

# Agricultural Research

## Economics of Limited Irrigation

pages  
12-13



# FORUM

## Feeding the World Through Food Technology Excellence

**Scientists** at the Agricultural Research Service's Eastern Regional Research Center (ERRC) in Wyndmoor, Pennsylvania, conduct research on a wide spectrum of agricultural commodities. Their scientific discoveries are transferred to industry stakeholders and clients with the help of the ARS Office of Technology Transfer. Private-sector partners then further develop and launch new commercial products.

ERRC scientists have been developing technologies and food-preparation processes since 1940. These discoveries have led to industry partners' developing new food products that help feed the world. These food-science innovations benefit not only the producers of agricultural commodities, but also the processors and handlers of food products. The story beginning on page 4 of this issue highlights ERRC's food science equipment, technologies, and processes that lead to the development of value-added new products.

In 2005, ERRC consolidated its industry-scale equipment, which is used to research modern food processes, and created the Center of Excellence in Extrusion and Polymer Rheology (CEEPR). The center is focused on improving and testing technologies, processes, and equipment that will eventually lead to new foods and food ingredients with value-added health and functional attributes.

The CEEPR scientists now work in a modern pilot plant where new product concepts and prototypes are ramped up for industrial production. Successful new technologies are passed on to industry through technology transfer collaborations. For example, CEEPR scientists have developed unique extrusion texturization processes that are used to produce new crunchy food products. Extrusion is an engineering process that applies pressure and heat to raw materials

and converts them into new forms with specific textures and properties.

A recently developed ARS-patented process incorporates a standard industry-scale machine called the "twin-screw extruder." The patented process can be used to make crunchy snacks that are enriched with whey proteins. As a result of the CEEPR-developed technology, a line of whey protein-enriched food products was commercialized by a food company. The new snacks made by the licensee could help meet the demands of health-conscious consumers.

CEEPR scientists have also collaborated with other U.S. Department of Agriculture agencies to resolve multiple food and agricultural problems. In particular, as featured in this issue, developments by CEEPR scientists have brought enhanced features to a traditional food ration called "corn-soy blend" that supplements foreign food-assistance meals, particularly for young children.

USDA's Foreign Agricultural Service administers the McGovern-Dole International Food for Education and Child Nutrition Program, which provides U.S. agricultural products for school feeding and other projects in more than 30 countries. USDA's Farm Service Agency purchases the U.S. commodities that help these foreign countries.

At ERRC, food technologist Charles Onwulata coordinates CEEPR projects. Onwulata spent his youth in Nigeria, Africa, where he received foreign food aid early in his life. Later, he developed a passion for solving problems related to hunger. Now, Onwulata has worked with a team of USDA scientists, program managers, policy administrators, and international aid agencies to deliver a new emergency-aid meal called "instant corn-soy blend" (ICSB).

The extrusion technology used to make ICSB cooks food completely and quickly, under high heat and high pressure. The

crunchy, fully cooked product exits the extruder through an opening at the end of the machine in less than 2 minutes. That product is then crushed and milled to form the ration.



Charles Onwulata, supervisory research food technologist.

Onwulata's efforts to improve corn-soy blend began in 1995, and the idea that resulted in the new product was developed from 2000 to 2005. For the first time in 50 years, the USDA Farm Service Agency has issued an invitation for a bid for a fully cooked corn-soy blend food ration that can be stirred with potable drinking water to make a porridge.

Members of a network of nonprofit agencies that participate in the federally sponsored AbilityOne program, which employs significantly handicapped individuals in the United States, have voiced their interest to food-aid administrators in manufacturing, producing, and packaging the new food-aid product. ICSB could soon be purchased for the McGovern-Dole program.

The ERRC technology significantly enhances the uniform distribution of added vitamins and minerals in a supplemental food ration that can be used for overseas delivery for mass feeding of young children and others. You'll read about the details on the development of this technology—and how it can be used by manufacturers to produce the new food-aid product—in this month's feature article starting on page 4.

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KENDRA HELMER, USAID



Thanks to the cooperative efforts of ARS, USAID, and USDA's Farm Service Agency, the children of Haiti may soon benefit from an instant corn-soy blend food aid developed by an ARS scientist at Wyndmoor, Pennsylvania. Story begins on page 4.

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Cover: Agricultural engineer Walter Bausch (left) and engineering technician Ted Bernard measure reflectance, canopy temperature, and ground cover of corn with their mobile platform. The wheels of the platform roll between the rows of fully mature corn while the sensors on the end of the telescoping boom look down on the crop from 15 feet above the corn canopy. Story begins on page 12. Photo by Peggy Greb. (D2099-1)

# Fully Cooked Emergency Aid Food



**We've all seen the images** on television from disasters overseas: Large white sacks of meal are unloaded from trucks as hungry families line up and await rations. The U.S. flag and the words “Gift of the United States of America” are prominently displayed in bright red and blue ink. These humanitarian efforts are the result of collaborations involving multiple national and international government managers, aid agency officials, and policy administrators.

Agricultural Research Service scientists have been working with collaborators to bring enhanced features to food rations—corn-soy blends that supplement meals, particularly for young children. A new, fully cooked food-aid product has been developed as a result of this team effort. The work was led by food technologist Charles Onwulata in the Dairy Processing and Products Research Unit at the ARS Eastern Regional Research Center (ERRC), in Wyndmoor, Pennsylvania.

The U.S. Department of Agriculture’s Foreign Agricultural Service (FAS) administers the McGovern-Dole International Food for Education and Child Nutrition Program, which provides U.S. agricultural products for school feeding and other projects in more than 30 countries. The U.S. Agency for International Development (USAID)—an independent federal agency—works in 100 developing countries to distribute commodity staples to people at risk of hunger and malnutrition.

USDA’s Washington, D.C.-based Farm Service Agency (FSA) purchases U.S. commodities that help foreign countries under the Agricultural Trade Development Assistance Act of 1954—or Public Law 480. This law was renamed the “Food for Peace Act” in 2008.

The FSA implements agricultural policies and oversees the procurement chain for multiple programs. These include purchasing U.S. commodity products using both FAS and USAID funds. These commodity foods are then provided to FAS programs and USAID Food for Peace programs, which direct the foods to recipients through a wide network of partners that includes the United Nations World Food Program.

A nutritionally fortified and processed corn-soy blend is provided as a supplement to a ration that provides staple foods. This

ration has been supplied as uncooked or partially cooked fortified corn- or wheat-soy blends that contain oil, vitamins, and minerals. But as with most food products, such blends go stale over time. Spoilage problems have been associated with uncooked corn-soy blends when fat components—oils—in the mix go rancid. Fine particles in the blends can segregate and fall to the bottom, and key vitamins and minerals can settle as well, making the mixture less nutritious and effective for the already malnourished.

These uncooked and partially cooked formulations were developed by ARS scientists nearly 50 years ago. During the past 15 years, Onwulata has worked on improving food blends and has now produced an enhanced ration that comes as a fully cooked commodity product. “The

new product is called ‘instant corn-soy blend,’ or ICSB, and it can be stirred with sanitized, potable drinking water” to make the ration, says Onwulata.

Periodically, FSA’s Kansas City Commodity Office in Missouri issues invitations for bids requesting particular commodities for procurement, as well as the requirements involved. The new ICSB could now be one of those commodities. FSA included the new extruded process that ARS developed into the specifications for an additional type of corn-soy blend. ICSB could be purchased for the FAS-administered McGovern-Dole program.

#### A Fully Cooked Blend

In early 1993, ARS national program leaders convened a meeting with ARS scientists to discuss ways that the vitamin and mineral content of food-aid rations

could be improved. In 1995, FSA food scientists contacted ARS food scientists to discuss specific problems with noncooked or partially cooked corn-soy rations becoming unstable over time. “Part of the problem was keeping partially cooked food fresh during long shipment and transportation periods,” says Onwulata. “We also discussed ways to remedy problems associated with nutrients settling to the bottom of bags.”

Onwulata then began developing a new food product using the same type of machines that are used to make puffed snacks and cereals. “Cheese puffs” and “cereal puffs” for example have been popular in the United States for more than 50 years. ICSB looks much like these popular puffed snacks when they exit the machinery—before it is ground—but with a creamy color and harder texture in its dry form.

The technology developed is referred to as an “instantized” process, which cooks foodstuff completely in a short time, under high heat and high pressure. The machine used to produce the food product is

Food technologist Charles Onwulata (left) inspects extruded instant corn-soy blend before milling and packaging while engineering technician Eric Tilman (right) scoops up a corn-soy blend intended for extrusion. In the background, chemist Audrey Thomas-Gahring monitors the control console.



PEGGY GREB (D2261-1)



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Employees working at Transylvania Vocational Services, Inc. (TVS), a private, nonprofit corporation in Brevard, North Carolina. TVS is in the process of manufacturing and packaging 20 tons of instant corn-soy blend food aid for a group of moderately malnourished children in Haiti. TVS provides career opportunities for people with significant disabilities.

nutrient distribution in corn-soy blend,” says Onwulata.

On the road to ICSB success, Onwulata published a study in which he evaluated the composition, vitamins and minerals, and other properties of corn-soy blends made by six processors. His findings showed nonuniform distribution of the added vitamins and minerals. The paper, “Variation in Corn Soy Blends for Overseas Distribution,” which was coauthored with six other ERRC scientists, was published in *Cereal Foods World* in 1999. The findings also drove



ABILITYONE PROGRAM (D2264-2)

called a “twin-screw extruder.” Cooking extruders are specialized food-manufacturing machines consisting of tightly fitting screws that rotate within a stationary barrel.

