United States
Department
of Agriculture

Agricultural
Research
Service

Midwest Area Research Highlights 2007–2009

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Foreword

In the following pages you will find articles about the recent scientific achievements of the Agricultural Research Service (ARS) in its Midwest Area. You may not be familiar with us, so let us take this opportunity to introduce you to ARS, the chief scientific research agency of the U.S. Department of Agriculture (USDA).

Congress first authorized federally supported agricultural research in the Organic Act of 1862, establishing what is now USDA. That statute directed the Commissioner of Agriculture "to acquire and preserve in his Department all information he can obtain by means of books and correspondence, and by practical and scientific experiments." In 1953, ARS formally came into existence. Today, the scope of USDA's agricultural research programs extends far beyond Congress's vision of 1862 and the vision for ARS in 1953. Agricultural research now has a direct impact on nearly all aspects of modern life.

ARS scientists within the Midwest Area conduct research at 12 locations across Illinois, Indiana, Iowa, Michigan, Minnesota, Missouri, Ohio, and Wisconsin. Working together with numerous cooperators across the country, our 1,300 employees, including 340 scientists, develop solutions to agricultural problems of high priority to the Midwest and the Nation. Our research supports ARS's mission to conduct research to develop and transfer solutions to agricultural problems of high national priority and provide information access and dissemination to:

- ensure high-quality, safe food and other agricultural products;
- assess the nutritional needs of Americans;
- sustain a competitive agricultural economy; and
- enhance the natural resource base and the environment, and provide economic opportunities for rural citizens, communities, and society as a whole.

Midwest Area scientists are leaders in all facets of ARS science programs. Their comprehensive research portfolio covers plant, animal, environmental sciences, and agricultural utilization research; and has impact from the field to the dinner table, from commodity production to product safety, from food to biofuels, and from DNA to the watershed. Our ultimate goal is to use the best science to develop new tools and products that are transferred to users, thus enabling scientists, producers, consumers, and policymakers to make informed decisions, reduce risks, and solve problems.

To achieve our mission as efficiently and effectively as possible, we partner with other Federal agencies, some of the largest and most dynamic public universities in the country, stakeholder groups, and State agencies, as well as other ARS laboratories across the Nation.

The research described in this book represents only a few of the ARS research programs dedicated to maintaining and enhancing the economic strength of U.S. agriculture, while improving the quality of life for every American. In this volume you will find some facts about the Midwest Area, a list of the research units, where they are located, and the people you can contact for further information. We are delighted that you are reviewing this volume and hope that you will find the articles useful and interesting. Please let us know if we can be of service to you.

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Contents

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February 2010

Cover: To increase the genetic diversity of U.S. corn, the Germplasm Enhancement for Maize (GEM) project seeks to combine exotic germplasm, such as this unusually colored and shaped maize from Latin America, with domestic corn lines. Photo by Keith Weller. (K7743-13)

ARS-Developed Riparian Zones Reduce Atrazine Contamination in Water

Native grass buffers (including switchgrass and gammagrass) were found to be most effective in reducing atrazine, a widely used herbicide, in runoff.

CHUNG-HO LIN (D1334-1)

Grass filter strips placed in riparian zones not only help curb soil erosion, but they can also help reduce transport and enhance degradation of a popular herbicide, Agricultural Research Service scientists report.

The herbicide atrazine has been used extensively to suppress weeds in corn production for decades. Because it's now commonly applied to soil without incorporation, it's especially prone to losses in surface runoff. In 2007, an estimated 36.3 million kilograms of atrazine were applied to more than 66 percent of all U.S. corn acreage.

Recently, contamination of surface water by atrazine and its breakdown components has raised public-health and ecological concerns.

Eastern gammagrass showed the highest capacity for promoting atrazine

Riparian zones are transitional areas of land between upland areas (for example, crop fields) and water bodies. Grasses and other vegetation in these zones help reduce pollution in streams and lakes. Previous field research has shown that vegetative strips reduce transport of both dissolved and sediment-bound atrazine in surface runoff.

Bob Lerch, a soil scientist in the ARS Cropping Systems and Water Quality Research Unit in Columbia, Missouri, is working with unit colleagues and with Chung-Ho Lin, a University of Missouri assistant professor for forestry, to study the effects of different grass species on herbicide transport and degradation in field and growth-chamber studies.

The study plots are located at the University of Missouri's Bradford Research and Extension Center. They were originally established in 2000, and the unit took over their maintenance and operation in 2004.

In the growth chamber, the grasses studied were orchardgrass, smooth bromegrass, tall fescue, Illinois bundleflower, ryegrass, switchgrass, and eastern gammagrass. A control treatment with no plants was also included. Plants were allowed to grow for 3 months, to maturity, in a mixture of 60 percent sand and 40 percent Mexico silt loam soil. The rhizosphere soil—that which surrounds roots and is influenced by them—was then separated from the plants and roots. Atrazine was then added to the rhizosphere soil and incubated in the dark for 100 days at 77˚F. Atrazine degradation and mineralization—conversion of atrazine to carbon dioxide—were measured.

Among the plant species, eastern gammagrass showed the highest capacity for promoting atrazine degradation. More than 90 percent of applied atrazine was degraded to less-toxic forms, compared to 24 percent in the control. Rhizosphere soil of orchardgrass, smooth bromegrass, and switchgrass also enhanced atrazine degradation.

The studies have shown that grass buffers reduce herbicide transport to shallow groundwater and in runoff. They do so by trapping sediment and increasing infiltration of water into the soil. The grasses have also significantly enhanced atrazine degradation.

In addition, soil enzymes that have been commonly used for assessing soil quality and microbial activity were shown to be good predictors of atrazine degradation potential in the rhizosphere.

Next, Lerch and Lin plan to introduce a microbe known to completely degrade atrazine. Key challenges with this approach are the microbe's ability to survive and the stability of its atrazine-degrading genes in the rhizosphere.

"With our new methods, we can track the number of copies of the gene in soil," said Lerch. "This will tell us how well the genes persisted and whether or not enhanced atrazine degradation is still likely occurring."—By **Alfredo Flores,** ARS.

This research is part of Soil Resource Management, an ARS national program (#202) described on the World Wide Web at www.nps.ars.usda.gov.

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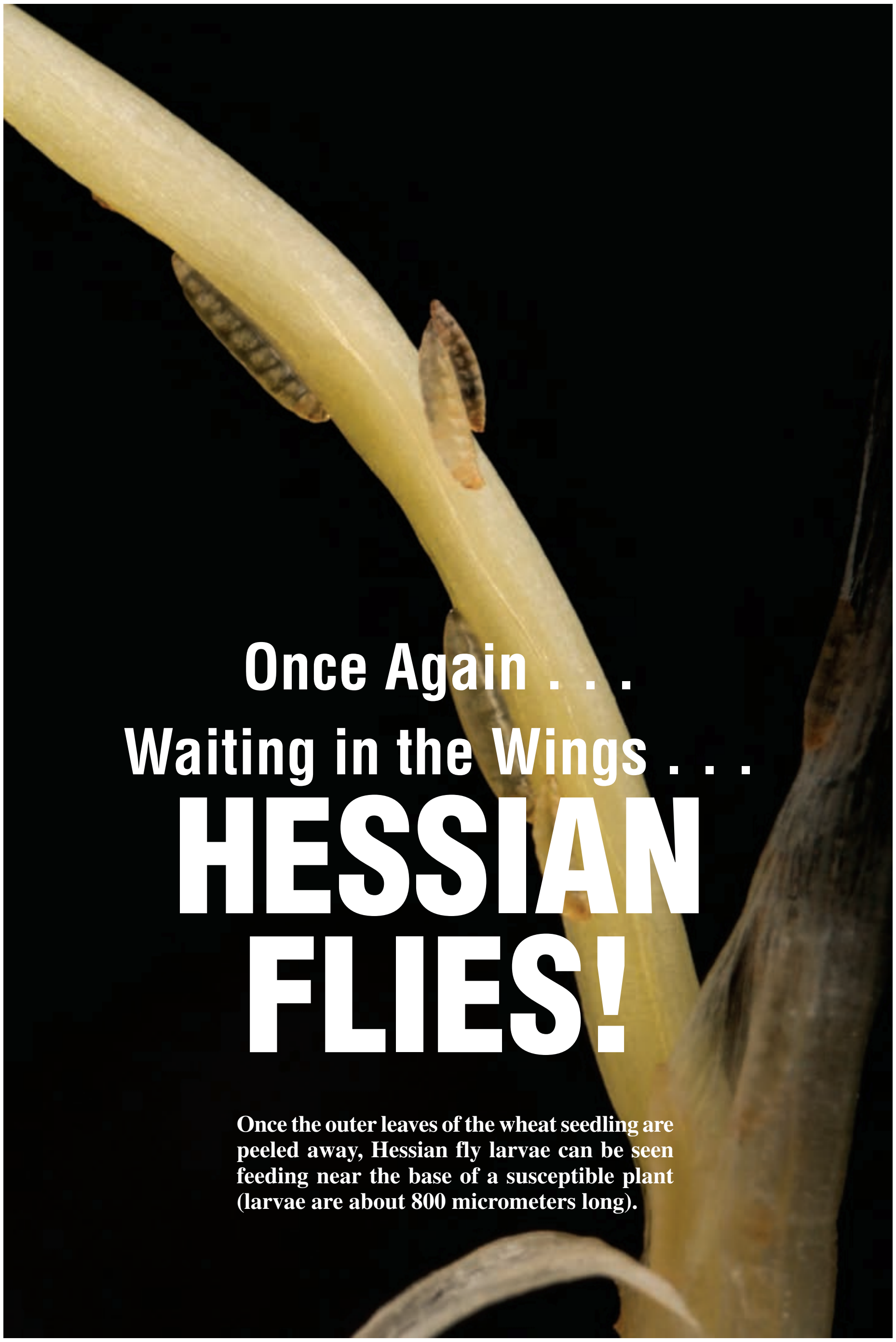
PEGGY GREB (D670-1)



Once Again . . . Waiting in the Wings . . . HESSIAN FLIES!

Once the outer leaves of the wheat seedling are peeled away, Hessian fly larvae can be seen feeding near the base of a susceptible plant (larvae are about 800 micrometers long).

ARS has done such a good job of combating the Hessian fly pest of wheat that many people think the problem is history.

Not so, says Brandon J. Schemerhorn, an ARS entomologist in the Crop Production and Pest Control Research Unit at West Lafayette, Indiana. That's where historic Hessian fly research was begun in the 1920s.

"We have been successful so far, but we also have to keep researching to stay one step ahead of it," says Schemerhorn. "We have a lot more tools to fight it today, but so does the fly."

Every year, wheat farmers across the nation send samples of fly-infested wheat to Schemerhorn and her colleagues—usually in late December, when winter wheat is maturing. In 2005, they received about a dozen samples.

In the Southeast, where infestations are more common, there have been unusually severe outbreaks in recent years. In 2006, the West Lafayette area saw its first Hessian fly infestation in more than a decade. Over the last 2 years, the flies showed up in Missouri for the first time ever, indicating their westward expansion.

"By 2000, they'd adapted enough to overcome the four primary wheat resistance genes," says Schemerhorn. "It took them just 14 years to overcome that defense. Now we use those four genes as a standard to judge the virulence of Hessian flies as they grow ever stronger."

Where have they been hiding between appearances? Schemerhorn says there is evidence that tall fescue grass is providing safe harbor for Hessian flies until they can find wheat fields.

Schemerhorn's collaborators include fellow ARS entomologist Rich Shukle; ARS molecular biologist Christie Williams; entomologist Jeff Stuart and wheat breeder Herb Ohm, both of Purdue University; and ARS entomologist Ming-Shun Chen at the ARS Grain Marketing and Production Research Center (GMPRC) in Manhattan, Kansas. The Manhattan group mainly covers U.S. areas growing hard wheat, while the West Lafayette unit focuses mostly on soft red winter wheat. Both labs send out Hessian fly-resistant wheat germplasm to wheat breeders.

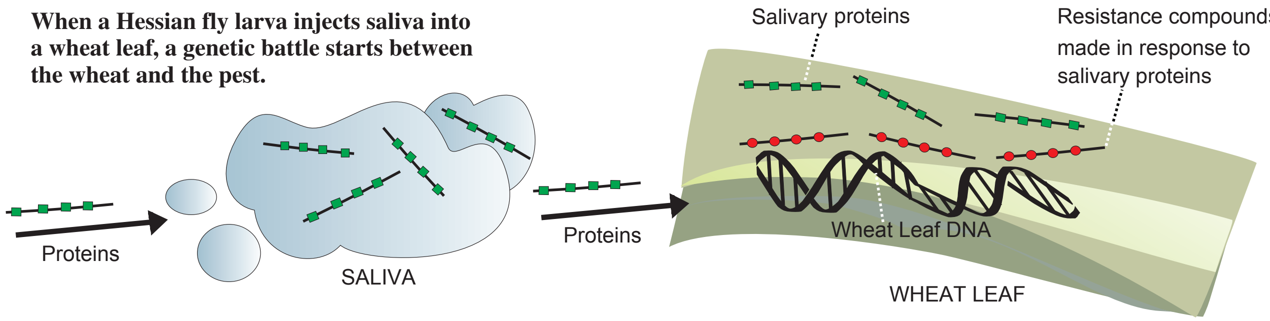
Monitoring Genetic "Conversations"

Williams, Shukle, and colleagues have assembled a custom biochip, or microarray, containing 2,000 genes expressed by the Hessian fly larva and 1,000 genes expressed by the wheat plant when the insect first pokes at it. These 1,000 genes are involved in wheat's resistance to Hessian flies. The West Lafayette researchers also have a commercial biochip to assay the expression of 60,000 wheat genes.

