

Drilling Deep into Plant Veins

WHAT cows do quite naturally does not come so easily for humans. Termites and ruminants such as cows digest cellulose—the main component of woody material in plants—with the aid of gut microorganisms, but scientists struggle to efficiently perform the same task for biofuel production. Researchers want to exploit cellulose, the most abundant organic material on Earth, from wood, grasses, or plant waste materials to create fuels to power our cars and cities. The crucial step in the biofuel production process is efficiently breaking down the long chains of sugars that comprise cellulose into simple sugars. This process has proved both challenging and expensive because of the complex structure of cellulose-yielding plants. *Zinnia elegans* is an annual plant with exuberant flowers and lacy leaves—not anyone’s idea of a green waste material. But the garden-variety zinnia’s lacy leaves have been put under the microscope in an effort to visualize plant cell structure and eventually develop more efficient ways to extract the raw ingredients for biofuels.

A team led by Livermore scientist Michael Thelen, in collaboration with scientists at Lawrence Berkeley National Laboratory and the National Renewable Energy Laboratory (NREL), uses a novel combination of imaging techniques to view the supportive structure of zinnia leaf cells at several length scales and to observe changes during the deconstruction of cellulose-yielding cells. The structure of supportive cellular elements in plants is rather poorly known, so this work has applications both to basic plant science and to biofuel production. According to Thelen, “We look at the heart of what makes up the woody materials in plants for use in biofuels.” While other research groups also study how to break down cellulose in plant tissues, this work differs because it uses single plant cells to fundamentally understand the initial stages of deconstruction.

Targeting Transformed Cells

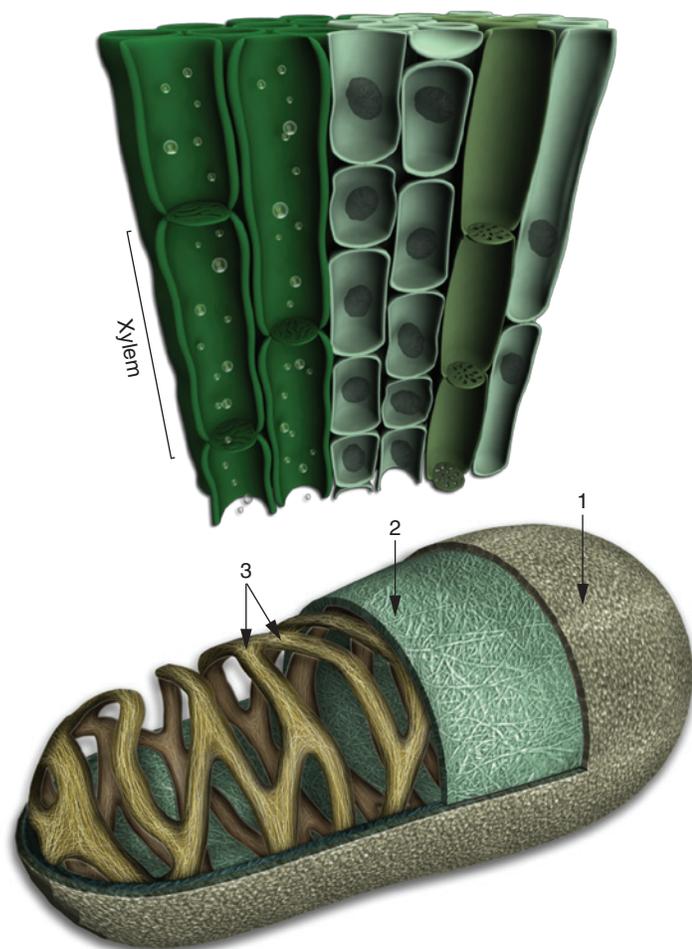
Plant scientists noted several decades ago that mesophyll cells (the primary site for photosynthesis) harvested from the leaves of zinnia seedlings will transform into xylem cells (the principal water-conducting tissue of vascular plants) when cultured in liquid for several days and exposed to plant hormones. Says Thelen, “Scientists previously have found that leaf cells in culture can be induced to give rise to structures and processes that normally happen in nearby, but different, cells.”

Mesophyll cells that transdifferentiate into xylem, or woody, cells have created a highly supportive secondary cell wall consisting mostly of cellulose, in organized patterns of hoops, spirals, or reticulated networks. Near the end of this process, lignin deposits form, and then the cells undergo a programmed