The uncooked corn-soy meal, the vitamin premix, and the mineral premix enter the barrel at one end and are heated and mixed by a variety of mixing and mashing screws. The cooked product then exits the extruder through an opening at the other end of the machine—and in less than 2 minutes has taken a new, fully cooked, expanded and textured form. The textured corn-soy blend is then crushed and milled to size to form the ICSB.

Onwulata conducted several experiments showing that twin-screw extrusion could be used to create an instant product that, when rehydrated with safe drinking water, turns into a porridge that meets all required nutritional specifications, color, and consistency.

#### Value-Added Emergency Food

The extrusion-based product that Onwulata and his team developed is based on the same formulation as traditional corn-soy blend, with the advantages being that the nutrients and oils are fully integrated into it. “This addresses the initial concerns of off-flavors, off-colors, and uneven

home the need to change to an instant, ready-to-eat blend. For the next 6 years, Onwulata worked on finalizing the new fully cooked, ready-to-eat product.

In 2005, Talari V. Rao Jude—who at the time was a lead food scientist with FSA’s Commodity Policy Procurement Analysis Division—spotted the corn-soy blend evaluation paper and contacted Onwulata to review and discuss ICSB. “The merits were so overwhelming that we initiated a joint collaboration in 2008 between FSA and ARS to produce fresh ICSB product,” says Jude.

Then in 2009, Jude contacted Food for Peace to discuss the merits of ICSB as an improved product for humanitarian feeding programs. This led to a key meeting in a series of in-person demonstrations of the new product. ARS attendees included ERRC’s Onwulata, technology transfer coordinator Robert Griesbach, and senior national program leader for utilization L. Frank Flora. Representatives of FAS,

FSA, and USAID also attended, along with representatives from the private and manufacturing sectors.

The group met in Washington, D.C. in June and November 2009. Onwulata and colleagues provided attendees a sample of the instant porridge made from ICSB mixed with water. “Other nutritious products made from ICSB were also served,” says Jude, “and all were liked by attendees.” Onwulata also provided nutritional fact sheets and a primer on industry’s capability to produce the product.

Jude had been working since 2000 on food-quality issues associated with food-aid products. He analyzed spoilage data on a bagged, partially cooked corn-soy blend that had been transported to foreign countries as food aid. He then recognized that particle size variation caused poor distribution of minerals and that the new ICSB could solve the problem.

“When food aid is sent overseas as a gift from the American people, it must be able to withstand a long journey during which storms and other challenges may occur,” says Jude, who is now an FSA domestic programs manager. “After the aid arrives, local transportation or storage begins, during which temperatures may swing drastically. Food rations must be of the highest edible quality when they reach those in need, and when they are damaged or go bad, the donor country’s reputation and image can be damaged as well.”

A new, stable, fully cooked product would require a new manufacturing network. This meant that further demonstrations to educate potential manufacturers on mass-producing fully cooked, extruded rations were needed. “The demonstration to administrators in June 2009 opened the way for further demonstrations to potential manufacturers on how the new extruder equipment and processes are used to produce the new product,” says Jude.

#### New Policies

By 2009, development of a new formula and specifications for instant corn soy blend had gained recognition among key food-aid sponsors. “This was accomplished in part through the continuing series of demonstrations that focused on policy officials, manufacturing managers, scientists, and aid-agency officials,” says Onwulata.

“Nearly 5 years ago, Onwulata described a new fully cooked, extruded food-aid product he was developing that would overcome some of the problems we’d been experiencing with traditional corn-soy blends,” says Cleveland Marsh, FSA’s assistant to the deputy administrator for commodity operations and also acting export program manager. “In late 2010, we amended our Commodity Requirements Document, or CRD, to include specifications for producing ICSB as an option, in addition to producing traditional milled grain as an option.” Once a request for food aid that cites the new CRD is received, FSA is in a position to procure the most competitively priced products.

ICSB was specified by FSA as a supplemental food for emergency rations, displaced-persons assistance, and as a weaning food in maternal and child health programs and other programs. It is to be composed of pre-gelatinized cornmeal; toasted soy flour; refined, deodorized, and bleached soybean oil; premixed minerals; and premixed vitamins and antioxidants.

The finished-product requirements stipulate that each 100-gram serving must have a uniform distribution of vitamins and minerals. The ICSB must also be manufactured so as to produce a fully cooked end product with a neutral to slightly nutty flavor and must have a light yellow to golden buff color. Particle size and nutritional labeling are also stipulated.



For more than 15 years now, FSA has been consulting with ARS’s Onwulata on resolving problems associated with uncooked corn-soy blend. Onwulata has provided direction for FSA policy on Food for Peace programs and, working with ARS national program leaders, has consulted regularly with USAID and FAS on Title II emergency feeding programs.

Food for Peace provides \$2 billion worth of commodity-based meals each year in times of emergency. To ensure that USAID can provide the best products for the Food for Peace programs, Food for Peace initiated a 2-year review of the nutritional quality of food aid through a contract with Tufts University Friedman

School of Nutrition and Policy in Boston, Massachusetts. A product similar to ICSB, but with a different formulation and micronutrient profile, may fulfill some of the recommendations of that review.

In addition to reviewing existing products, Food for Peace has been working with other university experts and USDA to develop the next generation of food-aid commodities, such as ready-to-use meal replacements for emergencies and foods for supplementary and therapeutic feeding programs.

It is expected that new products from both the Tufts review and the development of specifications for a variety of ready-to-use foods will be rolled out over the next 2 years.—By **Rosalie Marion Bliss, ARS.**

*This research supports the USDA priorities of ensuring food safety, promoting international food security, and improving children’s nutrition and health and is part of Quality and Utilization of Agricultural Products, an ARS national program (#306) described at [www.nps.ars.usda.gov](http://www.nps.ars.usda.gov).*

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Instant corn-soy blend (ICSB) is fully cooked. ICSB shown here is extruded, ground into a powder, and mixed with tepid water, producing a porridge.



Photo at top of page used by permission.

©Anne-Karine Brodeur/World Food Programme.

# Seeking Saponins and Other Compounds To Fortify Crops

**The perennial herb soapwort, *Saponaria officinalis*, owes its prized cleansing foam to detergent-like compounds called “saponins.” But soapwort isn’t the only plant that produces the compounds; nor are their properties limited to removing dirt and grime.**

In studies at the Agricultural Research Service’s National Center for Agricultural Utilization Research (NCAUR) in Peoria, Illinois, scientists are spiking laboratory diets fed to corn earworm and fall armyworm with saponins from soybeans, switchgrass, yerba maté, and other sources to determine what effects the compounds have on the caterpillar pests’ growth and survival.

ARS entomologist Pat Dowd says the studies are an integral part of a broader effort at Peoria to identify novel sources of resistance that can be put into corn—either through traditional plant breeding or biotechnological means. Ultimately, this could usher in new corn varieties that

sustain less damage from caterpillars, are less prone to infection by toxin-making molds, or require fewer pesticide applications. Another potential benefit is staving off the ability of pests like corn earworms to build tolerance to existing sources of resistance—such as that endowed by insecticidal proteins from the soil bacterium *Bacillus thuringiensis*, which is used in about 63 percent of U.S. corn, according to USDA’s Economic Research Service.

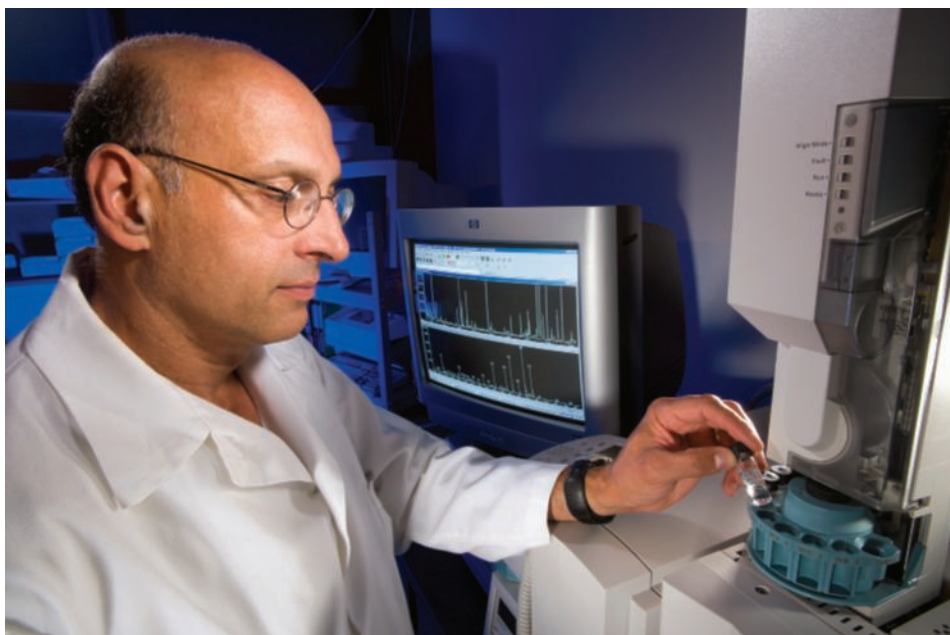
“Looking for natural methods of controlling pathogens and pests is a win-win situation for the environment, for businesses who want to grow their efforts in green technologies, and ultimately for the U.S. taxpayer, who benefits from a cleaner environment and a thriving economy,” remarks Alejandro Rooney, who leads NCAUR’s Crop Bioprotection Research Unit, where Dowd, ARS molecular biologist Eric T. Johnson, and others are evaluating the insecticidal properties of saponins and other natural compounds.