"Our biochip gives us a genetic record of the fly and wheat 'talking' to each

Battle of the Genes

When a Hessian fly larva injects saliva into a wheat leaf, a genetic battle starts between the wheat and the pest.



other," says Williams. She and colleagues have found that the Hessian fly-wheat interaction is very unusual for pest-host relationships. Their modern genetics work has proven what was thought for decades: The fly doesn't chew on wheat leaves. Instead it injects something into the plant that causes certain wheat genes to turn on and others to turn off.

Stuart focuses on virulence genes in Hessian fly larvae that trigger a wheat plant's resistance mechanisms when recognized. If the fly larvae win, the plant turns on a gene—not found in any other plant—that makes its leaves release a liquid the larvae sip on.

"It's bizarre," says Williams. "The plant has a gene for its own destruction."

Death by Saliva

Hessian flies don't just suck the liquid life out of wheat plants for their own nourishment. They also inject toxic substances that appear to create a more hospitable feeding environment for them and hasten their access to certain nutrients. It's an awfully cunning approach for a simple, gnat-sized fly. But considering that this insect has had thousands of years to perfect its assault on wheat, it's no surprise it's been so tough to swat.

Fortunately, that's about to change. Thanks to new genomics technology in the hands of ARS researchers at West Lafayette working with GMPRC colleagues, the fly's tactics are starting to be revealed genetically.

At the center of the researchers' study in Kansas—led by Chen—is the insect's most finely tuned weapon: its saliva. The fly's salivary glands, acting like tiny poison factories, churn out noxious compounds that cripple wheat plants.

After dissecting thousands of Hessian fly salivary glands and analyzing their DNA, Chen and his team found there's a lot riding on these salivary defenses. "We've identified 97 'superfamilies' of genes that encode secreted salivary gland proteins," says Chen. Each contains several families with about 10 members, or closely related genes. So far, the Kansas researchers have uncovered roughly 2,000 Hessian fly genes with some role in producing salivary proteins.

With so many genes to sift through and analyze, the researchers' best bet for pinpointing the most central gene players is the microarray technology. With the help of this powerful tool, they'll be able to scrutinize all the fly's salivary genes at once.

PEGGY GREB (D667-1)

ARS support scientist Sue Cambron and Herb Ohm, small grains breeder at Purdue University, examine plants that are resistant and susceptible to Hessian fly. Both plants are the same age, but the one Ohm is holding has had its growth stunted by Hessian flies.

Exploring Markered Territory

Schemerhorn says that researchers have only recently acquired enough genetic markers on the fly's genome to start searching for fly genes that correlate to the resistance genes in wheat plants. It is assumed that for every resistance gene there's a fly gene that overcomes it.

The West Lafayette researchers have 52,000 microsatellite markers for Hessian fly genes, with enough duplication and overlap to more than cover the entire Hessian fly genome. Schemerhorn and colleagues are working toward sequencing the entire genome. Tools like this allow a gene-for-gene approach that matches the fly's strategy of attack.

Schemerhorn is also doing something never done before: She's sampling all Hessian fly populations across the United States to determine the extent of their genetic variability. The more variable it is, the less likely that the limited number of genes in resistant wheat plants—like the standard complex of four resistance genes currently relied on—will work.

Speeding Breeding

It's not just fly genes that have captivated ARS researchers. They are also banking on the promise of exceptional wheat lines for fending off Hessian flies.

For example, the GMPRC greenhouses—where more than 5,000 lines of wheat are screened each year—are sprouting with possibility. Particularly exciting is a group of plants from the International Center for Agricultural Research in the Dry Areas, in Aleppo, Syria. In a mutually beneficial exchange, ARS scientists swapped wheat plant material

SCOTT BAUER (K4193-15)

A female Hessian fly, about one-eighth-inch long.

with Syrian researchers and found some samples resistant to Hessian flies in the United States.

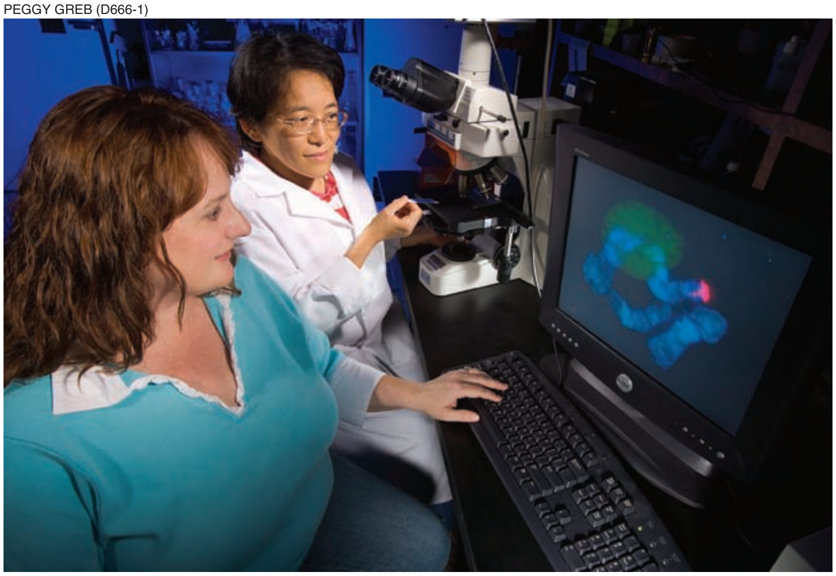
In addition to uncovering new resistance in wheat, GMPRC researchers are trying to find out exactly where the genes for that resistance are located. Of the 33 such genes described in the scientific literature, Chen's group has mapped several of the most effective ones. Many are clustered in and around the same chromosomes. For example, Chen's group has located 15 Hessian fly resistance genes on wheat chromosome 1A. "This means these genes may have come from the same ancestral gene in grass," says Chen. Williams and colleagues discovered and mapped the two newest resistance genes, *H31* and *H32*.

Knowledge of gene markers and locations enables breeders to conduct marker-assisted selection. "Using traditional methods, it takes about 6 to 10 years to develop a new wheat cultivar," says Chen. "With this technology, we can reduce that time. It also makes it much easier to combine, or pyramid, several different Hessian fly resistance genes into one cultivar." That's important, given the Hessian fly's troublingly nimble and adaptive nature.

PEGGY GREB (D664-1)

Entomologist Rich Shukle looks on while technician Alisha Johnson collects Hessian fly larvae from wheat plants for studies to discover the genes the larvae use in attacking wheat plants.

PEGGY GREB (D666-1)



Entomologist Brandon Schemerhorn (left) and technician Yan Crane discuss the location of a fluorescent-labeled marker through a confocal microscope. This marker is one of 52,000 identified on Hessian fly genes.

How Wheat Fights Back

Using microarray technology, Chen's group recently discovered that wheat's resistance genes jump-start activities in other genes that perform protective duties. Some barricade plant cells and tissues to deny the larvae food. Others fortify plant cell walls so they can't easily be penetrated by the fly's specialized mouthparts.

Williams found three genes turned on by plants in response to Hessian fly bites. They code for putting a protein called "lectin" into the oozed liquid, which causes the fly to get ulcers, lose its appetite, and starve to death.

"This slow killing of the pest is unusual for plants," says Williams. "They usually

STEPHEN BAENZIGER (D668-1)

"Using traditional methods, it takes about 6 to 10 years to develop a new wheat cultivar," says Chen. "With this technology, we can reduce that time."

ARS entomologist Ming-Shun Chen identifies resistant plants from wheat seedlings attacked by Hessian fly larvae.

RICH SHUKLE (D669-1)

Gut and salivary glands dissected from a Hessian fly larva as viewed through a microscope with dark-field illumination. The genes expressed in these tissues are critical to how the fly parasitizes wheat.

emit powerful toxins that will kill the pests rapidly."

Says Shukle, "We'll continue to try to stay one step ahead of the Hessian fly and use any down time to gain as complete an understanding of it as possible."—By **Don Comis** and **Erin Peabody,** ARS.

This research is part of Plant Genetic Resources, Genomics, and Genetic Improvement (#301) and Crop Protection and Quarantine (#304), two ARS National Programs available on the World Wide Web at www.nps.ars.usda.gov.

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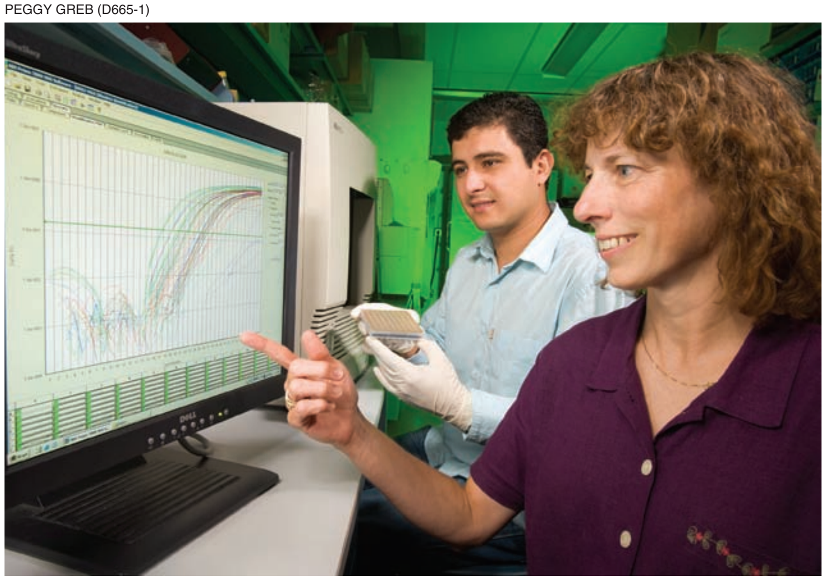
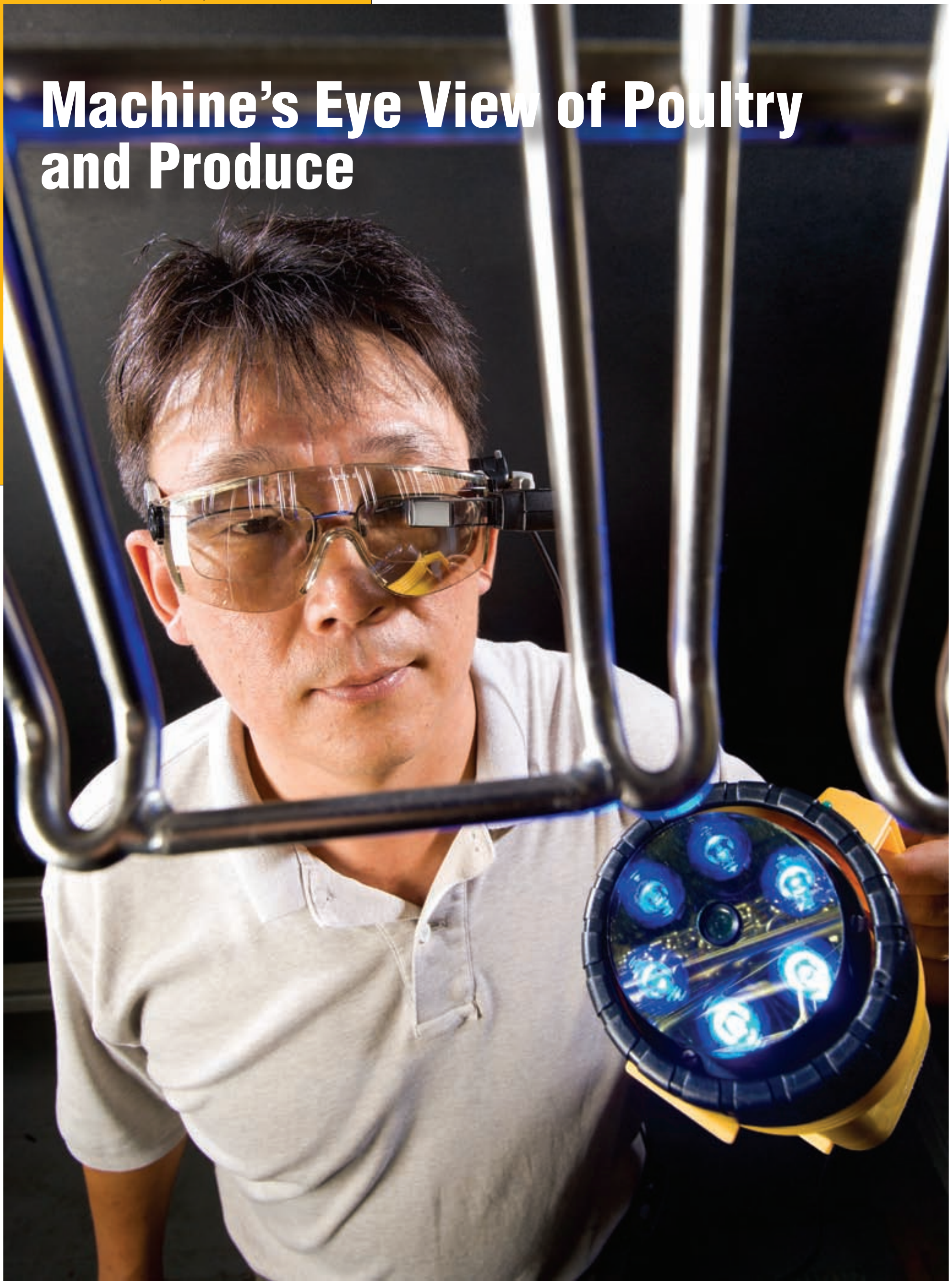
PEGGY GREB (D665-1)



Purdue University graduate student Marcelo Giovanini loads samples for analysis by quantitative real-time PCR to determine plant gene expression levels, while molecular biologist Christie Williams examines output. Gene expression data indicates which genes and defense mechanisms the plant uses in its battle against Hessian fly.