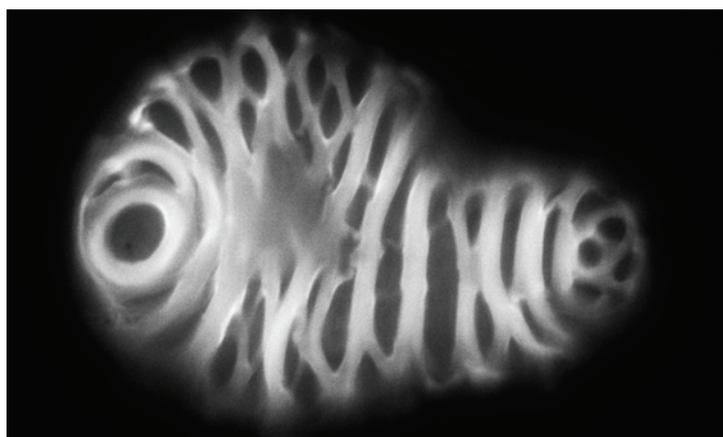
death, leaving behind only their shells. In plants, these “functional corpses,” as they are sometimes known, are linked end to end to form the sturdy, water-transporting veins that link the roots to the leaves of plants. “Those hollow shells made of cellulose and the lignin that sheaths it are the crucial part of the plant we are studying,” says Thelen. “They contain 70 percent of the lignocellulose in plants potentially available for biofuels processing.”

After isolating leaf cells and inducing secondary cell-wall formation, Thelen’s team applied a combination of imaging techniques to both primary and secondary cell walls to characterize their structure and their chemical composition. The structure





Researchers studied a zinnia xylem cell using a combination of imaging techniques. (top) Xylem cells link end to end in a plant to transport water. (bottom) The team observed three main layers in the xylem cell wall: (1) the outer granular matrix, (2) the primary cell wall, and (3) the secondary cell wall. (Rendering by Sabrina Fletcher.)



With fluorescence microscopy, the team observed that autofluorescent components of the xylem cell's secondary wall make the wall's hoops and swirls readily apparent.

of plant cells varies both by plant and cell type, so the team chose a single type of zinnia cell as a model system. According to Catherine Lacayo, a Livermore postdoctoral researcher who performed much of the hands-on work, this particular zinnia cell is known to perform the synchronized transdifferentiation process in culture. It is also easy to isolate and can produce large amounts of cellulose for its size. Lacayo notes that the team's work can be considered a starting point for industry-oriented biofuels research. "Zinnia is not practical for use beyond a research setting," she says. "The results will be applied to study other plant types—those plants known as feedstocks for biofuels."

A goal of this study was to gain a better understanding of how lignin and cellulose interact chemically and structurally within the secondary cell wall. Both primary and secondary cell walls contain a crystalline mesh of cellulose fibers that strengthen and support the cell and the overall plant. The secondary cell wall contains more cellulose but also lignin, a complex aromatic polymer. Lignin is an obstacle for biofuels scientists because the polymer makes it difficult to access and separate the cellulose targeted for biofuel production. "Lignin is linked to the cellulose fibers through chemical bonding," says Lacayo. Not only are lignin and cellulose chemically bonded, but lignin also is highly hydrophobic and resistant to breakage. Both are essential properties for a substance that lines the water-carrying veins of plants but pose challenges for deconstruction.

Zooming in for a Better Look

Thelen's group used three imaging platforms to study the zinnia cell-wall structures at different scales, which helped to visualize their organization and composition down to the nanometer scale. The structures under study are tiny, although they are about average in the world of plant cells. Generally, each secondary cell wall is 2 micrometers thick, and the xylem cells themselves are just 40 micrometers in length. For all three techniques, cells were divided into two groups. Cells in one group were examined in as close to their natural form as possible. Cells in the other group were chemically treated to strip off lignin and other materials before imaging.

Lacayo used fluorescence microscopy to peer inside the cells at the broadest scale. Ideally, for this imaging technique, the secondary walls in these cells will be highly autofluorescent because of their lignin content, making the hoops and swirls of the secondary cell wall easily discernable. To enhance visibility, Lacayo added to individual cells an NREL-engineered fluorescent protein that binds to cellulose. The cellulose-binding protein worked better when the cells were exposed to hot, acidified chlorite—a chemical treatment used to remove lignin and sugars from the wall. Lacayo explains, "We found that when lignin and some sugars are removed from the cell wall, cellulose becomes more accessible, which was confirmed using atomic force microscopy."