## Making Sense of Saponins

In nature, the saponins are produced in the stem, seed, roots, leaves, or fruit of plants belonging to more than 100 different families, including Allianceae, Caryophyllaceae, Rosaceae, and Gramineae (of which corn and switchgrass are both members). But until recently, no cross-cutting studies have been done comparing the degree to which saponins from different plant species or families confer resistance to pests and pathogens, note Dowd, Johnson, and colleagues in an upcoming issue of the *Journal of Chemical Ecology*.

“Most grain crops, except for oats, do not have saponins in them,” Dowd says. Why this is so remains a mystery. But studying close relatives of today’s grain crops may reveal important clues. For example, examining saponins that make some types of switchgrass less palatable to fall armyworms may reveal dormant genes or biochemical pathways in a distant relative like corn that can be activated via genetic engineering or conventional breeding.

“Theoretically, selecting plants for beneficial agronomic traits could have resulted in the loss of pest-resistance genes,” says Dowd. “If these resistance genes were located near alleles of genes that conferred undesirable agronomic traits, they may have been bred out along with the undesirable genes during the process of developing commercial lines.”



PEGGY GREB (D745-1)

Molecular biologist Gautam Sarath loads vials containing hydrolyzed switchgrass cell-wall samples for analyses of lignin content by gas chromatography-mass spectrometry. The data will be used to identify elite switchgrass plants for improvement through breeding.



### Investigating the Lignin Connection

A crucial first step in studying a compound is to determine its biological activity, structure, and expression levels. This past year, Dowd, Johnson, and ARS chemist Mark Berhow conducted experiments in which they force-fed 1 of 10 different kinds of saponins, caffeine, and other compounds to first-stage fall armyworms and corn earworms. The team purchased two of the saponins, a steroidal type called “diosgenin” and a related form called “protodioscin,” after being alerted to their presence in switchgrass by Ken Vogel at ARS’s Grain, Forage and Bioenergy Research Unit in Lincoln, Nebraska.

Vogel’s team is interested in learning whether the saponins played a part in the resistance of some low-lignin lines of switchgrass to fall armyworms. The group had previously developed the low-lignin lines to expedite fermentation of the plants’ sugars into ethanol. One concern was that reducing the lignin content would also make the plants more vulnerable to chewing by fall armyworms. But in trials, 7 of the 14 switchgrass lines in fact resisted the caterpillar pests, though this depended on the timing of the growing season.

“This suggests there is a temporal as well as a genetic component to expression of the fall armyworm resistance,” says Gautam Sarath, an ARS molecular biologist at Lincoln who is collaborating with Vogel and ARS agronomist Rob Mitchell to explore switchgrass’s potential as a commercial ethanol crop.

Sarath says it is possible that diosgenin, which has been linked to digestive problems in livestock, may have compensated for the reduced lignin by helping wreak similar havoc on fall armyworms that attempted to feed on the low-lignin lines during tests.

In the lab tests at Peoria, protodioscin had some activity against fall armyworms, as did saponins from maté, soap bark tree,

and soybeans. One type of soy saponin, called “soyasaponin B,” which has a sugar molecule attached, proved more effective than its sugar-free form against corn earworm caterpillars, reducing their growth by more than 50 percent.

Dowd notes that they evaluated different sources of saponins because some insect pests, like fall armyworms and corn earworms, are generalist feeders, but still have different host preferences. Thus, it was



Fall armyworm,  
*Spodoptera rugiperda*.

BARRY FITZGERALD (K2039-11)

expected that saponins the pests don’t typically encounter in nature would be more toxic to them. But this wasn’t necessarily the case. Rather, the difference seemed to come down to whether the saponins harbored certain sugar groups.

None of the saponins tested killed either of the two pest species. But a smaller caterpillar isn’t necessarily a failure: Under field conditions, plants that taste bad can cause the caterpillars to seek out other plants; this could translate to reduced kernel damage as well as easier pickings for hungry predators.

### Giving Pests the Blues

Besides saponins, the Peoria researchers are also evaluating the pest-fighting potential of phytochemicals such as anthocyanins, which give blueberries, plums, grapes, and flowers like petunias, for example, their blue and purple colors.

In feeding experiments, corn earworm larvae forced to feed on blue areas of petunia petals gained less weight than larvae that fed on white areas. Additional feeding experiments determined that anthocyanins isolated from the petunia petals also slowed the larvae’s growth. Cabbage looper larvae that fed on blue areas of one petunia cultivar’s petals died at higher rates than larvae that fed on white areas.

Although it’s unclear what petal compound or compounds were involved in looper mortality, the anthocyanins apparently increased the effectiveness of the toxic compound.

In other work, says Johnson, “We’re also very interested in proteins in maize that are produced at the seedling stage. The seedlings are quite resistant to insects, and this may be partly due to a combination of resistance biochemicals and proteins.”

If the proteins’ resistance role can be confirmed, then it may be possible to express the genes responsible for them at a later stage in the plant’s life cycle. “But this would be a matter of expressing them at sufficient levels,” Johnson adds.

### Pest-Fighting and People-Friendly

Berhow, who’s in NCAUR’s Functional Foods Research Unit, is pursuing another line of inquiry. Some of the same phytochemicals that plants make to cope with stress caused by insects and pathogens also benefit people and livestock. For example, studies by Berhow and University of Illinois (UoI) colleagues indicated that some soybean saponins have potential as cancer-fighting agents known as “chemoprotectants.” Indeed, in test-tube experiments, “group B” saponins reduced



Corn earworm, *Heliothis zea*.

cancerous human colon cell growth by 27 to 68 percent.

In addition to furnishing Dowd with purified material for the insect trials, Berhow has put his analytical chemistry skills to use assisting Elvira DeMejia, a UoI associate professor who is studying anti-inflammatory and other beneficial properties of saponins from yerba maté leaves, which are used to make a popular South American tea. Like other saponins tested, maté's saponins also deterred caterpillar feeding.

Ultimately, such multifaceted studies could converge, giving rise to new crop varieties that boast dual-use phytochemicals.

In furtherance of that possibility, scientists are continuing research to understand the full biological activity and effect of saponins in humans and livestock.—By **Jan Suszkiw, ARS.**

**Some of the same phytochemicals that plants make to cope with stress caused by insects and pathogens also benefit people and livestock.**

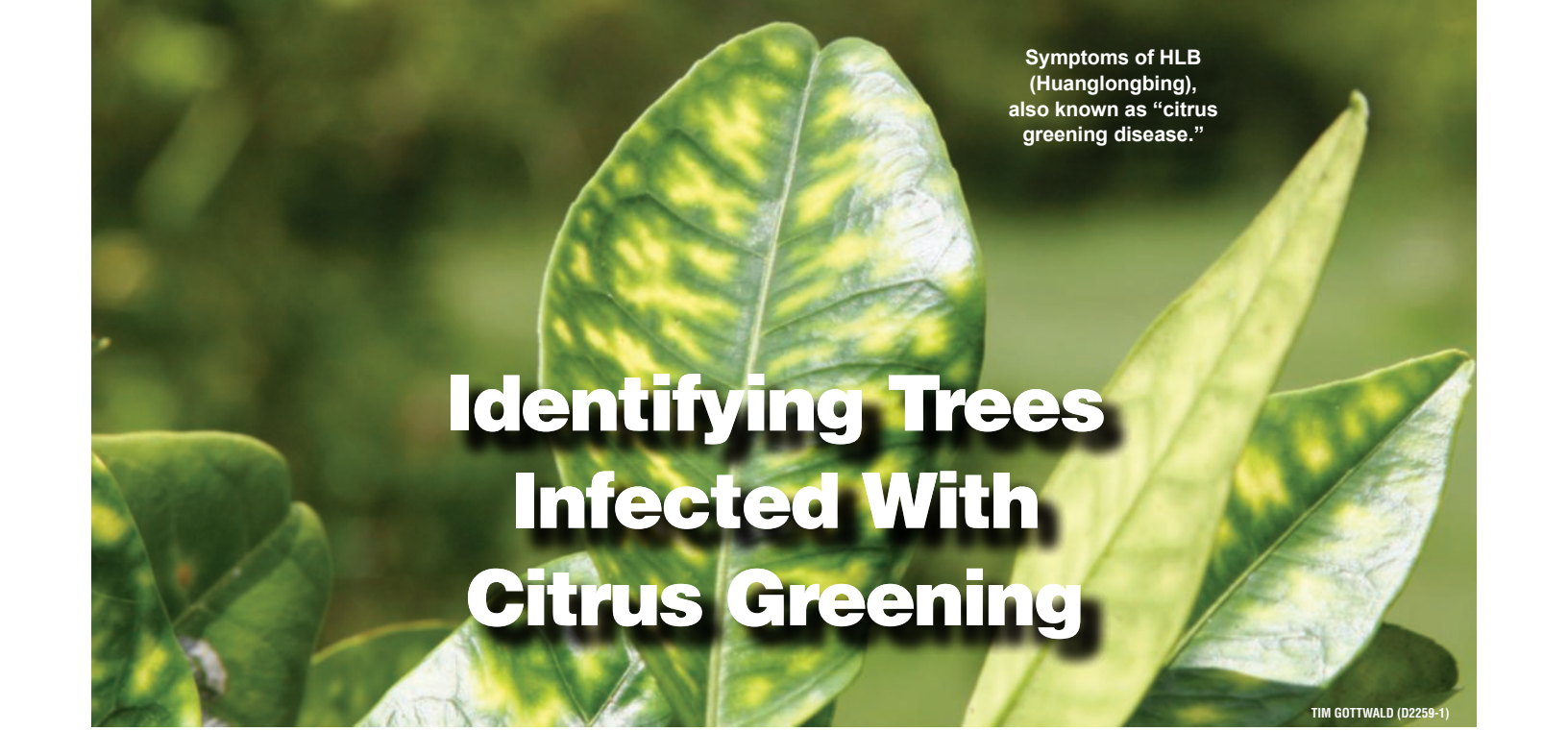
*This research is part of Food Safety (Animal and Plant Products) (#108) and Quality and Utilization of Agricultural Products (#306), two ARS national programs described at [www.nps.ars.usda.gov](http://www.nps.ars.usda.gov).*

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Switchgrass can yield almost twice as much ethanol as corn, estimates geneticist Ken Vogel, who is conducting breeding and genetics research on switchgrass to improve its biomass yield and its ability to recycle carbon as a renewable energy crop.