STEPHEN AUSMUS (D611-1)

Machine's Eye View of Poultry and Produce



Biophysicist Moon Kim tests a portable imaging device equipped with a head-mount display for sanitation inspection of food-processing equipment.

Take another look at the hardhat and safety glasses on the food safety inspector. Mounted on the hardhat is a small camera and a flashlight that gives off specially filtered light.

The safety glasses are actually a wearable miniature computer monitor that displays data from a miniature computer on the inspector's belt. The data tells the inspector whether there is any fecal matter on the processing equipment.

Another inspector might be looking through what at first looks like a pair of ordinary binoculars. But these binocular lenses filter special bands of light to check for disease, defects, or fecal matter on the meat, produce, or equipment.

There's also a hand-held device that shines filtered light to do a sanitation check of the processing plant. The device has a camera that sends images to another eyewear-mounted computer display. White specks on the image reveal fecal matter.

Although these gadgets sound like something dreamed up by James Bond's gadget man, cutting-edge prototypes like this actually exist in an Agricultural Research Service lab in Beltsville, Maryland.

At the Instrumentation and Sensing Laboratory (ISL), a team of scientists—led by Yud-Ren Chen and including biophysicist Moon Kim, agricultural engineer Kuanglin Chao, and visiting scientists from around the globe—design the portable inspection devices.

Chen, Chao, and visiting scientist Chun-Chieh Yang have finished work on a high-speed on-line imaging system for chicken inspection. They are turning over a prototype to industry as part of a cooperative research and development agreement with Stork-Gamco of Gainesville, Georgia, a major manufacturer of chicken-processing equipment. Chen and Kim and biomedical engineer Alan Lefcourt are working on a similar system for inspecting fruits and vegetables.

Because all these systems use optically filtered light and opto-electronics to "see," they are called "machine vision" or "optical sensing" systems. At the heart of these machine vision systems is a digital multispectral camera that can take photos at different wavelengths simultaneously and can even detect light invisible to the naked eye. The systems include the latest, fastest cameras of this type. All the systems rely on two or three wavelengths chosen to do the best job of seeing special features.

Fully funded by ARS—with additional funds from industry—Chen's team works with both industry and universities, such as the University of Kentucky and the nearby University of Maryland at College Park.

Sensing Remotely, Close Up

Machine vision using multiple images at selected wavelengths is also being developed for use in remote sensing of Earth by satellite imagery. But its potential for use in monitoring food safety and quality should be even greater, since the sensors are only inches away from the target object, and there is a wider range of applications.

The basic idea of machine vision is to supplement human inspectors with instruments that shine light on every

Agricultural engineers Yud-Ren Chen (left) and Kevin Chao make adjustments to a prototype line-scan multispectral imaging system for food safety inspection of chickens.

single fruit, vegetable, meat, or poultry product as it speeds by on the processing line faster than ever. Typical lines today can process about 360 fruits per minute or up to 180 poultry carcasses per minute, for example.

The system developed by Chen's team spots almost all biological conditions that cause inspectors to take a second look at chicken carcasses, such as signs of diseases that pose food safety risks or otherwise mar a chicken's consumer appeal.

Chen's team is now focusing its attention on apples, developing a system that could be used for other fresh produce as well. It can detect contaminants on the apple surface, such as fecal matter.

Stephen Delwiche, an agricultural engineer at the ISL, works with colleagues at the ARS Grain Marketing and Production Research Center in Manhattan, Kansas, on high-speed optical inspection of wheat and other grains. He uses near-infrared reflected light to detect proteins in wheat as well as scab and other molds.

Quality More Than Skin Deep

In East Lansing, Michigan, ARS engineer Renfu Lu, who originally worked with Chen at Beltsville, is leading a research team that uses similar optical technologies to judge taste and other quality aspects of produce. He has worked with apples, peaches, and cherries using a prototype optical detector he and colleagues devised that uses laser beams to detect fruit sweetness and firmness.

The team consists of research associates Diwan Ariana and Hyunkwon Noh, visiting assistant professor Yankun Peng, engineering technician Benjamin Bailey, and a Ph.D. graduate student, Jianwei Qin, of Michigan State University (MSU) at East Lansing.

Lu and colleagues are refining the mathematical equations and the imaging sensor used by the prototype to judge the internal quality of fruit. "We should have an improved machine vision prototype for 'tasting' apples and other fruit very soon," Lu says.

He is now expanding the machine vision inspection system to pickling cucumbers, inspecting for bruises and other defects as well as internal quality factors—such as firmness, dry matter content, and color.

"We want to select the best cucumbers—those that are firm, have a fresh green color, and aren't too soggy," says Lu.

In terms of acreage planted and crop yields, Michigan is the top state for pickling cucumbers by far, with a value of $30 million a year. And at $150 million a year, pickling cucumbers are also one of the most valuable vegetables in the United States, competing only with tomatoes and sweet corn.

Lu's research program is also fully funded by USDA, with additional funds from industry, and it partners with MSU's Biosystems and Agricultural Engineering Department to address priority needs of the produce industry in Michigan and the nation.

When commercialized, Lu's optical sensors would be used by the fruit industry to sort fruits and vegetables just after they had been picked and again on the processing line. The equipment would likely be blended into existing industry sensors that nondestructively assess superficial visual traits, including size, color, and bruising.

"Such technologies would ensure a consistent premium quality, increase consumer satisfaction, and enhance the U.S. produce industry's competitiveness and profitability," Lu says.—By **Don Comis,** ARS.

This research is part of Food Safety (#108) and Quality and Utilization of Agricultural Products (#306), two ARS National Programs described on the World Wide Web at www.nps.ars.usda.gov.

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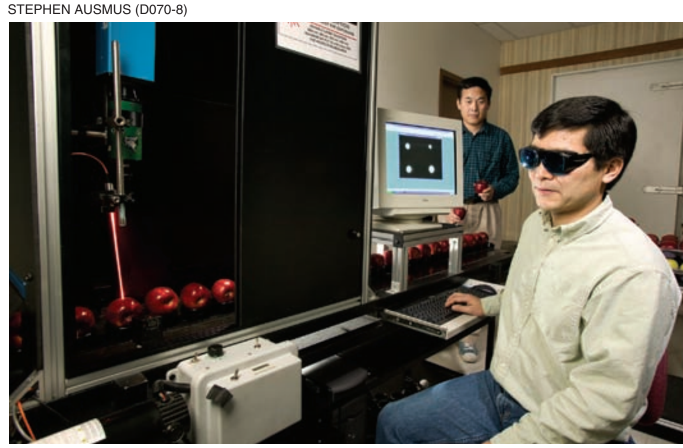
STEPHEN AUSMUS (D070-8)



Agricultural engineer Renfu Lu (front) and visiting assistant professor Yankun Peng (from Michigan State University) test a laser-based multispectral imaging prototype that detects apple firmness and sugar content.

Veggies Reinvented

Breeding vegetables with more flavor and nutrients

No matter how you slice it, Americans just aren't eating their veggies. According to a recently published USDA study, less than half of 8,000 people surveyed in 1999 and 2000 got their recommended daily servings of fruits and vegetables.

To meet the federal government's MyPyramid dietary recommendations, individuals should munch on anywhere from 2 to 6-1/2 cups of fruits and vegetables each day, depending on their age, gender, and activity levels. For instance, a 50-year-old man who exercises 30 minutes a day should consume about 3 cups of veggies and 2 cups of fruit daily.

Are you making the cut?

If not, take comfort in knowing that Agricultural Research Service (ARS) researchers are on your side. They're working to make the nation's veggie-crunching goals more reachable. And they're taking more of a carrot—and less of a stick—approach.

You've Got To Dazzle 'Em

How do you get people to eat more brussels sprouts, cauliflower, and beets? You excite their senses. Surprise them, say, with unexpected color and explosive flavor. It's a worthwhile tack to take, says Philipp Simon, plant geneticist at the Vegetable Crops Research Unit in Madison, Wisconsin. And he should know.

Simon, who heads the ARS laboratory on the University of Wisconsin campus, helped elevate the humble carrot to its current prestigious position. Thanks to work he did with colleagues more than 25 years ago, the carrot is now an even better source of dietary vitamin A.

Using classical breeding methods, they helped boost the veggie's already abundant stores of beta-carotene by 75 percent. Beta-carotene is what our bodies use to make all-important vitamin A, which is crucial for good eye health and a strong immune system. It's also responsible for the carrot's orange hue.

Simon would like to sneak in other nutrients too. That's why, several years ago, he got to wondering: Why settle for just orange? After all, 700 years ago Western Europeans were feasting on carrots that ranged in color from lemon-yellow to burgundy to purple. We can have the same variety today—and the healthful antioxidants associated with those brightly colored pigments.

In addition to breeding yellow, red, deep-orange, purple, and even white carrots, Simon aims to create a "rainbow" carrot—a multi-pigmented root that naturally contains several antioxidants, such as lycopene, lutein, and anthocyanin.

Other ARS researchers at the Madison lab are just as innovative. Inspired by nature's genetic diversity, they're dreaming up all kinds of eye-catching and palette-tempting veggies.

STEPHEN AUSMUS (D726-33)

Geneticist Philipp Simon (left) and graduate student Mehtap Yildiz take measurements of garlic to evaluate flowering differences in diverse germplasm.

Potatoes: Mix Them Up!

Given its universal appeal and popularity (it's the most consumed veggie in America), the potato is one vegetable that's not being passed over in the pantry. But that doesn't mean it couldn't stand a little improvement.

STEPHEN AUSMUS (D724-36)

Inside a cage that keeps potential pollinating insects outside, geneticist Mike Havey collects onion seeds during studies to develop new inbred lines of onions. Flies are used as pollinators inside the cage.

Just ask geneticist Shelley Jansky, who works in Simon's research unit. "Think of apples," she says. "We've got a staggering array of choices when it comes to this fruit. There's Red Delicious, Golden Delicious, Gala, Granny Smith, and so on." She thinks consumers deserve similar variety when it comes to potatoes.

Tapping genetic diversity as it exists in nature or in seed-preserving genebanks is one way to dress up the common potato. Taking advantage of environmental influences is another.

Exploring both routes, Jansky investigated how several factors, including potato variety, production site, and production method, influenced the sensory experiences of a group of volunteers eating baked potatoes.

"I was pretty surprised," says Jansky, "to see how dramatic the link was between flavor differences and the environment."

A Better Baker

Her study involved 13 potato varieties—some heralded as excellent bakers, some considered "so-so"—planted in four locations across Wisconsin. Some were grown in accordance with organic farming standards, others in a conventional manner. A panel of trained taste-testers evaluated the baked potatoes for texture, sweetness, flavor, and overall appeal.

"The panelists detected variations in texture among potatoes grown in different locations," says Jansky. "And some were able to group the organically raised potatoes together, based on taste or texture."

Jansky is repeating the sensory evaluation to see if she gets the same results. If so, she will begin the challenging task of linking desirable qualities with specific entities in the potato or its growing environment.

Even if the current sensory evaluations don't reveal consistent flavor differences among common varieties, Jansky is confident that she'll find fresh, new flavors and textures for pleasing potato enthusiasts. "In a new study, I've included some colorful potato selections with strong South American ties," she says, "and I can always add more."