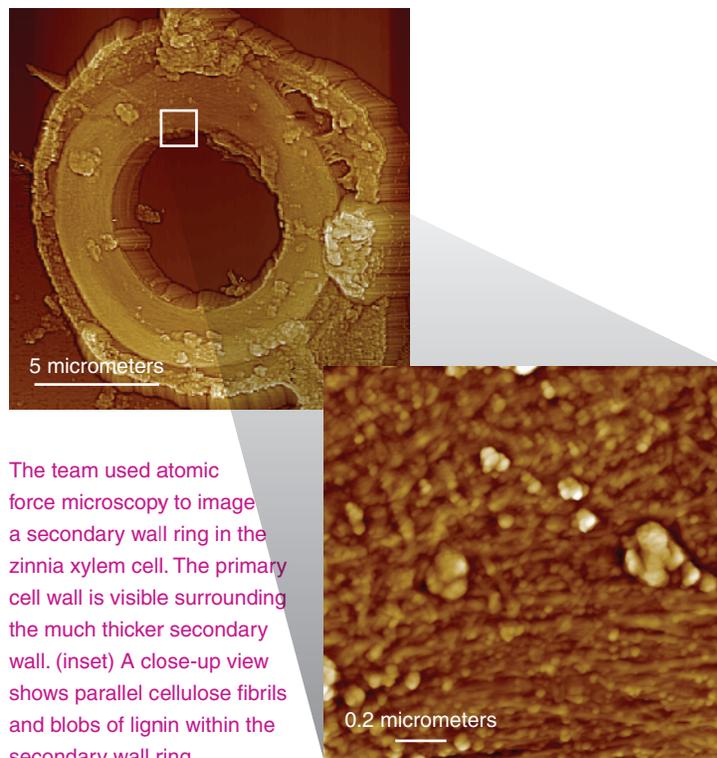
The Livermore team used atomic force microscopy (AFM) to study the cell-wall surface and structures in finer detail. Funding for developing this capability was provided by the Laboratory Directed Research and Development Program. AFM is an imaging and measuring technique, where information is gathered by “feeling” the surface with a mechanical probe. The researchers determined that a granular material with structures a few tens of nanometers thick covers the outer surface of the cells. Drilling down a bit deeper, cells treated with hot, acidified chlorite and imaged with AFM revealed a networked structure of cellulose fibrils comprising the primary cell wall. By breaking the cells apart, they obtained fragments of the secondary cell wall to image as well. These fragments consisted of granular deposits of lignin within cellulose fibrils arranged in parallel. The fibrils were observed to sometimes form thicker groupings up to 100 nanometers wide. These direct observations allowed Thelen’s group to develop a model of the zinnia cell-wall architecture. According to Alexander Malkin, a Livermore scientist who lent his AFM expertise to the project, “This is the first time that high-resolution imaging has been done of several structural layers of a cell wall.”

Imaging at the most minute scale was performed for the project by Lawrence Berkeley scientist Hoi-Ying Holman. Using synchrotron radiation Fourier-transform infrared (SR-FTIR) spectromicroscopy, Holman looked at the chemical composition of individual cells. Spectromicroscopy identifies and measures substances through the spectrum those substances emit or absorb. Once the cells were treated with chemicals to remove components from the cell walls, SR-FTIR was used to image cell composition. In recorded changes in the relevant absorbance spectra, SR-FTIR imaging showed that the cellulose remaining in each cell was more accessible after lignin was removed with the acidified chlorite treatment.

Platform for Research Growth

The results from this trio of cell imaging techniques are likely to benefit plant biologists and biofuels scientists alike. The Livermore team and collaborators have emerged with a better understanding of the fine structure of the zinnia xylem cell walls, especially the structure of the secondary cell wall with and without lignin. They have developed a chemical and structural model of cell walls and have gained insight into the deconstruction and removal of cell-wall components. By demonstrating a comprehensive and effective combination of imaging techniques on plant cell structures, the researchers have established a new standard in their field.

The Department of Energy (DOE) Genome Science Program and the DOE Joint BioEnergy Institute (a six-institution partnership led by Lawrence Berkeley in which Thelen is also involved) have supported this work as part of a larger research effort to turn biomass into fuels using microbial enzymes. For



The team used atomic force microscopy to image a secondary wall ring in the zinnia xylem cell. The primary cell wall is visible surrounding the much thicker secondary wall. (inset) A close-up view shows parallel cellulose fibrils and blobs of lignin within the secondary wall ring.

Thelen’s group, research continues. Building on what they have learned, the Livermore scientists are now working on imaging the deconstruction of zinnia cell walls using various enzymes extracted from microbes. “Our goal is to systematically break down the plant cell wall and to monitor that process using high-resolution imaging in real time,” Lacayo says. The next step will likely be to select microbes with desirable characteristics, such as adaptability to high temperatures, and study how effectively each particular microbe’s enzymes break down cellulose and other sugars.

The successful characterization of the zinnia cell-wall structures has provided a more comprehensive understanding of the supportive structures comprising zinnia leaf veins and how decomposition of complex sugars occurs in these regions. But perhaps most importantly, Thelen’s group has discovered a winning combination of techniques to peer deep within a cell, a research platform that will likely prove useful to many other plant scientists. Biofuels scientists, too, may benefit from the brilliantly hued zinnia plant, which is helping to unlock the secrets to plant cell-wall construction and deconstruction.

—Rose Hansen

Key Words: atomic force microscopy (AFM), biofuel, cellulose, fluorescence microscopy, Joint BioEnergy Institute, lignin, lignocellulose, mesophyll, synchrotron radiation Fourier-transform infrared (SR-FTIR) spectromicroscopy, xylem.

For further information contact Michael Thelen (925) 422-6547 (thelen1@llnl.gov).