Symptoms of HLB  
(Huanglongbing),  
also known as “citrus  
greening disease.”

# Identifying Trees Infected With Citrus Greening

TIM GOTTWALD (D2259-1)

**Citrus plants** are highly susceptible to a disease commonly called “HLB” (Huanglongbing), also known as “citrus greening disease.” Agricultural Research Service scientists in the Quality and Safety Assessment Research Unit in Athens, Georgia, and the Subtropical Plant Pathology Research Unit in Fort Pierce, Florida, joined forces to use a technology known as “Fourier transform infrared-attenuated total reflection” (FTIR-ATR) spectroscopy to identify citrus greening-infected plant leaves with 95-percent accuracy.

Citrus greening was discovered in Florida in 2005 and is rapidly spreading in the citrus-growing regions of the state. The disease is transmitted by the Asian psyllid, an insect found throughout Florida. Fruit from infected trees drops prematurely or fails to ripen.

A 2007-2008 survey conducted by the University of Florida, in collaboration with USDA’s National Agricultural Statistics Service and the Florida Department of Agriculture and Consumer Services, included 153,000 net acres of oranges and 17,676,000 orange trees. The survey revealed that growers—to prevent spread of disease—removed 847,208 infected trees during the survey period. Plants can be infected for up to several years before showing symptoms, and during this period the psyllid can transmit the disease agent to nearby plants.

Currently, the best method for detecting citrus greening-infected trees is a type of DNA testing called PCR (polymerase chain reaction), which is both costly and time consuming.

FTIR uses light to identify chemicals and reactions in a sample. This technology has the potential to detect the disease before visible symptoms occur and is cheaper and faster. The mid-infrared region of the electromagnetic spectrum reveals dramatic changes that occur in leaves of infected trees compared to leaves from noninfected trees. The results from this technique’s application were published in the journal *Applied Spectroscopy* in 2010.

To create the new technology, experts from both labs were needed. “ARS is great in this respect because it has so many experts with which to collaborate,” says ARS researcher Gavin Poole in the Fort Pierce unit. “Research leader Tim Gottwald and I had an idea to use spectroscopic methods to identify citrus greening before symptoms develop in the field, and our colleagues in Georgia were able to use their expertise to help us attack this problem.”

The Athens team included chemist Samantha Hawkins, engineer Bosoon Park, physiologist William Windham, and research leader Kurt Lawrence. Hawkins used an FTIR spectrometer with an ATR crystal accessory to test for the presence of citrus greening.

“We used a leaf removed from the citrus tree, dried it out in a microwave, and ground it into a powder—a simple protocol developed by Windham,” says Hawkins. A very small sample—just 0.1 milligrams—of the leaf powder was placed on top of an ATR plate. The system clearly discriminated HLB-infected leaves from healthy leaves. More work will be needed to discriminate HLB from other citrus diseases.

“This is a great method because the sample preparation is faster and easier than PCR, which is typically used to get a value of how much disease is in the leaves,” says Hawkins. “Growers currently send leaf samples out for PCR analysis that costs about \$6 to \$8 per sample and takes several days. The FTIR technique is done in seconds.”—By **Sharon Durham, ARS.**

*This research is part of Plant Diseases, an ARSnational program (#303) described at [www.nps.ars.usda.gov](http://www.nps.ars.usda.gov).*

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# Growing Crops and Sa

**Colorado** is typical of much of the western United States in that the era of expanding irrigated agriculture has come to an end. In fact, the number of irrigated acres is declining.

The State of Colorado estimates that this decline will continue, because the current number of irrigated acres requires an amount of water that falls short of supplies by 10 percent.

## Buy and Dry

Municipal and industrial users in cities along the Rocky Mountains are buying up farmland to get its water rights, then leaving the land idle—a practice called “buy and dry.”

An alternative to this is to have farmers limit their irrigation and sell or lease only the rights to their unused water, rather than sell the land as well. But that requires documentation of water saved that is sufficient for Colorado’s “Water Court” and approval by the State Engineer’s Office.

For this reason, Tom Trout, research leader of the Agricultural Research Service’s Water Management Research Unit (WMRU) in Fort Collins, Colorado, is measuring crop water-use efficiency not by the traditional measure of crop yield per drop of irrigation water applied, but rather by yield per drop of water actually taken in by the crop. That measure is called a “crop water productivity function.” It eliminates all water that does not enter a plant’s roots.

“This shows the actual strain on groundwater supplies, because water used by crops is effectively lost, while most unused rain and irrigation water returns to ground water or flows into streams for use downstream,” Trout says.

Trout is in the third year of a study to determine how much water four crops common to the High Plains region—corn, wheat, sunflower, and pinto beans—actually use. He is growing these crops in rotation and using drip irrigation on a 50-acre limited irrigation research farm near Greeley, Colorado. ARS operates the farm collaboratively with Colorado State University (CSU) at Fort Collins, which is about 30 miles northwest of Greeley.

Trout’s ARS colleagues in this study include agricultural engineer Walter Bausch and plant physiologists Dale Shaner and Lori Wiles, all at WMRU.

The data from this study will be used by the ARS Agricultural Systems Research Unit in Fort Collins to develop a computer “decision support” model to provide farmers with documentation of water savings and information on the economic viability of limited irrigation, crop by crop, to help farmers make decisions.

## Water as a Crop

Regenesis Management Group, LLC, in Denver, Colorado, has signed a cooperative research and development agreement (CRADA) with both research units to create monitoring instruments and software for a web-based application being designed by the company and known as “SWIIM,”

for Sustainable Water and Innovative Irrigation Management. This would allow farmers to treat water as a commodity, like corn, while sustaining economic and environmentally sound irrigation.

As part of the CRADA, the study will be expanded to include one of the main irrigation methods farmers use: running water down furrows between crop rows. ARS and CSU scientists have installed instruments on a 15-acre furrow-irrigated field to measure irrigation applications, runoff, and water percolating down through the soil. Regenesis Management Group will partly fund the research with the goal of developing the underlying science to legally support water transfers.

## Will Limited Irrigation Save Water?

Farmers using limited irrigation do not give crops the full irrigation amounts needed for maximum yields. Instead, they use partial irrigations timed to critical growth stages.



Plant physiologist Dale Shaner measures photosynthesis of a corn plant in a deficit irrigation study.

PEGGY GREB (D2100-3)



In a deficit irrigation study, corn on the left side was fully irrigated and produced about half as much corn.

# Saving Water in the West

Trout and colleagues designed the original study to see whether limited irrigation is best economically for each of the four crops and to help farmers with irrigation timing and amounts and other options. The four crops are being grown with six levels of irrigation, from full irrigation down to only 40 percent of full.

The research farm was set up to enable precision water control and accurate field measurements of water consumed. Use of drip irrigation eliminates the many variables found in furrow and overhead sprinkler irrigation. A series of meters and valves measures irrigation amounts.

A field weather station helps scientists predict the rate at which water is consumed—both transpired through plant leaves and evaporated from the soil surface. Actual soil water depletion is measured by moisture sensors down to 6 feet. Irrigation timing is based on both the predicted rates of crop water use and the soil water depletion measurements.

A “high boy” platform with digital cameras, infrared



irrigated while that on the right received only half as much

TOM TROUT (D2260-1)

detectors, and an infrared thermometer is driven through the plots weekly to monitor crop growth and leaf temperature, an indicator of crop water deprivation.

So far, Trout has results for three seasons of limited-irrigation studies. For example, he found that corn yields varied from 210 bushels an acre for full irrigation down to 130 bushels for the lowest irrigation level.

## Sell One Bushel of Corn or 2,500 Gallons of Water?

Trout found that the corn plants on 1 acre of land need to consume about 600,000 gallons of water—from irrigation and rain—to produce 200 bushels of corn. “After an initial amount of water to get the corn growing, the consumption rate stayed about the same through all six levels of irrigation—about 2,500 gallons per bushel of corn,” he says.

This flies in the face of the traditional belief that crops use water less efficiently as they get more of it. But in this experiment, Trout found that while that is true in terms of drops of irrigation water applied, it is not necessarily true in terms of drops of water consumed. In other words, there is no reduction in the amount of water corn takes in to produce each bushel, despite the reduction in the amount of irrigation water applied. This may make limited irrigation less attractive financially, at least for corn in this region.

“Corn farmers might do better financially to use full irrigation on a portion of their irrigated acres, rather than limited irrigation spread over all the acres,” Trout says. “They could then sell or lease the water rights on the nonirrigated acres. Another option would be to grow a different crop that requires less water, if the economics of limited irrigation work for that crop.”

These results are preliminary and may vary with changes in the timing of water applications, type of crop, or variety of corn.

The scientists plan to use computer models to test the results beyond the climate and soils on the research farm to a wide range of conditions throughout the central High Plains.