Jansky's pie-in-the-sky goal? A baked potato that has all the taste of one that's "loaded," but requires little salt, pepper, or sour cream. Because potatoes have hundreds of aromatic compounds that can be subtlety plied and tweaked by expert breeders, she thinks it's altogether possible.

Tradition—With a Twist

But what if you've grown accustomed to classic white potatoes and want to keep them that way, without skimping on nutrition?

That happens to be a goal of ARS geneticist John Bamberg, who manages the agency's U.S. Potato Genebank in Sturgeon Bay, Wisconsin.

"There's a lot of exciting research going on with colored potatoes," he says. "But for many consumers, those might seem like a totally different food compared to the potatoes they're used to."

Just 12 potato varieties account for 90 percent of the spuds grown in the United States. This is in contrast to the 5,000 potato samples—representing nearly 140 species, from the southwestern United States to as far away as southern Chile—held in ARS's U.S. Potato Genebank.

Bamberg and collaborator Creighton Miller at Texas A&M University in College Station are trying to tap some of this wild character. The potatoes they're interested in have ordinary white flesh but are loaded with good-for-the-body antioxidants, including phenolic compounds such as chlorogenic and caffeic acid—and salicylic and p-coumaric acids, too.

"Like most wild species they have tubers the size of marbles," says Bamberg. "Their beneficial traits can eventually be bred into larger potatoes appropriate for a commercial crop."

Bamberg also has a passion when it comes to potatoes and potassium—a mineral that's been shown to lower blood pressure. Along with physiologist Jiwan

Palta at the University of Wisconsin's Department of Horticulture, he's screening the genebank's genetic diversity for stocks naturally higher in potassium.

"Potatoes are already high in potassium, and since we eat a lot of them, they account for a significant part of the average American's daily intake," he says. "So upping potatoes' potassium content, even just a bit, ought to be a practical way to increase the country's overall potassium intake without asking consumers to do anything different."

How Sweet It Is!

The onion has a reputation for being rather unpleasant or difficult. It's been associated with teary eyes, bad breath, flatulence, and indigestion.

That's a real shame, says Michael Havey, a geneticist in the Madison lab, because the onion is a nutritional knockout, containing three different groups of health-enhancing compounds: thiosulfinates, fructans, and flavonoids.

Thiosulfinates are what gives onions their pungent, sulfurlike taste and aroma. Despite their occasional sting and stench, these compounds are some of our bloodstream's best allies: They can bust up platelets that might otherwise form life-threatening troublesome plugs at sites of vascular damage.

Fructans, the most abundant soluble carbohydrate in onions, are a type of dietary fiber shown to reduce rates of colorectal cancers. And onions' flavonoids, such as quercetin, have proven antioxidant activities.

While this trio of impressive compounds is found in today's onions, you've got to be a fan of strong-tasting, ultra-firm varieties to access them. The onions that consumers generally find most pleasant, the so-called sweet onions, are heavily diluted with water and possess fewer health-enhancing compounds.

"And cooking or lightly sautéing onions to soften their bite can reduce thiosulfinates' effects too," says Havey. He aims to develop an onion that's mild in

STEPHEN AUSMUS (D725-6)

Geneticist Mike Havey juices onions to prepare for an analysis of flavor and health-enhancing fructans.

STEPHEN AUSMUS (D728-22)

Horticulturist Jack Staub (center) evaluates melon color with University of Wisconsin graduate research assistant Hugo Cuevas (right) and undergraduate Eric Wiesman. The color rating gives them a measure of relative carotene content and vitamin A content.

taste but still chock-full of heart-healthy nutrients. Before he can do this, though, he has to pinpoint the genetic differences between sweet onions and carbohydrate-dense ones.

Havey and colleagues are most keenly interested in fructans, which also happen to affect thiosulfinate concentrations. In other words, the more fiberlike fructans there are in an onion, the more platelet-busting thiosulfinates there'll be too.

In a recent paper published in the journal *Theoretical and Applied Genetics,* Havey and colleagues report a valuable gene that appears closely involved in fructan accumulation. They've identified its effect and mapped it, placing it on onion chromosome 8.

Havey's also discovered a gene that helps orchestrate sucrose concentrations in onion bulbs. This means it may be possible to boost their natural sweetness while increasing the carbohydrates linked to good health.

The major limiting factor for Havey? The onion's sluggish reproductive cycle. "It takes 2 whole years to get a new generation of onions after performing a cross between two plants," he says. But most consumers would probably agree that a sweet and healthful onion is definitely worth the long wait.—By **Erin Peabody,** ARS.

This research is part of Plant Genetic Resources, Genomics, and Genetic Improvement, an ARS National Program (#301) described on the World Wide Web at www.nps.ars.usda.gov.

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STEPHEN AUSMUS (D730-30)

Cucumbers: Encumbered by Lean Genes

Could the cucumber lose its cool? According to ARS geneticist Jack Staub, it just might.

He says this salad favorite suffers from an overly narrow genetic base. That means if disease strikes or severe drought settles in, it's possible that not just a few cucumber plants will suffer—they all could.

Staub is working to invigorate cucumbers' dismal DNA base with the help of wild relatives from southern Asia, the veggie's birthplace. "It hasn't been easy though," he says, "since domestic cucumbers don't cross easily with wild ones."

Twelve years ago, the Madison, Wisconsin-based researcher got lucky when an unusual wild cucumber species was discovered in China. Excited by its rarity and unlikely Chinese origins (the vegetable originated in India), Staub and a Chinese colleague tried crossing it with a domestic cultivar.

At first, they weren't able to recover any offspring. Then, with the help of a technique called "embryo rescue," some plants survived. And they were fertile, producing valuable seed for additional research.

Staub says that his advanced hybrids now "cross freely with domestic cucumbers." He's evaluating these hybrids for their horticultural potential in hopes of sharing the unique germplasm with breeders all over the world.

Now Staub faces a new obstacle: trying to cross the wild cucumber with its cousin, the melon. Why? Because untamed melons have a lot to offer domestic melons and cucumbers, including resistance to drought and pests. But Staub hasn't been able to achieve a successful cross, even though the plants should be compatible reproductively, since they share the same number of chromosomes.

One of only two public cucumber breeders in the country, Staub will continue trying to solve the mysteries of why the cucurbit cousins won't cross and how such a rare cucumber plant arose in China.

The future of crunchy dill pickles and refreshing cucumber slices could depend on it.—By **Erin Peabody,** ARS.

You Say Tomato . . .

Solanaceae (Soe-luh-NAY-see-ah). It's a mouthful, for sure, but this Latin-derived name describes a plant family that could be considered royalty. It embodies not just one, but two of our most valued vegetables: the tomato and the potato—not to mention other culinary notables such as the eggplant and chili pepper.

This group of premier plants was the focus last July at the 6th International Solanaceae Conference in Madison, Wisconsin. ARS played a key role in organizing the special event, which united more than 550 researchers from 30 countries. Researchers from at least six ARS laboratories attended.

The group's common goal is to use ever-evolving genetic tools to evaluate and organize the diverse Solanaceae family. For the layperson, this research promises new-and-improved fruits and veggies, with a greater zing, zest, and connection to their roots.

According to David Spooner and Shelley Jansky—the ARS researchers who helped organize the conference—the event was especially timely, since the genomes of both the tomato and the potato are currently being sequenced.

To learn more about the conference, go to: www.horticulture.wisc.edu/PAA-Solanaceae.—By **Erin Peabody,** ARS.

NEWELL KITCHEN (D721-3)

Light reflectance sensors mounted on the front of an applicator are used to measure nitrogen deficiency so that nitrogen can be applied as needed (at a variable rate) when the applicator moves through the field.

NEWELL KITCHEN (D721-1)

Two commercial soil electrical conductivity (EC) sensors were used to map soil variability and develop productivity zones. One is mounted on the back of the tractor and uses disk coulters contacting the soil to measure EC at two depths. The other, attached to the small vehicle behind the tractor, does not require soil contact but measures EC at only one depth.

Precision Agriculture Systems

Maximizing Benefits With Better Management

Good cooks don't toss Worcestershire sauce in the ice cream or mint in the mashed potatoes. Instead, they season each dish with the best portions and combinations of spices to enhance its natural flavor.

That's the concept behind precision agriculture, the practice of modifying management techniques to meet within-field variations that affect crop growth.

The premise of precision agriculture systems is that farmers should tailor their management to fit specific areas of their farms instead of using a blanket treatment for everything. This means recognizing areas that have productivity and environmental problems and selecting the best solution for each one. The outcome is a system that increases profitability and conserves environmental resources.

Researchers in the ARS Cropping Systems and Water Quality Research Unit (CSWQ) at Columbia, Missouri, are determining which combinations of precision-agriculture methods work best. Plenty of scientists are investigating the benefits of precision agriculture, but only a few are using an integrated approach, says soil scientist Newell Kitchen.

"Many scientists look at one variable, whereas we examine the interaction of many variables. We're evaluating the system's effects on production, profitability, and environment," he says. This approach lets them assess, modify, and evaluate the effectiveness of integrated precision agriculture management practices.

Mind the Map

Between 1991 and 2003, CSWQ researchers monitored an 89-acre, conventionally managed claypan-soil field with a corn-soybean rotation, a common crop combination for that region of Missouri. Kitchen, with soil scientist Robert Lerch and agricultural engineer Ken Sudduth, measured soil and landscape variables and determined the causes of yield differences.

"We found that topsoil loss from the last two centuries of erosion on the field was a key factor in reduced productivity. Areas within the field varied in soil loss, and that had a major influence on patterns of soil quality, water quality, and crop yields," Sudduth says.

In 2004, the CSWQ team analyzed the data they'd collected to determine how precision-agriculture management could best promote soil and water quality and profitability. Accurate representation of yield variability is a keystone of precision agriculture. So the scientists used different kinds of maps to identify areas where changes were needed to improve the field's economic output.

Using profitability maps, yield maps, soil maps, and other data, the team created a management plan for the field. Because the maps indicated that corn could be grown profitably only in one section of the field, that area continued in a corn-soybean rotation while the rest of the field was switched to a wheat-forage-soybean rotation.

"We hope that our work will lead to information that farmers, consultants, and researchers throughout the country and around the world can use to make better, more informed decisions," Lerch says.

EC-Zone Grown

One way to assess fertilization needs and soil variability is to use soil electrical conductivity (EC)—a measure of how easily soil allows an electrical current to flow through it. The CSWQ team was one of the first to apply EC readings to precision agriculture. Working with university and ARS colleagues, they

NEWELL KITCHEN (D721-2)

To develop the integrated precision agriculture system that best promotes profitability and soil and water quality, ARS researchers used various tools, including water quality measurements from groundwater wells (foreground), runoff data from surface water weirs (see photo on right), and yield maps.

NEWELL KITCHEN (D721-4)

An instrumented water weir used to measure water runoff.

investigated how EC relates to soil properties and grain yields. They also used EC to map where erosion had occurred within a field.

The team discovered that EC measurements and elevation (gauged with Global Positioning System—or GPS—equipment) were extremely helpful in identifying management zones. In fact, Sudduth says, zone maps created with this information were more accurate predictors of yield than maps made with traditional soil surveys. Using yield mapping and profitability analysis, the scientists identified areas of the field that were generally unprofitable. By monitoring those areas and analyzing computer models, scientists and growers can select the best management practices.

Making Sense of Nitrogen

While environmental conservation is an important potential benefit of precision agriculture, scientists recognize that growers will only adopt new technology if it cuts production costs or improves efficiency.

"Many farmers hear 'precision agriculture' and think about tools, such as mapping tools, reflectance spectrometers, or GPS. Our objective is to demonstrate how these tools can add value and how farmers can make sensible management changes," says Sudduth.

Nitrogen application is one area where more precise management has multiple benefits. Growers don't want nutrient-deficient crops, but excessive fertilization results in unnecessary expenses and can lead to leaching, runoff, and water pollution. The CSWQ team developed two management approaches to help growers identify fertilizer needs of specific sites within a field, allowing for more accurate application.

The first method assesses nitrogen deficiency by measuring crop-canopy reflectance. Scientists estimate that using this method in a cornfield might increase profits about $15 per acre. The second method—still in development—relies on an automated soil sampling and analysis system, which could quickly and economically predict the soil's nitrogen-supplying capacity.

The researchers believe that combining the first assessment method with variable-rate fertilizer applications could benefit both the economics of corn production and the environment of corn-growing regions. By limiting fertilizer application to the amount required, growers could avoid many of the potential problems associated with nitrogen leaching.

During the next 5 years, the CSWQ team hopes to show that their methods decrease nutrient and sediment losses, increase profitability, and improve soil quality. Above all, they hope to demonstrate that precision agriculture can be an economically viable tool for farmers.

"This research directly benefits farmers by identifying technologies and methods they can use to improve efficiency of nutrients and pesticides, thereby increasing their profits," Sudduth says.—By **Laura McGinnis,** ARS.

This research is part of Water Resource Management (#201), Soil Resource Management (#202), and Integrated Agricultural Systems (#207), three ARS National Programs described on the World Wide Web at www.nps.ars.usda.gov.

