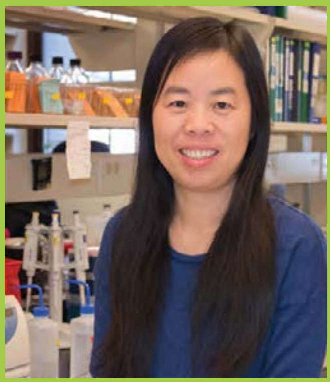
In the 1990s, Cosgrove's lab discovered a class of proteins that somehow loosen the bonds that hold the wall components together just enough to let the cell wall grow, creating a bigger space inside for the cell's gel-like innards to fill. He called the proteins expansins.

"To this day, the mechanism of action of expansins is mysterious," says Cosgrove. "They work in a way that seems contradictory to our notions of how the cell wall is structured—they turn the cell wall from something that behaves like a solid to one that behaves like a liquid and starts to flow. Then the walls can extend and the cells grow."

And yet, even while growing, the walls remain strong. Their constituents remain linked together in a way we don't yet understand—and those are the bonds that need to be broken to release the energy stored within.

## SEEING IS BELIEVING

To learn more about how cell walls are made, **Charles Anderson**, assistant professor of biology, and **Ying Gu**, assistant professor of biochemistry and molecular biology, use techniques that allow them to actually see many of the building blocks of cell walls and the cellular machinery that puts them together.


Patrick Mansell

Ying Gu

"I like to see things," says Gu. "Seeing is believing!"

Her lab is studying how the orientation of cellulose microfibrils is controlled. When cellulose chains first emerge from the cell, they lie crosswise, perpendicular to the long axis of the cell. They later rotate and end up arranged lengthwise.

In an experiment with seedlings of a small flowering plant, Gu's research associate **Shundai Li** and graduate student **Lei Lei** showed that the Cellulose Synthesizing Complex is linked to supportive structures inside the cell, and that the linkage helps control how the cellulose fibers are oriented outside the cell.

They marked specific proteins with compounds that fluoresce when exposed to a laser. They then photographed individual cells of the seedling—which was still alive and growing—through a laser confocal microscope.

Under one wavelength of light, a red glow revealed the location within the cell of the supportive struts. Under a different wavelength of light, a green glow showed the location of a protein, CSI1, that Gu had previously found is attached to the cellulose-making machinery. Time-lapse recordings of the living cell under both wavelengths showed CSI1 shuttling back and forth along the struts.

Because the struts are oriented crosswise in the cell and CSI1 is connected to both them and the cellulose machinery, that motion would result in new cellulose chains being oriented crosswise to the cells.

Now colleagues in the Center can use other techniques to study how the microfibrils rotate and how that movement is related to the cell's ability to grow.

"We have good collaborations across the Center," says Gu, "so we can do our thing, which we are good at, and then send our samples to other people to analyze, to measure the physical properties and see how they relate to cell structure."

## THE PAYOFF

Solving the mystery of cell walls could do more than provide an alternative fuel source, says Cosgrove. Farmers could sell the crop residue they currently discard, gaining extra income with little or no additional investment. In some areas, getting rid of the leftover plant matter may be even more important than gaining income.

"Down in Brazil they grow sugar cane," says Cosgrove. "They chop up the stalks, they extract the sugars, but then they're left with a huge residue of cell wall material. It's very much like cornstalks, just bulkier. They used to burn it but it created air pollution problems, so they banned burning it. So they're left with this problem, what do you do with all this stuff?"

Raising crops specifically for fuel production, already under way on an experimental basis, could become efficient enough to be profitable.

The research could even translate into a wide range of other products. "Cell walls go into all kinds of things," he says. "It's cotton, it's wood, it's fibers, and it's used in a lot of industrial processes. Knowledge lets you improve all of that. You never know where this will be picked up by engineers who say, OK, we now see a way that we can tweak this to make a product better, different, cheaper."

Last year, the DOE renewed the Center's funding for another four years. Cosgrove says he'll be happy to let others develop specific applications from cell wall research. He just wants to learn how walls are made and how they work.

"We knew a whole lot more about cell walls 20 years ago!" he laughs. "We were totally misinformed. We thought we knew, and we didn't. We keep coming upon surprises, all the time, as we learn more. The wall is a much more sophisticated structure than anyone had believed."