Irrigated agriculture in the central High Plains will continue and will help meet the food needs of a growing world population, but it must be carefully managed to maximize crop yields for each drop of water that passes through a plant.—By **Don Comis, ARS.**

*This research is part of Water Availability and Watershed Management, an ARS national program (#211) described at [www.nps.ars.usda.gov](http://www.nps.ars.usda.gov).*

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Agricultural engineer Tom Trout checks instruments on a weather station that predicts water consumption through soil surface evaporation and transpiration through plant leaves.

PEGGY GREB (D2098-1)

# Where the Wild Bees Are



LEAH LEWIS (D2233-1)

Hunt's bumble bee, *Bombus huntii*, a native to the intermountain west.



STEPHEN AUSMUS (D2212-7)

## The search for more pollinators

The western bumble bee, *Bombus occidentalis*.

**B**umble bees, like their well-known honey bee cousins, are important pollinators of agricultural crops and native plants. But bumble bees are mainly used to pollinate greenhouse plants like peppers and tomatoes rather than field crops.

Anecdotal evidence over the past 10-15 years has suggested that several bumble bee species are disappearing and that their range is constricting. Coupled with the current declines in honey bee populations, the decline in some bumble bee species sparked urgent action for finding suitable pollinators so that we can continue to enjoy the various fruits, vegetables, and flora that play important roles—nutritionally and aesthetically—in our daily lives.

Entomologist James Strange is doing his part to help find new pollinators that can rise to the task of working in the

greenhouse and the field. From his post in the Pollinating Insects Biology, Management, and Systematics Research Unit in Logan, Utah, Strange is studying various bumble bee species that could serve as the next generation of proficient commercial pollinators.

### Searching for the Western Bumble Bee

Strange has traveled all over the country in search of bees. Over the past few years, one bumble bee in particular, the western bumble bee, *Bombus occidentalis*, has caught his eye.

The range of this native species is one that Strange and other scientists believe has declined over the years. “Although most of the preliminary observations of species decline were anecdotal, recent studies in North America have documented both decreased bumble bee abundance and

absence of some species where they were once fairly abundant,” says Strange.

Just 20 years ago, *B. occidentalis* was one of the most common bees found in western North America. Its native range includes Alaska and the Aleutian Archipelago, south to the mountains in Arizona and New Mexico. The bee could also be found from the Pacific coastline of the United States and Canada east to the plains of central Canada and central Colorado. It has also been detected in the Big Horn Mountains of Wyoming and the isolated Black Hills of South Dakota.

Companies used to rear colonies of *B. occidentalis* for commercial greenhouse pollination. The bee is known as a generalist forager, which means it doesn't have a narrow preference for which type of plants it pollinates, making it ideal for

use on a wide variety of crops. But in the late 1990s, commercial colonies were affected by disease, causing companies to stop rearing the bees.

Strange and colleague Jonathan Koch, a graduate student at Utah State University, sought to examine the extent of *B. occidentalis*'s disappearance. But to do that, they first had to comb through numerous historical records in museum collections to gather information about the insect's native range and abundance so they could accurately compare it to the bee's current range and abundance. For this task, they enlisted the help of fellow ARS entomologist Terry Griswold, curator of the U.S. National Pollinating Insects Collection.

After combining their data with information collected by a team of University of Illinois scientists led by entomologist Sydney Cameron, the researchers now have a large database containing information on more than 80,000 *Bombus* specimens representing 10 species throughout the country, including *B. occidentalis*. Coupled with geographic information system (GIS) modeling technology, Strange and colleagues were able to construct historical and current range maps of several bumble bee species. Details of the mapping process are described in *Proceedings of the National Academy of Sciences*.

"We also sampled for 3 years specific sites where, according to our data, *B. occidentalis* is supposed to occur," says Strange. "We have good evidence that the range and abundance of this species have declined, especially when compared to two other bee species found in that same native range."

Genetic testing conducted by Strange and University of Illinois colleagues also confirmed that *B. occidentalis* is

not as genetically diverse as other bee species, and it has a higher prevalence of pathogens than that found in species with stable populations.

Lee Solter, the research team's pathologist at the University of Illinois, is currently conducting more pathogen tests on colonies of *B. occidentalis* that Strange is rearing in his lab. With her help, we may finally find the culprit behind the bee's decline.

#### A Potential New Pollinator

Since the collapse of commercially reared *B. occidentalis* in the 1990s, companies and growers have turned to *Bombus impatiens*, a generalist pollinator native to the midwestern and eastern United States and Canada. Companies are shipping colonies of *B. impatiens* from rearing facilities in the midwestern United States for use in greenhouses in the western part of the country.

But scientists are becoming increasingly concerned about using a bee outside of its native range, because doing so could have drastic environmental implications. For example, if *B. impatiens* escapes from the greenhouse and forms wild colonies in the western United States, it could compete with native bees there for food and resources. Additionally, nonnative *B. impatiens* could expose native bumble

bee species to pathogens to which they are not adapted. This is a serious concern, as evidenced by some western states restricting the import and use of nonnative bees.

Strange has been researching a possible solution: a pretty, orange-striped bee named *Bombus huntii*. The insect is another generalist pollinator that could be used in greenhouse pollination.

"The bee forms robust colonies," says Strange. "And because it's native to the western half of the country, it could be a suitable replacement for *B. occidentalis*, accommodating both growers' and states' requirements."

Strange is determining how to best rear *B. huntii* in a laboratory environment, a vital component in commercializing this bee species. With continued research, this bee may soon be making its debut in greenhouses throughout the western United States.—By **Stephanie Yao**, formerly with ARS.

*This research is part of Crop Production, an ARS national program (#305) described at [www.nps.ars.usda.gov](http://www.nps.ars.usda.gov).*

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LEAH LEWIS (D2232-1)



ARS entomologists James Strange (left) and Terry Griswold (right) and Utah State University graduate student Jonathan Koch inspect a nest of the western bumble bee, which is being reared as part of an effort to understand the effects of the pathogen *Nosema bombi* on colony development.

# A Tale of Freeze-Drying Fish

Health-conscious consumers are constantly looking for ways to improve their diets, and one way is to eat more fish. For those who don't have time to bake, broil, or steam fish, a crispy, freeze-dried snack made from salmon may be ideal.

With a little digging, limited supplies of freeze-dried clams, crabs, shrimp, and lobster good for snacks and other meals can be found in some food markets, but even smaller amounts of freeze-dried fish are available. A new method that produces freeze-dried salmon cubes could be used to make tasty snacks, salad toppings, and ready-to-eat soups.

Scientists at the University of Alaska-Fairbanks (UAF), in collaboration with the Agricultural Research Service's Subarctic Agricultural Research Unit (SARU), which has offices at the Fishery Industrial Technology Center in Kodiak, Alaska, developed a freeze-dry process that produces acceptable food characteristics such as good product color, quick rehydration, and limited shrinkage in less time than the traditional freeze-dry method.

This quicker, less energy-demanding process could potentially be applied to lower value meat components, says ARS

food technologist Peter Bechtel, who is located at SARU. Salmon cubes could offer delicious, healthful alternatives for unused portions of fish muscle.

For example, Bechtel says, "sometimes when the salmon gets too close to spawning season, the roe is of high quality and value, but the muscle quality has deteriorated and often has limited uses. Therefore, it's considered a byproduct; but a freeze-dried product would be a way to use edible portions of meat and add value."

Freeze-dried salmon products also fit into the recommendations of the 2010 Dietary Guidelines for Americans. For the first time, these guidelines—released in 2011—stress the importance of increasing seafood in the diet, particularly seafood rich in omega-3 fatty acids, such as salmon.

The novel drying process consists of two stages. The first stage involves freezing small cubes of salmon and then putting them into a freeze-dryer to take out a certain percentage of moisture. During the second stage, the product is again put into a dryer to remove the rest of the moisture.

Chuck Crapo, seafood technology specialist with the UAF Marine Advisory Program, and Duy Nguyen, visiting scientist from the University of Nha Trang in Khanh

Hoa, Vietnam, used ARS freeze-dryers in Kodiak to establish drying parameters for salmon fillets. The key was to establish a process that would produce freeze-dried cubes with less than 10 percent moisture.

By manipulating temperature and time, Crapo, Nguyen, and UAF professor Alexandra Oliveira created a process that took only 9 hours—traditional processing takes 20 hours or more. About 97 percent of the moisture was removed from fillets of Alaska's most abundantly harvested Pacific salmon species—pink, sockeye, and chum.

Experiments were conducted using an average of three fillets that were frozen, partially thawed, cut into cubes, arranged into freeze-dry trays, and processed immediately. To reduce the time and remove moisture from the salmon cubes at a faster rate, the temperature during the freeze-drying process was raised from  $-40^{\circ}\text{C}$  to  $0^{\circ}\text{C}$ .

"We took about 50 to 60 percent of the time off the traditional drying process," Crapo says.

In addition to requiring a shorter processing time, cubes retained their Pacific salmon color, shrank by only 12 percent, and rehydrated quickly—a desirable trait for use in dry soups.

Bechtel is looking at nutritional quality characteristics of freeze-dried fish through analyses of amino acids and minerals. These findings will be used to identify food products that can be developed for health-conscious consumers.—By **Sandra Avant, ARS.**

*This research is part of Aquaculture (#106) and Quality and Utilization of Agricultural Products (#306), two ARS national programs described at [www.nps.ars.usda.gov](http://www.nps.ars.usda.gov).*

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ARS and collaborators have developed a new freeze-drying process for salmon. Here freeze-dried salmon cubes are added to a fresh garden salad.