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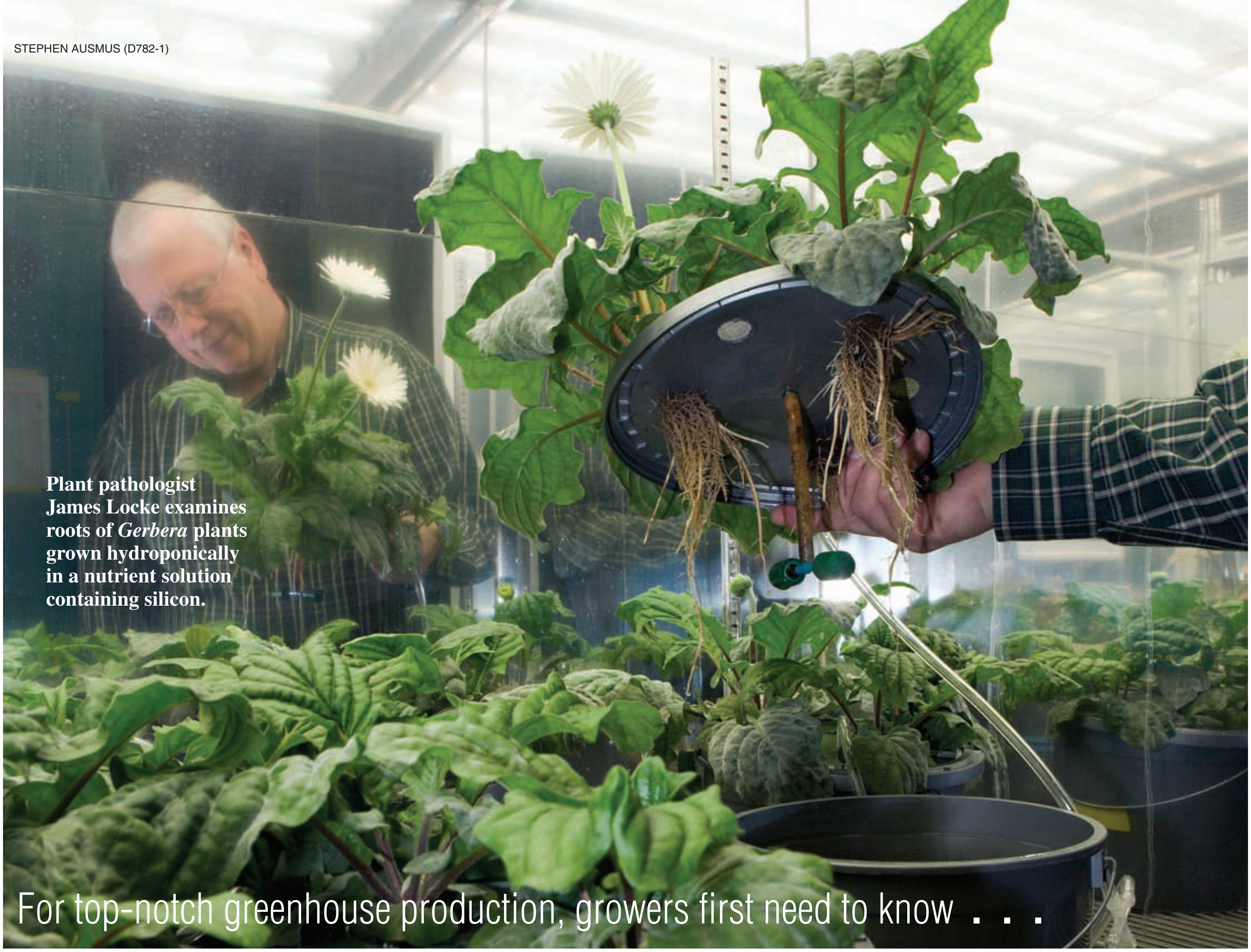
STEPHEN AUSMUS (D782-1)



Plant pathologist James Locke examines roots of *Gerbera* plants grown hydroponically in a nutrient solution containing silicon.

For top-notch greenhouse production, growers first need to know . . .

What Plants REALLY Want!

The greenhouse manager of the future walks around the greenhouse, pointing an infrared flashlight at potted plants. A tiny screen tells whether each plant has too much, too little, or just the right amount of nutrients. The manager doesn't worry about water because he lets a computer worry about that for him. The computer reads moisture sensors that trigger irrigations only as needed.

The top two concerns of greenhouse operators are to make sure their valuable plants aren't ruined by too little or too much water and to provide them with optimal nutrients. And these issues should also be the top two research priorities for Agricultural Research Service scientists working on commercial greenhouse production.

That's what ARS plant pathologist Jim Locke and ARS horticulturist Jonathan Frantz learned a few years ago, after extensive contact with Ohio's booming greenhouse and nursery industry. In 2001, in response to a congressional initiative, Charles Krause, research leader of the ARS Application Technology Research Unit (ATRU) at Ohio State University (OSU) at Wooster, Ohio, formed a team to research ways to overcome priority problems faced by the floricultural greenhouse industry in the Great Lakes region, to make American producers more competitive globally.

In 2002, Locke relocated to Toledo, Ohio, from the Henry A. Wallace Beltsville (Maryland) Agricultural Research Center. Six months later, Frantz joined him there. By 2003, the ARS Greenhouse Production Research Group (GPRG), a worksite of ATRU, was fully operational, working to shape the industry's automated future.

The group operates out of a complex of labs, offices, and greenhouses on the University of Toledo's main campus. It also leases about 8,000 square feet of greenhouse space from the nearby public Toledo Botanical Garden. The garden provides ARS and university researchers with meeting space for grower focus sessions and offers expertise in transferring research information to growers. The garden also houses 18 county horticulture organizations, including OSU Extension, Urban and Consumer Horticulture, the Master Gardener Volunteer Program, and Green Industry Education.

Scoping Out the Industry's Needs

At the very beginning, Locke and Frantz toured greenhouses throughout northern Ohio to talk with industry people about priority problems and to observe the operations themselves.

"When we made a short list of the top problems that could be researched to find solutions, we realized that all of them had nutrition or water as a common theme," Frantz says. "So we made those the top research areas for our group to focus on."

"Plants need good nutrition to grow well and avoid diseases," adds Locke. "And healthy plants need fewer chemicals, such as fungicides, insecticides, or growth regulators. We're researching use of soilless media as a way to further protect plants against disease and hold water and nutrients for them. When you're growing plants in pots, you have the opportunity to replace the soil entirely and eliminate possible soilborne pathogens—if you can find a cost-effective way to do it."

"Seeing" Nutrient Needs, Molecularly

Locke and Frantz have made a lot of progress toward their goals in the past 3 years. Frantz is testing commercial nutrient sensors as he tries to design improved portable sensors. "Devices like these can give growers a few extra days to correct nutrition problems before their plants are seriously damaged," he says.

To develop better portable sensors, he and colleagues are testing ways to spot nutrition problems by identifying key proteins or other molecules associated with stress. One of these ways is bouncing infrared light off plants to analyze the molecules present. "You can see these proteins start working before you ever see any evidence of damage in the stressed plant," Frantz says.

The GPRG's current research with silicon offers a window into how the group operates with all the nutrients it studies, such as nitrogen, phosphorus, potassium, and trace elements like magnesium. This is true even though—unlike those other nutrients—the research community doesn't yet agree on whether silicon is even an essential plant nutrient. Frantz and Locke want to find out whether it is essential and, if so, just how much it can benefit plants. To ascertain this, they use various research tools such as hydroponic culture—growing plants in a nutrient-water solution.

For example, in a recent experiment with zinnias, the scientists delivered silicon in irrigation water given to plants growing in potting mixes; they added silicon to the hydroponic solution in which other plants were growing. Then they exposed the seedlings to powdery mildew, a common disease of greenhouse plants.

The scientists examined leaf tissue using scanning electron microscopy with energy dispersive spectrometry x-ray analyzers at Wooster to determine silicon content and location. They assessed the mildew visually and documented their observations with digital photography, which was analyzed with special software to pinpoint the areas of powdery mildew development.

After observing the zinnias daily for 4 to 6 weeks, the scientists then harvested them and determined the final total silicon content of leaf tissue using inductively coupled plasma (ICP) spectrometry. With ICP, plant tissue samples are burned in very hot plasma, which is created as argon gas becomes electrically conductive after passing near a coil that generates high-energy radio waves. The combustion creates a light spectrum that makes it possible to identify elements including silicon, phosphorus, potassium, copper, magnesium, and boron.

Frantz and Locke found that significant amounts of silicon had accumulated in the harvested zinnia leaves. The silicon also decreased the severity of symptoms of powdery mildew infections in the zinnias. Next, the scientists will use similar tests to see whether silicon accumulates in the leaves of begonias, geraniums, and other ornamental crops.

"We want to see which crops put nutrients where they are most useful to the plant. It will help breeders choose promising lines for creating new varieties of flowers and ornamental plants that will need fewer pesticide applications," Locke says.

How Do Nutrients Protect?

Locke says that he and colleagues have found that silicon helps to reduce both insect and disease problems in geraniums and begonias as well as in zinnias. In fact, "an ARS postdoctoral researcher working with these plants has found that silicon can reduce the incidence or severity of the two most common foliar diseases of horticultural plants—powdery mildew and *Botrytis cinerea,"* Locke says. Now they're using these diseases as models to evaluate the role of mineral trace elements on plant disease resistance.

JAMES LOCKE (D786-2)

A retail greenhouse section of a production greenhouse facility in northwest Ohio shows some of the diversity of floricultural plants produced in that region.

"Foliar fungal diseases of herbaceous bedding plants pose a serious management challenge to greenhouse growers," says Krause. "Disease can spread rapidly in a greenhouse, where so many plants are so close together. We want to find out how nutrients protect plants from diseases. For example, do they build protection in the cell walls, or do they activate plant defense mechanisms?"

Locke, working with Krause and OSU researchers, found that a potting mix of composted hardwood bark, peat moss, and certain types of the beneficial fungus *Trichoderma* could combat *Botrytis* gray mold on plant leaves. "In begonias, it reduced this mold more than the standard fungicide chlorothalonil did," Locke says.

But Locke and colleagues have found that more isn't necessarily better with regard to applying nutrients. In tests on begonia and New Guinea impatiens grown in sphagnum peat moss/perlite potting mixes, they applied various rates of nitrogen and then infected the plants with gray mold. They found that it doesn't pay to add more than 100 parts per million of nitrogen.

"After that, you can green up the plants just before sale, but you do so at the possible expense of more disease and poorer overall plant growth and appearance," says Locke.

In addition to the University of Toledo and OSU, the ARS scientists work with colleagues at North Carolina State University, the University of Florida, Michigan State University, and the Cooperative Extension Services in Michigan, North Carolina, and Ohio.

"We're the new kid on the block, so we take advantage of the years of expertise at the more established laboratories in those institutions," Frantz says.

Wired!

Krause warns visitors that "if you are at the Toledo Botanical Garden and see government vehicles, individuals in white lab coats, or plants growing with unusual equipment attached, you should think of it as the plant equivalent of the University of Toledo's medical college.

"To do our research, we have many strange-looking sensors, gadgets, wires, and computers connected to the potted plants so we can measure and record everything from nutrient levels in their leaves to moisture in the soil or potting media," says Krause. "We've deliberately inoculated pathogens into some plants to help us study the various stress symptoms resulting from nutrient deficiencies, moisture conditions, and disease interactions.

"We advise observers to think of this research area as an intensive care unit for plants—one that's similar to hospital units where patients are monitored with wires, tubes, and other devices to facilitate recovery. Similarly, we need to carefully monitor the research plants to obtain the information we need to develop better recommendations for growing ornamentals more efficiently and economically."

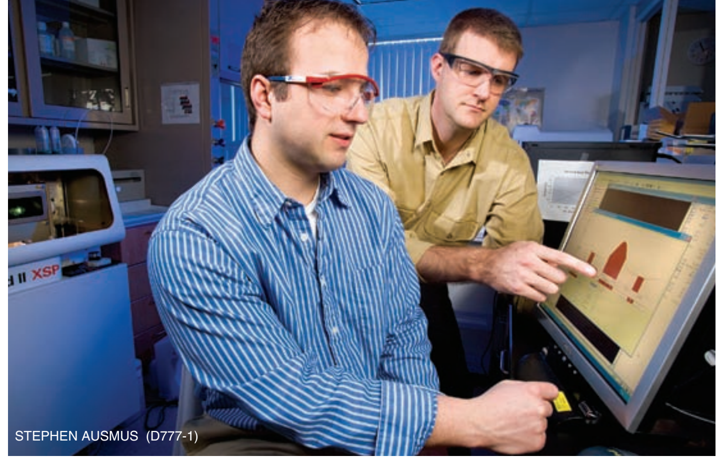
Toward Totally Automated Production

In one greenhouse, there's a small scale, called a "lysimeter," under each potted plant. The soil gets lighter as water moves through and out of the plant. Some of the water applied to the plant is also captured in the lysimeter box and sampled periodically for quality. The researchers also test the quality of the water before it's applied.