PEGGY GREB (D2271-3)



# Predicting Switchgrass Ethanol Yields

The truck pulls up to the ethanol biorefinery with a load of switchgrass. A technician greets the driver, inspects the load, and samples several switchgrass bales on the truck. The samples are brought to the nearby office lab and quickly analyzed for biomass composition and the maximum and expected ethanol yields per ton of switchgrass. This information enables the biorefinery to pay switchgrass producers for both the tonnage and the quality of their crop. A biorefinery could even reject a subpar shipment of switchgrass before the truck is ever unloaded.

That future has been made possible by a team of Agricultural Research Service scientists, including Ken Vogel, Rob Mitchell, and Steve Masterson at Lincoln, Nebraska; Hans Jung at St. Paul, Minnesota; Bruce Dien at Peoria, Illinois; and Michael Casler at Madison, Wisconsin. They developed the use of near-infrared sensing (NIRS) to measure 20 components in switchgrass biomass that determine its potential value to biorefiners. These components include cell wall sugars, soluble sugars, and lignin.

With this information, 13 traits can be determined, such as the total theoretical or maximum ethanol yield as well as the

efficiency of the conversion from sugars to ethanol. Measuring cell wall sugars can also be used to calculate the maximum yield for fermentation to other biofuels and chemicals.

This is the first use of NIRS to predict maximum and actual ethanol yields of grasses from a basic conversion process. This capability already exists for corn grain using NIRS. “Corn is the easiest crop to grade for ethanol yield,” says Vogel, who is in the ARS Grain, Forage and Bioenergy Research Unit in Lincoln.

Predictions of actual ethanol yields were based on hexoses, or six-carbon sugars, present within the plant cell wall and as soluble sugars. Since additional ethanol could be produced from pentoses, or five-carbon sugars, as conversion technology improves, the NIRS method can be used to estimate what the total potential yield of ethanol or other biofuels would be if all sugars in the plant were converted.

The scientists used NIRS to test switchgrass varieties and experimental lines adapted to the Midwest and found significant differences for actual and potential ethanol yield per ton and per acre. The lowland variety Kanlow had the highest crop yield but the lowest

ethanol conversion efficiency, resulting in the lowest predicted ethanol yields per ton—but the largest ethanol yields per acre. An experimental upland strain, NE 2229, had the highest predicted ethanol yields per ton, with the third highest crop yield.

Vogel says, “The importance of this study is that we’ve shown it’s feasible to use NIRS to estimate ethanol yields of switchgrass at about \$5 a sample, instead of \$300 to \$2,000 a sample using conventional analytical methods. The low price makes the method available for use in research by breeders, agronomists, and biorefineries.”

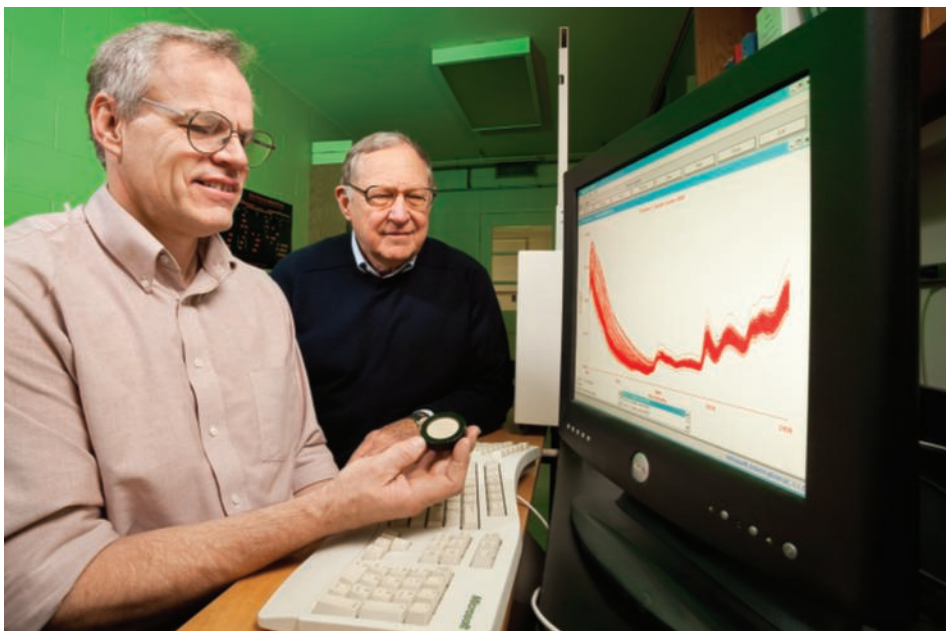
The calibrations developed in this study—and improved future versions—can be used in all aspects of plant research, including basic genetics, harvest, and storage for a variety of perennial grasses, including those used for forage as well as those used for ethanol production. Casler says, “The NIRS equations are already being used for developing new cultivars in the ARS breeding programs in Nebraska and Wisconsin.”

“This method can also be used to develop management practices that can result in improved ethanol yields per acre,” Vogel says.—By **Don Comis, ARS.**

*This research supports the USDA priority of developing new sources of bioenergy and is part of Pasture, Forage, and Rangeland Systems (#215) and Bioenergy (#213), two ARS national programs described at [www.nps.ars.usda.gov](http://www.nps.ars.usda.gov).*

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Biological technician Steven Masterson (left) and geneticist Ken Vogel examine the near-infrared reflectance spectral profiles of switchgrass samples collected from switchgrass bales. The near-infrared sensing calibration equations developed for determining switchgrass composition and ethanol conversion are being used to determine the effects of switchgrass bale storage conditions on ethanol conversion. Masterson is holding a sample ring with the ground switchgrass sample.



STEPHEN AUSMUS (D2231-1)



# Battling the Nematodes That Threaten Potatoes

LYNN CARTA (D2266-1)

## The potato cyst nematode (PCN) is a formidable adversary.

It can survive in soils for more than 20 years at depths of up to 40 inches and cause crop losses of up to 70 percent. Two species of PCN exist, and both are evolving threats. The pale cyst nematode, *Globodera pallida*, was discovered in Idaho in 2006, and though its presence in the United States is currently confined to only about 1,000 acres in Idaho, it remains a major threat in Europe. The golden nematode, *G. rostochiensis*, has been a problem in New York State since its discovery there in 1941. While it has been contained to nine New York counties through a coordinated pest management program, it has also been found in key potato-producing regions in Canada.

Potatoes and seed potatoes are exchanged across international boundaries, so a PCN outbreak in Canada or Europe raises concern about the potential threat not only to U.S. potatoes, but also to tomatoes and eggplant. The combined losses to potatoes, tomatoes, and eggplant from

PCN could reach an estimated \$4.8 billion each year if left unchecked, so keeping PCN from spreading in the United States remains a top priority.

## An Essential Safeguard: Testing New Potato Varieties for Nematode Resistance

Xiaohong Wang, a molecular biologist with the Agricultural Research Service's Biological Integrated Pest Management Unit at the Robert W. Holley Center for Agriculture and Health in Ithaca, New York, is battling PCN on several fronts. She has filed a patent application on a molecular diagnostic test that can identify the type of PCN infesting a grower's field from a tiny amount of nematode material. She and her colleagues also test new potato clones and varieties for resistance to golden nematode, and in collaboration with USDA's Animal and Plant Health Inspection Service (APHIS), she helps monitor New York potato fields for particularly damaging races of golden nematode.

Differential interference contrast (DIC) light microscope image of an infective juvenile of the pale cyst nematode, *Globodera pallida*, about 0.2 mm long.

Potato cyst nematodes are a quarantine pathogen, and researchers must obtain permission from APHIS before they can work with them. Such restrictions mean that Wang's lab is the only facility in the United States that works with live golden and pale cyst nematodes. There, she and her colleagues use the nematodes to test potato clones developed by partners at Cornell University as part of its potato breeding program. They also conduct small-scale testing of clones from other breeding programs. To test clones, they grow them in a secure greenhouse, inoculate them with the nematodes, and count nematode cysts on the roots of the potato plant.

Wang also identifies specific races of golden nematode from cyst samples collected by APHIS as part of its effort to help monitor New York potato fields and prevent the spread of golden nematode. The identification work is important because there are two races of golden nematode present in the United States, Ro1 and Ro2. While growers can plant potato varieties that resist the common Ro1 race, there are no commercially available varieties that resist Ro2. When the Ro1 race is found in a field and a potato variety resistant to Ro1 is grown there, the nematode effectively cannot reproduce on the potato plants. When the Ro2 race is detected, growers have to switch to a nonhost crop, such as corn or soybeans.

## A Diagnostic Tool To Tell Them Apart

Since breeders have yet to develop potatoes that resist pale cyst nematodes, it's



DEMOSTHENIS CHRONIS (D2235-1)

Molecular biologist Xiaohong Wang and support specialist David Thurston check golden nematode cysts on the root system of a potato clone developed by the Cornell University potato breeding program.



Yellow- and brown-colored golden nematode (*Globodera rostochiensis*) cysts (about 1/3 to 1/2 mm in diameter) on potato roots.

XIAOHONG WANG (D814-1)

important to be able to distinguish the pale cyst from the golden nematode. “The pale cyst nematode is genetically more diverse than the golden nematode, and that makes it harder to come up with resistant germplasm in potato breeding programs,” Wang says.

Traditional methods used to distinguish between the two PCN species have relied on morphological analyses and PCR (polymerase chain reaction) assays that are time-consuming and require extensive training in nematode morphology and relatively large samples of nematode cysts.

Wang has developed a way to distinguish between the two species of PCN that uses a new genetic marker and is more sensitive and reliable. The system is capable of testing much smaller samples of cyst material, making it a thousand times more sensitive than the current PCR technologies. “If you only have one cyst, you can still use our method to confirm whether you have golden nematode or pale cyst nematode, and our method is relatively easy to perform,” Wang says.

Wang and her colleagues developed their system by cloning the parasitism gene that these nematodes use to produce

an enzyme, chorismate mutase, that plays an important role in nematode infection. The researchers then sequenced those chorismate mutase genes, compared the sequences, and identified unique regions in each sequence. They then developed a “TaqMan probe” capable of recognizing the unique regions in the nematode’s DNA. “It’s the first time that a parasitism gene has been used as a diagnostic marker for identifying plant-parasitic nematodes,” Wang says.

She has filed a patent application on the technology and described it in a paper recently published in the *European Journal of Plant Pathology*. The system is one of several new technologies developed to distinguish the golden nematode from the pale cyst nematode. But with its high degree of sensitivity and reliability, it should prove to be a valuable tool

Juvenile golden nematode, about 1/2 mm long.

for nematode regulatory and quarantine programs throughout the United States.—  
By **Dennis O’Brien, ARS.**

*This research supports the USDA priorities of ensuring food safety and promoting international food security and is part of Plant Diseases, an ARS national program (#303) described at [www.nps.ars.usda.gov](http://www.nps.ars.usda.gov).*

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LYNN CARTA (D2268-1)



# Making Bioenergy Yeasts Strong

Bales of corn stover (stalks, leaves, husks, and cobs) in a field in York, Nebraska, after harvest. Corn stover and other harvest leftovers such as wheat or rice straw may soon be more economical to ferment into cellulosic ethanol, thanks to ARS research with a promising new yeast for biorefineries.

Your favorite breads and brews may, in part, be the work of the versatile yeast *Saccharomyces cerevisiae*. In a perhaps lesser known role, this helpful yeast works in fermenters at biorefineries, converting cornstarch, for example, into ethanol.

Tomorrow, improved strains of this yeast might be employed at what are known as “advanced biorefineries.” There, the microbe would help convert rough, fibrous, harvest-time leftovers—such as cornstalks or straw—into cellulosic ethanol.

A renewable fuel, cellulosic ethanol is regarded by many as a cleaner, more environmentally friendly energy source than petroleum, which is nonrenewable.

But life inside a fermenter at an advanced biorefinery wouldn’t necessarily be easy for a yeast.

Perhaps the biggest problem stems from the fact that the agricultural leftovers have to be pretreated before they can be converted into cellulosic ethanol. That step makes it easier to break down the cellulose, hemicellulose, and lignin in plant cell walls, speeding release of simple sugars that are readily fermentable into ethanol. The problem is that the pretreatment, done with dilute acid, typically generates compounds that inhibit and stress the yeast, interfering with its ability to do its job.

“These inhibitors are one of the major obstacles to low-cost, large-scale, sustainable production of cellulosic ethanol,” says Zonglin Lewis Liu, a molecular biologist. He’s based at the Agricultural Research Service’s National Center for Agricultural Utilization Research in Peoria, Illinois.

### For Hapless Yeasts, Stress Is Serious

Liu says damage caused by two key inhibitors, furfural (short for 2-furaldehyde) and HMF (which stands for 5-hydroxymethyl-2-furaldehyde), is representative of the havoc that inhibitors can wreak on biorefinery yeasts. Inhibitors can damage yeast cell walls and membranes, disrupt yeast genetic material such as DNA and RNA, inhibit yeast growth, and interfere with the fermenting activity of the yeast’s enzymes.

The result?

Yeast performance slows.

Ethanol yields lessen.

Sometimes, the yeast dies.

Remedial steps, such as removing the inhibitors from the fermentation slurry, can reduce their effects. But these options require time and materials and thus add to the overall cost of producing cellulosic ethanol.

“Essentially, removal is economically impractical,” says Liu.

### The Quest for Stress-Tolerant Yeasts

Liu and his coresearchers have been tackling this problem since 2003. In one line of study, they have worked with dozens of strains of *S. cerevisiae* yeasts, housed at the Peoria center’s world-renowned collection of microbes. In their quest for hardier biorefinery yeasts, they have recently focused on NRRL Y-12632, an industrial *S. cerevisiae* already used for producing ethanol from cornstarch.

Using a laboratory approach known as “evolutionary engineering,” the scientists speeded up the microbe’s natural adaptation to the hostile environment created by the inhibitors. The result is NRRL Y-50049, a promising new yeast for the Peoria collection.

In tests with a 2-liter fermenter, this new strain has demonstrated an impressive ability to shrug off the debilitating effects of furfural and HMF. Further tests with a 100-liter fermenter are planned, says Liu.

The researchers are discovering more about the genes and networks of genes that are likely responsible for the notable tolerance shown by this new yeast.

Liu and colleagues Jaewoong Moon, at Peoria; Mingzhou Song, at New Mexico State University-Las Cruces; and Menggen Ma, previously an ARS research associate affiliated with the university, are curious

about these genes. Their discoveries might prove useful in enhancing other promising yeasts for tomorrow's biorefineries.

Studies by Liu and colleagues suggest that of the nearly 7,000 genes in the *S. cerevisiae* genome, more than 350 may be involved in counteracting stress. Their work has brought some of these genes, and their complex interactions with many other genes, into sharper focus.

For instance, Liu and the Las Cruces scientists compared the new, stress-tolerant NRRL Y-50049 with its parent, the comparatively stress-intolerant NRRL Y-12632.

### What Genes Help New Yeast Handle Stress?

To discover what genes the new strain expresses, or turns on, when it needs to cope with the problematical inhibitors, Liu and colleagues grew cells of both yeasts in their laboratory and exposed the cells to sublethal amounts of furfural and HMF. Using procedures known as a "microarray" and a "qRT-PCR array assay," they captured what is essentially a series of snapshots of gene responses at different points in time, from 0 through 65 hours. The time-span covered periods of yeast growth and of fermentation performed by the yeast.

In some instances, the new strain turned on certain genes that allowed it to withstand the inhibitors, while the parent strain did not activate those genes. In other instances, the inhibitors successfully repressed certain genes in both strains at early stages of the time-course experiment. However, "genes in the new strain eventually recovered from this lag phase, or downtime," says Liu. "NRRL Y-50049 adapted, survived, and produced normal yields of ethanol." In contrast, certain genes in the parent strain did not recover.

ARS molecular biologist Z. Lewis Liu holds a yeast genome microarray, or "gene chip." Microarrays help Liu and his associates determine which genes, and networks of genes, enable next-generation biorefinery yeasts to tolerate stressful conditions and to efficiently ferment sugars from straw or other biomass to make cellulosic ethanol.

### Gene Families Get Involved

Details about the most relevant interactions among key stress-tolerance genes are also emerging from the research. Genes and networks of genes that the team has pinpointed as being of major importance include many that act under the influence of *YAPI*, a master gene.

"We have shown that this gene acts as a regulator that cues interactions of many related genes," says Liu. "These regulated genes work together, enhancing production of enzymes needed for reducing furfural and HMF into less toxic compounds."

Also important: Members of the *PDR* gene family. "*PDR1* and *PDR3* regulate other genes in the *PDR* family that help the yeast to pump out the toxins' harmful byproducts," Liu explains. "Ridding itself of the byproducts helps the yeast recover and get back to work."

The genes exert their effects through various molecular pathways. Liu and co-workers have published detailed preliminary diagrams of these pathways for other scientists' scrutiny.

"This is new knowledge," Liu says. "We may be able to use it to further improve stress tolerance in NRRL Y-50049 or to develop stress tolerance in other candidate yeast strains."

Why examine so many genes and their pathways at once?

Says Liu, "It is impossible to fully explain complicated functions such as stress tolerance if you use the single-gene approach. We've shown that stress

tolerance involves multiple networks of genes and complex interactions of those genes. A single-gene approach would make it too difficult to see the big picture."

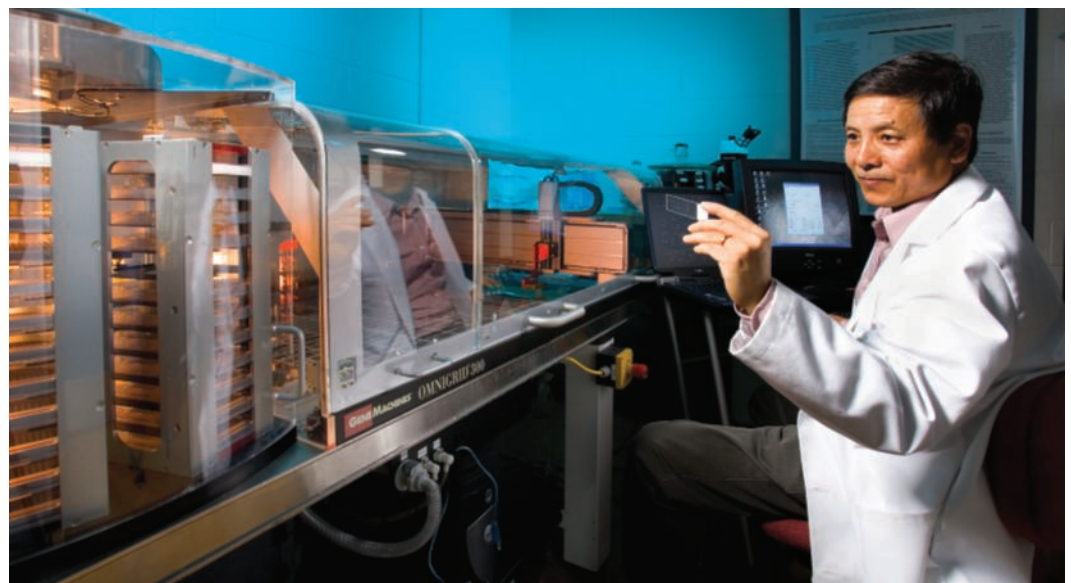
Liu and collaborators pioneered research on yeast's ability to—while in the fermenter—detoxify inhibitors and ferment sugars to make cellulosic ethanol. This microbial multi-tasking is known as "in situ detoxification."