"From these lysimeters, we gain an understanding of how much water plants need so we can give them just the right amount and at the right timing and pace," Locke says. "We will eventually automate watering based on the data we get from these lysimeter experiments."

Thanks to research findings to date, Frantz, Locke, and Krause have published "Virtual Grower" software, which is available on the World Wide Web. It can help growers manage their greenhouses for greater productivity at lower costs. The current version focuses on energy requirements, helping growers choose the best fuel and heating schedules. It is available, free of charge, by going to www.ars.usda.gov/services/software/software.htm and scrolling down to "Virtual Grower."

Frantz and Locke will gradually expand the software to include all aspects of greenhouse management, including applications of nutrients, water, growth regulators, and pesticides. Ultimately, it


STEPHEN AUSMUS (D777-1)

With inductively coupled plasma emission spectroscopy, technician Doug Sturtz (left) and horticulturist Jonathan Frantz can detect and measure many elements in plant tissue, soil, and solution samples at concentration levels in the parts per billion. Here, they're verifying the instrument's calibration.

STEPHEN AUSMUS (D784-1)

Plant pathologists Charles Krause (left) and James Locke inspect poinsettias for nutrient stress (stressed plant is on right).

STEPHEN AUSMUS (D781-1)

Using a nondestructive infrared temperature sensor, James Locke (left) takes leaf temperature measurements to predict plant root health, while plant pathologist Medani Omer measures chlorophyll fluorescence.

STEPHEN AUSMUS (D783-3)

To evaluate the role of silicon in plant health, a hydroponic study is being prepared in a Toledo Botanical Gardens greenhouse by (left to right) James Locke, University of Toledo students Tera McDowell and Kurt Thomas, and ARS technician Ann Widrig.

STEPHEN AUSMUS (D785-1)

A severe case of powdery mildew on a zinnia.

will also help growers to manage labor, optimize plant productivity, and set sale prices.

According to Frantz, "There are many individual programs like this, but none that considers all these factors interacting together as this one will."—By **Don Comis,** ARS.

This research is part of Crop Production (#305) and Plant Diseases (#303), two ARS National Research Programs described on the World Wide Web at www.nps.ars.usda.gov.

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STEPHEN AUSMUS (D779-1)

Jonathan Frantz (left), James Locke (center), and IT specialist Byron Hand make modifications to Virtual Grower, a software program developed by the Greenhouse Production Research Group in Toledo, Ohio.

A focus at Ames

Helping Corn Face Tomorrow's Challenges

KEITH WELLER (K7746-15)

ARS plant pathologist Marty Carson (right) and maize geneticist/ breeder Major Goodman (NCSU) inspect different maize varieties for resistance to southern leaf blight infection.

The future of U.S. corn has been long in the making at Agricultural Research Service units in Ames, Iowa.

The past quarter century has seen scientists there drive two landmark programs aimed at preparing corn—maize—for a tomorrow that now seems destined to involve energy as well as food production.

These programs—the Latin American Maize Project (LAMP) and the Germplasm Enhancement of Maize Project (GEM)—have helped produce scores of unique corn varieties that can be bred to help farmers maintain profits as they combat blights and pests.

Plant geneticist Linda Pollak of ARS's Corn Insects and Crop Genetics Research Unit has been involved with both programs, serving as GEM's first and founding coordinator during the 1990s. Now she's spearheading another initiative: Breeding High-Quality Corn for Sustainable, Low-Input Farming Systems, or HQ-LIFS.

Low Nutrient Input

"My focus now is on providing smaller scale maize producers with plants containing specific traits that will soon be in high demand," she says. "Crucial are varieties for feed and specialty markets that can be grown using small amounts of fertilizers. Varieties that meet these market needs will allow small family farms and seed producers to remain independent and profitable."

Pollak says programs such as these are important because consolidation in the seed industry has decreased diversity and stock choice for small seed companies.

Pollak explains that HQ-LIFS is novel because of its goal: boosting corn's nutritional content while making it more compatible with sustainable farming systems.

"Specifically, we're selecting for responses to two factors: slowly available forms of nitrogen and weed pressure."

Because some states regulate use of nitrogen fertilizers, all growers could benefit from corn varieties that yield well with slowly available nitrogen sources, such as organic manures, or with lower amounts of applied fertilizer. "It's time to start selecting corn that yields well under such environments," says Pollak.

Also, she says, recent hybrid breeding efforts have not focused much on nutrient content, such as high levels of carotenoids and vitamin E or enhanced protein quality. "We also need more corn varieties that depend less on herbicides. Especially helpful would be corn that naturally establishes an early competitive edge over weeds."

Pollak says new varieties from the 3-year-old program can also contribute traits required for reliable production under alternative farming systems, such as organic farming. "We're breeding specialty varieties, too—blue corn, white corn, high-methionine corn for organic poultry producers, and corn with slowly digestible starch for native and ethnic foods—that will provide new market possibilities."

Collaborating with ARS in HQ-LIFS are Iowa State University's Corn Breeding Project in Ames; the Michael Fields Agricultural Institute in East Troy, Wisconsin; and the Practical Farmers of Iowa, for on-farm testing.

Pollak wants to form groups involving farmers, seed companies, and processors to grow, test, and evaluate varieties resulting from the program.

A GEM of a Project

Meanwhile, GEM remains a significant force in research and development of corn's genetic resources, or germplasm. Launched in 1994—with support from plant pathologist Marty Carson at ARS's Plant Science Research Unit in Raleigh, North Carolina, and other collaborators—GEM has released 135 lines to cooperators over the past 5 years.

The project is currently administered by ARS's North Central Regional Plant Introduction Research Unit in Ames and by the Raleigh unit, now led by plant pathologist David Marshall.

"GEM's mission is to increase the diversity of U.S. maize germplasm by providing an array of useful genetic material that contains superior traits for human and animal consumption, crop protection, and bioenergy and industrial uses," says Michael Blanco, the plant geneticist who now directs the Ames part of the project.

"It's a collaboration involving universities, private industry, and international and nongovernmental organizations."

The germplasm collection—gained from exotic sources—holds key characteristics such as agronomic adaptability; abiotic stress tolerance; resistance to mycotoxins, diseases, and insects; value-added traits; and superior silage yield and quality.

The most recent releases, which are all publicly available, represent about 20 races of maize, Blanco adds. "Within them, you'll see resistance to threats such as *Fusarium* ear rot, anthracnose stalk rot, and corn rootworm."

He explains that Ames's GEM studies focus on germplasm adapted for the Midwest, while Raleigh scientists concentrate on later-maturing germplasm adapted for the southern Corn Belt and Southeast.

NUO SHEN (D818-1)

Geneticist Mike Blanco pollinates tropical exotic maize as a first step in breeding corn with improved disease resistance, nutritional quality, and bioenergy potential.

Built on Past Success

The germplasm initially used in GEM was identified during LAMP, an ARS-administered, multinational program financed by Pioneer Hi-Bred International of Des Moines, Iowa, and launched during the 1980s.

LAMP focused on Central America and South America because modern corn was originally domesticated there, and the region still contains wild relatives of U.S. corn with potentially useful genetic traits. Of more than 12,000 Latin American and U.S. corn varieties evaluated under LAMP, 260 accessions were chosen for development.

"GEM's original focus included breeding crosses made with the top 51 picks from LAMP," says Blanco. "But we've expanded the germplasm base significantly with additions from Thailand, Mexico, and Brazil, and improved exotic lines and superior tropical hybrids donated by public and private cooperators."

The germplasm GEM uses at Ames is about 25 percent exotic tropical and 75 percent temperate, while Raleigh's is about 50 percent tropical, Blanco says. "Materials from tropical breeding crosses have been excellent sources of resistance to *Fusarium,* gray leaf spot, and southern rust and of value-added traits such as unique starch properties, high protein and oil content, and silage quality. The diversity of these materials may enhance our abilities to support bioenergy and other biobased products."

Since its inception, GEM has spurred release of more than 1,000 sources of germplasm to cooperators who develop them further within their own breeding programs.—By **Luis Pons,** formerly with ARS.

This research is part of Plant Genetic Resources, Genomics, and Genetic Improvement (#301) and Agricultural System Competitiveness and Sustainability (#216), two ARS national programs described on the World Wide Web at www.nps.ars.usda.gov.

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New Leptospirosis Discovery!

Changes seen in pathogen's ability to survive outside a host.

JAMES FOSSE (D838-1)

Veterinary medical officer David Alt processes *Leptospira* cultures for plasmid preparation. The plasmids will contain fragments of *Leptospira* DNA and help scientists assemble its genomic sequence.

The bacterial species that commonly causes leptospirosis in cattle has lost much of its ability to survive in water.

This change, discovered by scientists at ARS's National Animal Disease Center (NADC), in Ames, Iowa, and at Monash University in Melbourne, Australia, is likely affecting the ability of this microbe, *Leptospira borgpetersenii* serovar Hardjo, to spread through the environment.

Leptospirosis is among the most widespread of zoonotic diseases, which are infections transmitted naturally from domestic or wild animals to people.

L. borgpetersenii is one of two leptospiral species associated with most cases of bovine leptospirosis worldwide, and it is responsible for most cases in North American cattle. The other species is *L. interrogans.*

"Our results suggest that *L. borgpetersenii* is now spread mainly, if not solely, through close contact with infected animals," says NADC microbiologist Richard Zuerner. "The research also shows that *L. interrogans* is still able to spread easily through contaminated water."

What's the Difference?

Zuerner says that, before this study, evidence was lacking that *L. borgpetersenii* and *L. interrogans* differed in ways that would affect their survival in the environment. "Now we have a foundation for studying how the disease-transmission processes of both species differ," he says. "It's a step toward better disease-control strategies that may include changes in farm management and vaccine design."

There's great demand for better control of leptospirosis, in large part because current vaccines effectively guard only against infection by the serovars, or subgroups, that are used in making them. "This provides limited cross protection," says Zuerner. "A benefit of obtaining the *L. borgpetersenii* genome sequence is that we can use it to identify proteins that may be incorporated into new, more effective vaccines."

Leptospirosis is broadly distributed in wildlife and persists in livestock. Each serovar appears to be adapted to a particular animal species in which it causes problems with pregnancy and fertility but usually doesn't cause severe disease. For example, serovar Hardjo is less likely to cause severe infections in adult cattle—its normal maintenance host—but it can cause abortion, stillbirth, or weakened offspring.

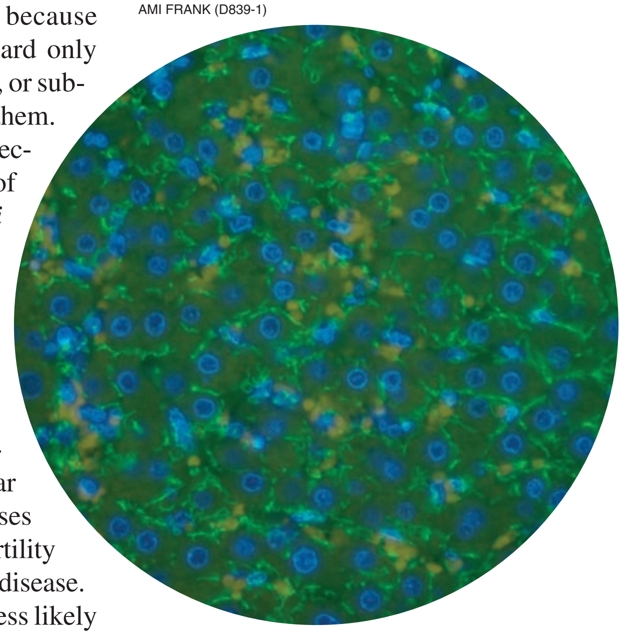
"In contrast, when *Leptospira* infect humans, the disease ranges from mild, flulike symptoms to a potentially lethal infection resulting from pulmonary hemorrhage or organ failure," says Zuerner.

People and animals are commonly infected with *Leptospira* by exposure through water contaminated with urine from infected animals. Direct contact with body fluids or tissue from infected animals is another common way to contract the disease.

Sequencing Opens the Door

The recent finding was the result of genomic sequencing studies of *L. borgpetersenii* conducted at NADC by Zuerner and veterinary medical officer David Alt, at Monash by scientists Ben Adler and Dieter Bulach, and at the University of Queensland's Australian Genome Research Facility under the direction of Elizabeth Kuczek. Zuerner and Bulach analyzed the data with support from Torsten Seemann, a research

AMI FRANK (D839-1)



Corkscrew-shaped *Leptospira borgpetersenii* serovar Hardjo appear bright green. The bright-blue areas are nuclei of hamster liver cells. Red blood cells present in the tissue are light orange.

fellow at the Victorian Bioinformatics Consortium at Monash.