The scientists have documented their findings in *Applied Biochemistry and Biotechnology*, *Applied Microbiology and Biotechnology*, *BMC Genomics*, *BMC Microbiology*, *Gene*, *Industrial Microbiology and Biotechnology*, and *Molecular Genetics and Genomics*.

Development of superior, inhibitor-tolerant yeasts for efficient, economical, and environmentally sound production of cellulosic ethanol "is a hot area of research not only for us, but for academic and industry researchers around the world," says Liu. "We think our research can help everyone reach this shared goal."—By **Marcia Wood, ARS.**

*This research supports the USDA priority of developing alternative sources of energy and is part of Bioenergy, an ARS national program (#213) described at [www.nps.ars.usda.gov](http://www.nps.ars.usda.gov).*

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SCOTT BAUER (D2234-1)

**S**outhern Idaho is known for its prodigious yields of potatoes and sugar beets, but the state's growing dairy industry is also supporting the production of a different crop—corn. So David Tarkalson and David Bjorneberg, who work at the ARS Northwest Irrigation and Soils Research Laboratory in Kimberly, Idaho, conducted a 2-year study to see whether farmers who use conventional methods of tillage and fertilizer application could increase corn yields by banding fertilizer with strip tillage instead.

Farmers using strip tillage make just one pass through fields before planting. When they use strip tillage, they remove residue from a 6- to 12-inch-wide strip and till the soil 6 to 8 inches deep with a knifelike shank, which can also inject fertilizer.

Tarkalson and Bjorneberg studied corn yields from one field in 2007 and one in 2009, both of which had been planted with alfalfa the year before. (Research was not

conducted in 2008 because a field was not available for study.) In both years, one of the study areas was located at the top of an eroded slope, and the other was located at the bottom of a slope, where soils eroded from higher elevations had accumulated.

The scientists used either conventional tillage or strip tillage and applied nitrogen and phosphorus to the fields either by broadcast application or by subsurface injection with the strip-till shank.

The scientists found that using strip tillage to place fertilizers 6 to 8 inches directly below the seed increased corn grain yields on the nutrient-depleted eroded area by 12 percent in 2007 and 26 percent in 2009, compared to the other fertilizer-placement treatments. This translated into yield increases of 11 to 26 bushels per acre.

Though band placement of nitrogen and phosphorus on higher slopes was especially beneficial to boosting yields, at lower elevations the technique resulted in the same yields as broadcast fertilizer applications. These findings suggest that Pacific Northwest corn growers who apply fertilizer in bands with strip tillage could help lower production costs by reducing tillage while maintaining or increasing yields.—By **Ann Perry, ARS.**

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## In Idaho, New Tillage For a New Crop

**C**onventional and organic farmers know that plastic or fabric ground covers can help suppress weeds and retain soil moisture. But using these ground covers as a chemical-free weed control can be complicated for organic farmers who need to till composted manure into their crop fields after planting.

Agricultural Research Service soil scientist Larry Zibilske, who works at the Integrated Farming and Natural Resources Research Unit in Weslaco, Texas, set out to see how these ground covers limit water penetration and affect carbon and nutrient levels in soils. He conducted a soil chamber study using two types of commercial ground covers: One was a needle-punched, double-layer fabric, and the other was a tightly woven material made of flat polypropylene strands. Two types of compost—poultry litter pellets or a compost mix of cattle manure and other organic materials—were used in the research.

Zibilske monitored the movement of nutrients from the two types of composted materials through the two types of ground covers for 30 days. Water was able to pass freely through the fabric cover, but the polypropylene cover limited the movement of water for the first 2 weeks. However, water was able to pass through the polypropylene cover much more easily by the end of the study, perhaps because the cover was becoming coated with organic molecules from the compost.

Levels of beta-glucosidase are sometimes used as a soil quality index to assess how the influx of soluble carbon affects soil microbial activity. Zibilske found that beta-glucosidase levels were essentially the same in soils protected by fabric covers, soils protected by polypropylene covers, and control soil samples without a ground cover. This similarity suggests that these ground covers did not significantly alter or limit biological activities in the soil.

But Zibilske did note links between fabric covers and reduced soil levels of carbon and nutrients. For instance, soil covered by fabric contained only 84 percent of the carbon that the control sample contained, and the soil protected by the polypropylene material contained only 80 percent as much of the carbon as the control sample. Soil samples from the covered columns also had somewhat lower nitrogen and phosphorus levels than the controls.

These results, which were published in 2010 in the *International Journal of Fruit Science*, show that some organic farmers who need to periodically amend their soils with composts after planting can still control weeds—and costs—by using fabric ground covers.—By **Ann Perry, ARS.**

*Larry Zibilske is in the USDA-ARS Integrated Farming and Natural Resources Research Unit, 2413 E. Highway 83, Weslaco, TX 78596-8344; (956) 969-4832, [larry.zibilske@ars.usda.gov](mailto:larry.zibilske@ars.usda.gov).\**

## Good News About Ground Covers for Organic Gardeners

The Agricultural Research Service has about 100 labs all over the country.

## Locations Featured in This Magazine Issue



### **Northwest Irrigation and Soils Research Laboratory, Kimberly, Idaho**

1 research unit ■ 39 employees

### **Logan, Utah**

3 research units ■ 100 employees

### **Fort Collins, Colorado**

5 research units ■ 143 employees

### **Fairbanks, Alaska**

1 research unit ■ 22 employees

### **Kika de la Garza Subtropical Agricultural Research Center, Weslaco, Texas**

4 research units ■ 113 employees

### **Lincoln, Nebraska**

2 research units ■ 81 employees

### **St. Paul, Minnesota**

3 research units ■ 82 employees

### **Madison, Wisconsin**

5 research units ■ 140 employees

### **National Center for Agricultural Utilization Research, Peoria, Illinois**

7 research units ■ 226 employees

### **Robert W. Holley Center for Agriculture and Health, Ithaca, New York**

3 research units ■ 62 employees

### **Eastern Regional Research Center, Wyndmoor, Pennsylvania**

6 research units ■ 205 employees

### **Athens, Georgia**

9 research units ■ 210 employees

### **U.S. Horticultural Research Laboratory, Fort Pierce, Florida**

3 research units ■ 133 employees



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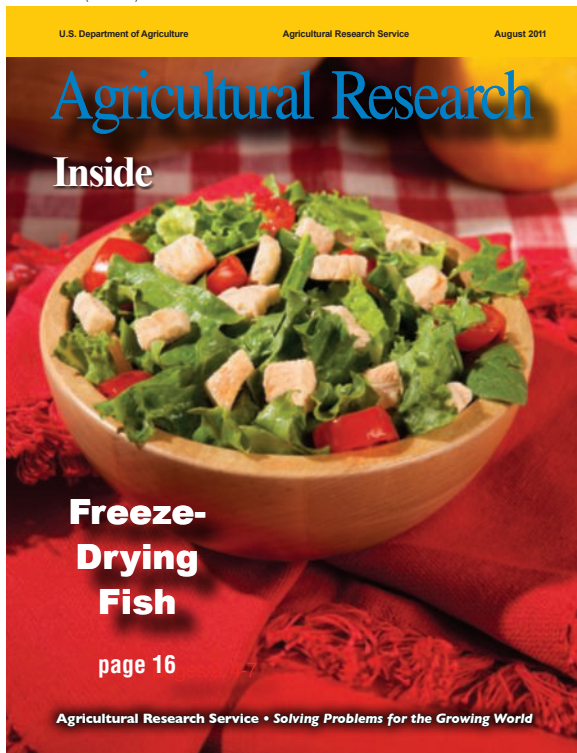
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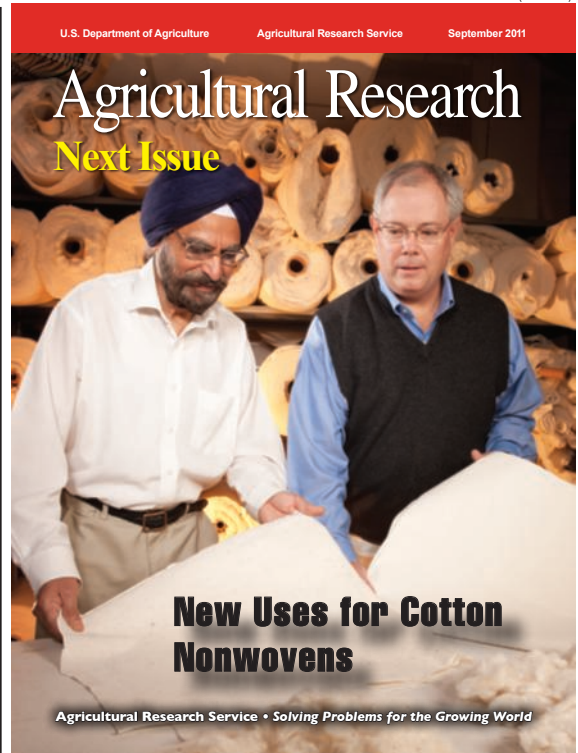


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