In the report describing their discovery, the researchers explained that the *L. borgpetersenii* genome is decaying.

"The species carries a large number of defective genes as compared to *L. interrogans*," says Zuerner. "This loss of functional genes is thought to impair both the bacterium's ability to sense changes in the environment and its capacity to acquire nutrients and survive outside a mammalian host. We concluded that *L. borgpetersenii* is evolving toward dependence on a strict host-to-host transmission cycle."

In contrast, Zuerner says, most other *Leptospira* species can be transmitted through surface water. "This is a contributing factor to epidemics in human populations that coincide with heavy flooding, especially in underdeveloped countries with inadequate wastewater control," he says.

Zuerner explains that while *L. interrogans* strains are also found in livestock, they're often associated with disease occurring during seasonal flooding from chronically infected rats.

He says the initial steps involved in the evolutionary process leading to host dependence are unclear, but identifying them is important.

"If we can characterize the changes that lead to this reliance, it should help identify early events that lead to host dependence among a large variety of other bacterial pathogens," says Zuerner.

The microbiologist, who works in NADC's Bacterial Diseases of Livestock Research Unit, is a seasoned leptospirosis researcher. A decade ago, he developed new tests that precisely identify *Leptospira* strains from different host-animal species. That key advancement made it possible to pinpoint the source of infections from a variety of animal species.

An Outbreak in Sea Lions

In 2004, Zuerner worked with many scientists spread along the Pacific Coast to investigate the deaths of more than 300 sea lions. NADC studies ultimately showed that the animals were infected with *L. interrogans* serovar Pomona.

"That was a notable outbreak because it killed a significant number of marine mammals, including endangered or protected species," says Zuerner. "It also helped show how animal migration may spread leptospirosis."

Zuerner says that the new findings with *L. borgpetersenii* may eventually have application outside of leptospirosis.

"Presumably, the mechanism that impaired its ability to survive outside the host and started it on its path toward host dependence is shared by other bacterial genera," he says.

"It seems that an early step in the process of becoming dependent on a host for survival is the disruption of sensory functions. If bacteria can't differentiate between being in the host and being in water, it's likely they'll be ill-prepared for the challenges of living in a nutrient-deficient environment."—By **Luis Pons,** formerly with ARS.

This research is part of Animal Health, an ARS national program (#103) described on the World Wide Web at www.nps.ars.usda.gov.

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JAMES FOSSE (D837-1)

In preparation for sequencing *Leptospira's* genome, technician Rick Hornsby loads a 96-well plate into a machine known as a "colony picker."

Where'd That Boll Weevil Come From?

DNA-marker technology helps keep tabs on legendary insect pest.

ROB FLYNN (K2742-6)

Boll weevil, *Anthonomus grandis,* on a young cotton boll.

TOM SAPPINGTON (D841-2)

Research associate Mark Arnold—with the Cotton Entomology Research Program, Texas Agricultural Experiment Station, Lubbock—releases marked boll weevils on freshly harvested cotton. Recapture of weevils a day later enables researchers to determine survival rate.

The devastation wrought by the boll weevil, *Anthonomus grandis,* on the U.S. cotton industry during most of the 20th century is the stuff of social, economic, and agricultural legend.

Though evicted from more than 12 million acres of southern farmland by eradication efforts started in the 1970s, the pest is still found in portions of the mid-South and South Texas.

"In Texas and surrounding states, it's common for a zone that's far advanced in boll weevil eradication to share borders with zones still harboring substantial weevil populations," says entomologist Tom Sappington, in ARS's Corn Insects and Crop Genetics Research Unit at Ames, Iowa. "So the threat of reintroduction remains a constant concern."

Bill Grefenstette, national coordinator for boll weevil eradication for USDA's Animal and Plant Health Inspection Service, says that these remaining areas should be free of the pest within 4 years. But Sappington knows that until that time—and well past it—vigilance will be a key weapon against the weevil.

Using modern DNA-marker technology, Sappington and colleagues are keeping close tabs on this nemesis of cotton farmers. They are combining conventional microsatellite analysis with population-assignment techniques to pinpoint the migratory patterns and origins of dispersing boll weevils.

They Get Around

"Identifying recent boll weevil migrants in local populations and knowing where they came from can greatly enhance insect-pest management," says Sappington. "It results in better strategies for monitoring, and responding to, pest introductions. Knowing the source of a reintroduction into an eradication zone helps us determine appropriate actions to stem the flow."

Sappington has studied the boll weevil's movements for more than 8 years. While at ARS's Kika de la Garza Agricultural Research Center in Weslaco, Texas—where weevils were marked with enamel paint or fluorescent powder and then recaptured—he collected valuable data about the insect's ability to spread via cotton-transport and ginning operations.

"We did some important genetic studies there," he says, "but it wasn't easy to use microsatellites, because we didn't have the right equipment." Upon arriving in Ames in 2003, Sappington used his new lab's genetic-studies tools to expand the research.

Microsatellites are repetitive, short sequences of DNA. Also called "short tandem repeats," they provide scientists with a way of locating genes.

"They're seeing extensive use in the entomological community, especially in tracing inheritance patterns," says Sappington. "But there are relatively few instances of microsatellites being applied to characterize movement of insects. We've found that they're very useful when applied in statistical population-assignment tests."

These population tests have helped scientists trace invasion routes of other insect pests, including gypsy moths, Mediterranean fruit flies, and western corn rootworm. "Linking information from microsatellite analysis to animal movement is a relatively new and powerful approach that's being widely used in conservation genetics and fisheries studies," says Sappington. "But it's an approach that's been underused by entomologists."

South of the Border

Sappington's latest study, conducted with ARS molecular biologist Kyung Seok Kim from his Ames unit, was the result of a request from Pedro Cano-Ríos, a scientist at Mexico's National Institute for Forestry, Agronomy, and Animal Research.

Cano-Ríos caught several boll weevils in pheromone traps in 2004 near Tlahualilo, Durango, next to a Mexican eradication zone. Weevils had not been reported in that part of Mexico for about 10 years. "Our goal was to determine whether a small boll weevil population had been there all along or the captured weevils had migrated in from somewhere else," says Sappington.

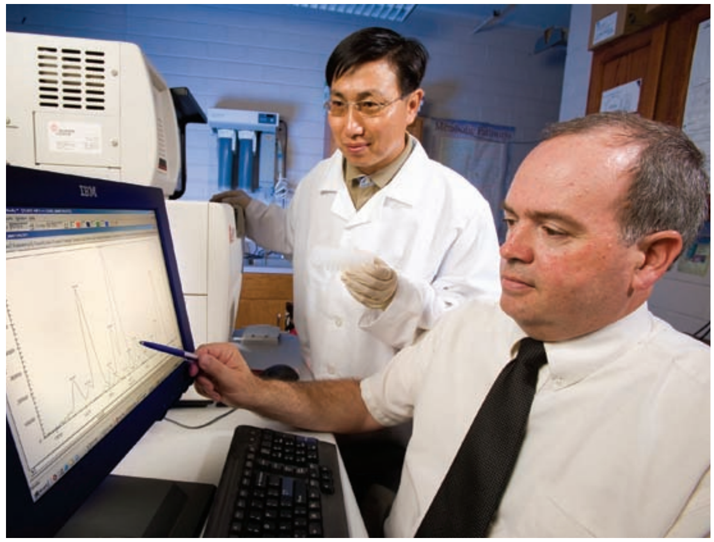
To identify the weevils' most likely origin, Sappington, Kim, and Cano-Ríos compared microsatellites in weevils caught in Tlahualilo to microsatellites in three other populations in northern Mexico and from one in southern Texas.

The microsatellites revealed that boll weevil migrants from the state's still-infested Lower Rio Grande Valley were greatly hindering final eradication of the pest in the Lower Coastal Bend, near Kingsville. The researchers also found strong genetic evidence of boll weevil migration from the Tampico, Mexico, area to the valley.

The results from Tlahualilo suggested that, while some of the boll weevils caught there in 2004 were immigrants, the bulk were residents whose relatively low numbers probably increased that year because of higher-than-usual rainfall.

"Both studies demonstrate that microsatellite markers and population assignment techniques are practical tools for determining the most likely origins of boll weevils reintroduced to eradication zones in the United States and Mexico," says Sappington. "These techniques also hold promise for replacing conventional mark-and-recapture studies of insect dispersal."

STEPHEN AUSMUS (D842-1)



Using an automated sequencer, molecular biologist Kyung Seok Kim (left) and entomologist Tom Sappington study DNA samples from a boll weevil to identify the weevil's origin.

TOM SAPPINGTON (D840-1)

In a darkened storage shed, assistant research scientist Stan Carroll—with the Cotton Entomology Research Program, Texas Agricultural Experiment Station, Lubbock—uses a blacklight to search for boll weevils marked with fluorescent powder on freshly harvested cotton. He is counting the number of weevils still on the cotton after simulated transport from field to cotton gin.

Mark and Recapture

Sappington had made effective use of the mark-and-recapture technique while in Texas. Working with agricultural engineers Alan Brashears and Roy Baker of ARS's Cropping Systems Research Laboratory at Lubbock and colleagues at Texas A&M University and the Texas Agricultural Experiment Station, he used it to study boll weevils' dispersal from harvested cotton and their survival through the cotton ginning process.

Early in the 20th century, cotton gins contributed to the spread of boll weevils throughout the United States. "That's not the case today," says Sappington. "Today's gin stands use more closely spaced saws that operate at higher speeds. Our studies show that the probability of boll weevils entering the gin stand and surviving intact in ginned lint is very close to zero."

In fact, Cano-Ríos's original concern was that the boll weevils had migrated to Tlahualilo in cottonseed imported as cattle feed from the United States and other parts of Mexico.

"Fortunately, our previous ginning research showed that it is virtually impossible for weevils to survive the gin and be transported alive in cottonseed," says Sappington. "Mexican authorities used that research to decide the seed did not require fumigation, saving them and U.S. exporters a lot of money—and the environment a lot of needless insecticide input."—By **Luis Pons,** formerly with ARS.

This research is part of Crop Protection and Quarantine, an ARS national program (#304) described on the World Wide Web at www.nps.ars.usda.gov.

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Fungal Foam Seeks and Destroys Termites

SCOTT BAUER (K8210-10)

Formosan subterranean termites feed on trees and wood structures. To combat them, ARS scientists have developed a foaming fungal biocontrol treatment that kills the insects in a few days.

About 850 miles separate Peoria, Illinois, from New Orleans, Louisiana. But that hasn't stopped a team of Agricultural Research Service (ARS) scientists in the two cities from plotting ways to sabotage pesky subterranean termites.

On behalf of the scientists—Christopher A. Dunlap, Mark A. Jackson, and Maureen S. Wright—ARS applied for a patent in September 2006 on a first-of-its-kind foam they developed to control the pests biologically. Rather than deliver a slow-acting insecticide, as do other foam products now sold, the scientists' formulation exposes termites to spores of the fungus *Paecilomyces fumosoroseus.* On contact, the fungus sends threadlike filaments called "hyphae" into the termites' bodies. It then starts to feed and grow, killing its hapless victims within a few days.

It's a gruesome end for sure, but one not likely to earn the sympathy of homeowners, building managers, or others whose property has been ravaged by the pests.

Formidable Formosans

Each year, termites cost an estimated $1 billion in U.S. property damage, preventive measures, and structural repairs. Among the worst offenders—and top target on the scientists' hit list—is *Coptotermes formosanus.* In the southern and southwestern United States, this termite is unrivaled in the size of its colonies, tunneling, and appetite for cellulose in wood materials and living trees. In New Orleans alone, this nonindigenous species causes an estimated $300 million annually in losses.

But if ongoing field studies in New Orleans are any indication, the innovative fungal foam could make life a good bit more difficult for the Formosan termite. The scientists developed the concoction to improve the fungus's capacity to biologically control this foreign pest and its native subterranean brethren.

Some insecticide compounds simply repel the pests, which then go forage elsewhere. Other insecticides are nonrepellent and are applied as either liquids or baits, where they serve as slow-acting poisons.

Today's pesticides must be reapplied after a few years to maintain a barrier around the foundations of homes and other structures. Earlier termite treatment chemicals, such as chlordane, persisted in the environment for long periods. But all the old standards have been discontinued because of environmental and human health concerns.

Paecilomyces and other insect-killing fungi that the team is considering are just as lethal as chemical pesticides. And as biological control agents, they are better for environmentally sensitive areas, say Dunlap, a chemist, and Jackson, a microbiologist. Both are in the Crop Bioprotection Research Unit at ARS's National Center for Agricultural Utilization Research in Peoria. Wright, a microbiologist, is in the Formosan Subterranean Termite Research Unit at the agency's Southern Regional Research Center (SRRC) in New Orleans.

Operation Fungal Foam

Paecilomyces was the team's first choice for use with the foam because of Jackson's extensive experience in mass-producing and formulating its spores for use against silverleaf whiteflies and other crop pests.

As a host-specific fungus, it only infects members of certain insect families. It poses little known danger to beneficial insects such as bees, or to humans, pets, or other animals.

In the lab, Dunlap examined more than a dozen foaming agents—some synthetic, others food grade—for compatibility with *Paecilomyces.* That meant finding one that wouldn't kill the fungus or diminish its ability to form spores and grow (germinate).

After extensive testing, he chose a commercially available protein called "keratin hydrolysate." It's a smaller, water-soluble version of the keratin that's found naturally in animal hooves and horns, fish scales, hair, wool, feathers, and other sources.

Dunlap traces keratin's first industrial uses to fire-fighting foams of the 1940s. During World War II, for example, it served as a substitute for petroleum, which was in short supply. Today, petroleum-based foaming agents are once again the norm, including for insect-control applications.

Besides checking for compatibility with *Paecilomyces,* the ARS team observed that the protein has a beneficial effect on the fungus's ability to control termites. That is, fungi in foam killed more termites than fungi in water. The scientists note that the foam causes the spores to germinate faster than they normally would—a feature that could improve *Paecilomyces's* effectiveness. The foam's chemical properties also allow the spores to stick better to the termites.

To create the foam, the scientists mixed keratin hydrolysate with water, fungal spores, nutrients, and ingredients called "adjuvants," which help the spores cling to treated surfaces.

A fiber-optic video camera, supplied by collaborators from the New Orleans Mosquito and Termite Control Board, enabled the ARS team to watch the foam in action and to check for its impact on termite activity in trees they had treated for the outdoor phase of their studies.

Treatment involves drilling some small holes in a tree's trunk and then injecting the foam inside those holes, where it can creep and expand into any cavities or tunnels the pests have made in the heartwood. After about 25 minutes or so, the foam collapses, depositing the fungal spores to act like thousands of tiny, termite-killing landmines.

With the fiber-optic camera, says Dunlap, "You can see the termites running, with the foam coming in behind them." If not directly coated with spores, the termites later pick them up while resuming their foraging or grooming of one another back at the nest. A sign the spores have taken effect is the termites' failure to plug the drill holes several days after treatment, notes Dunlap. Another is moldy cadavers.

PEGGY GREB (D865-1)

Technician Bridgette Duplantis and chemist Chris Dunlap prepare *Paecilomyces fumosoroseus* fungal powder and keratin solution for field application. This liquid will turn into a foam when placed in a pressurized foaming machine.

PEGGY GREB (D862-1)

Microbiologist Maureen Wright saturates filter paper with the foam solution for termite bioassays.

Of Hurricanes and Survival

Despite such high-tech surveillance, the scientists still had many questions about the all-natural biological control they were developing. How slow acting is the fungus? Can it be easily passed from termite to termite? What formulation is most enticing to foraging termites?

But of all their questions, they never guessed that they'd find answers to this one: How will the fungal foam fare under hurricane conditions?

Wright was in the midst of carrying out long-term field studies on *Paecilomyces* when Hurricane Katrina struck in August 2005. Several months before, she'd injected the fungal formulation into several termite-infested trees located in City Park, a 1,300-acre green space situated near the heart of New Orleans.

City Park contains hundreds of cherished tree specimens, including the largest collection of mature live oaks in the world. Some of these moss-draped giants predate the city by three centuries or more. While more than 1,000 trees in City Park were toppled or suffered wind damage, most survived Katrina. But the same may not be said about their ability to outlive the wood-hungry Formosan termite.

New Orleans's termites are known as hardy underground dwellers, but even ARS researchers were surprised to learn how many persisted through the flooding and upheaval inflicted by Katrina. SRRC entomologists Mary Cornelius and Weste Osbrink tracked the pests before and after the storm, across City Park and elsewhere, and found that around 80 percent of their research traps were still crawling with termites just a month after the hurricane had struck.

Fortunately, *Paecilomyces* has been equally tenacious. Despite Katrina's impact on her City Park study area, Wright reports that she's still seeing significant control of termites.

"Even after Katrina," says Wright, "we still have seen little to no termite activity in the treated trees."

"Even after Katrina, we still have seen little to no termite activity in the treated trees."—Maureen Wright

The researchers experienced one major drawback, though: Katrina wiped out trees serving as Wright's controls. Other of her research trees were badly damaged and must now be removed by city officials to make space for new plantings. For these reasons, Wright won't be able to continue to monitor the trees, as she'd hoped.

But to follow up, she kicked off another field study this past spring. "Also taking place in City Park, this study will eventually involve many more trees, which will give us more confidence in our findings," she says.

What excites Wright about the fungal foam is that in addition to terminating termites, the method uses all-natural components. "Treatments currently being used on trees and in buildings are largely chemical," she says. "Our method is a nice option for consumers who like knowing that the termite treatment being used in their homes or yards is biologically based."

PEGGY GREB (D867-1)

Ed Freytag, an entomologist with the New Orleans Mosquito and Termite Control Board, drills a tree for monitoring of termite activity and injection of the fungal foam.

The fungal foam is just one of many control methods being developed by ARS researchers. Ultimately, they'd like to have ready an entire toolbox of termite treatments for use in various scenarios. As Hurricane Katrina proved, they'll need all the help they can get in outwitting the Formosan subterranean termite, which seems uniquely programmed for survival.—By **Jan Suszkiw** and **Erin Peabody,** ARS.

This research is part of Crop Protection and Quarantine (#304) and Veterinary, Medical, and Urban Entomology (#104), two ARS national programs described on the World Wide Web at www.nps.ars.usda.gov.

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DON FRASER (D1431-1)

Chemist Mila Hojilla-Evangelista tests the breaking point of plywood laminated with vegetable-based glues to determine the strength of the glue bond.

CORN THE LATEST GLUE INGREDIENT?

After oil is extracted from it, corn germ meal is typically sold to farmers for use as a nutritious feed for poultry and other livestock.

But a surplus of corn germ could be looming on the horizon. That's if America's biorefineries can increase corn-ethanol production from the current 9 billion gallons (2008) to 15 billion gallons by 2015. But prescient researchers aren't waiting. Take, for example, Milagros P. Hojilla-Evangelista.

In studies begun in 2005 at ARS's National Center for Agricultural Utilization Research in Peoria, Illinois, the chemist determined that corn germ can be used as a protein extender for plywood glues, potentially opening a new, value-added market for the byproduct. Glue extenders reduce the amount of the main binder, or resin, that's used, thus lowering production costs. They also enhance adhesive action and impart textural properties to the glue.

The conventional extender for most plywood glues is wheat flour. Hojilla-Evangelista has sought to expand the list of agricultural extenders to include not only corn germ protein, but also sorghum flour and soybean meal. The price and performance of industrial-grade wheat flour make it a dominant extender, but alternative sources are still important to consider.

DON FRASER (D1430-1)

Technician Debra Stamm (right) feeds a sheet of wood into a glue spreader where it will receive an even coating of vegetable-based glue before being laminated to another sheet to form plywood. Mila Hojilla-Evangelista watches and waits to receive the piece as it exits.

"If something should happen—say, the price of wheat flour goes too high—glue manufacturers would like to be able to have something else they can use that's comparable," says Hojilla-Evangelista, who's in the ARS center's Plant Polymer Research Unit.

In earlier work there, she developed a soy-flour-based plywood glue formula for foam-extrusion applications. This process pumps out evenly spaced, pastalike, foamed strands of adhesive onto plywood veneers, which are then pressed together. The corn germ formula she's now testing is for sprayline coating, in which the liquid adhesive is applied by overhead nozzles to wood surfaces.

"I chose a sprayline formulation because it's more tolerant of nonprotein components such as oil, which is a defoamer," says Hojilla-Evangelista. "But since foaming isn't critical to sprayline applications, the presence of oil isn't an issue."

For tests, she applied the required amount of glue onto one side of 12-inch x 12-inch southern pine veneers, then hot-pressed them following industry-standard conditions to produce three-ply panels. She found the bonding strength of the corn germ-based glue to be the same as that of the wheat flour-based formula.

The corn germ glue's viscosity and mixing properties also compared well with the industry adhesive, notes Hojilla-Evangelista, who, in June 2008, presented her work at the Corn Utilization and Technology Conference in Kansas City, Missouri.

"Next, I plan to increase the amount of corn germ in the glue," she says. "If I can do that, I can reduce the amount of resin that's used, which cuts down on the costs of glue manufacturing."—By **Jan Suszkiw,** ARS.

This research is part of Quality and Utilization of Agricultural Products, an ARS national program (#306) described on the World Wide Web at www.nps.ars.usda.gov.

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A Prairie Land's Companion

Which switchgrasses are best suited for reseeding our native grasslands? An ARS researcher finds out.

STEPHEN AUSMUS (D854-1)

Switchgrass—a lean, mean, growing machine that would tap most ceilings if grown indoors—is on the verge of a major comeback.

Until the grass was mentioned in the 2006 State of the Union address for its bioenergy potential, most Americans had never heard of *Panicum virgatum.* Wandlike, as its Latin name implies, the grass was an integral part of the tall-grass prairie that dominated much of America's Midwest until about 150 years ago.

For a while, it looked as if native switchgrass was going the way of the buffalo, routed out by westward expansion. Now, there appears to be a growing effort to bring back this pivotal prairie plant and others like it.

Michael Casler, a plant geneticist who works at the ARS U.S. Dairy Forage Research Center in Madison, Wisconsin, can attest to the recent zeal over native grasses. In fall 2006, he attended an Eastern Native Grass Symposium in Harrisburg, Pennsylvania, that he describes as "growing by leaps and bounds."

"Months later, I still have people contacting me, excited to tell me about native prairies they know of in places as far south as Tennessee and Mississippi," Casler says.

Plant breeders, conservationists, landscaping firms, homeowners—they all see a future that's deeply rooted in lanky, softly flowing grasses. Renewable energy, a desire to "go native," and a longing for easy-to-grow, drought-resistant garden plants are a few of the driving forces.

But the task of reestablishing a plant that's been largely missing from its home range for hundreds of years poses many questions.

Casler, who's been breeding switchgrass plants for the past 10 years, has found at least a few answers. His is the first study to delve into the genetic legacy of this king of grasses.

"Remnant" Plants Versus "Bred"

There are basically two worlds when it comes to switchgrass: grass that grows unhindered on fragments of pristine prairie land and grass that's been cultivated by humans to encourage more positive agricultural traits.

For those wishing to restore prairie lands using native grasses like switchgrass, the general rule of thumb has been to tap local stocks—those from no farther than 50 to 100 miles out. It's believed that these plants are less likely to genetically contaminate other native or restored prairies in the area.

In other words, switchgrass growing in a native prairie in central Ohio isn't thought to be fit for planting in Minnesota. Even if that grass seed were more plentiful, easier to access, and cheaper, the prevailing thought is: Its genes would be too different.

"This kind of 'purist' thinking has often meant that switchgrass cultivars, which tend to be more readily available and less costly, get passed over for restoration projects," says Casler.

Casler, who's mostly focused on switchgrass's value in making biofuel, decided to pursue this offshoot of his research with assistance from ARS plant geneticist Kenneth Vogel of Lincoln, Nebraska. No one had ever before examined the genetic similarity between native switchgrass plants and their contemporary cousins.

Natural Land, Never Farmed

In the summers of 1996 and 1997, Casler and colleagues traveled from western Minnesota to New York and down to Indiana and over to Ohio in search of prairie lands that had never been "under the plow." This was Casler's cue for locating still-living slices of genuine prairie.

They collected more than 75 switchgrass samples from dozens of locations. Since most sites had been set aside by county or state departments of natural resources or were owned by private land-acquisition organizations, they contained essentially preserved grasses left over from the days of the great sprawling prairie.

After cultivating the accessions in his Madison laboratory, Casler extracted their DNA. He then extracted DNA from common current-day cultivars like Blackwell, Cave-in-Rock, Pathfinder, and Shawnee and compared them all for genetic differences and likenesses.

The results were surprising.

Still Rooted to Its Ancestors

Casler's switchgrass subjects had their differences, but hardly any were attributable to broad geographic disparities. Actually, aside from subtle differences owed to variations in soil, climate, and slope, the broad switchgrass pool sitting before Casler was pretty homogeneous.

"Plants from each individual population were as variable as those from geographically distant populations, and the remnant populations were very similar to the cultivars," he says.

Part of this can be explained by the fact that people have been breeding switchgrass for only about 50 years, compared to the thousands of years of domesticating modern wheat or corn.

"In fact, the most advanced cultivars I analyzed are only three to four generations removed from wild switchgrass," says Casler. He adds that these breeding-induced changes are small, since breeders only exploited a small amount of genetic variation already existing in native switchgrasses.

The good news about these findings is that so-called "improved" switchgrass cultivars are, genetically speaking, very similar to populations of plants being used for native restoration. "The difference between native and cultivated switchgrass," says Casler, "is probably due to changes in the frequency of just a few genes that have little overall impact on switchgrass gene pools."

A Grass That Can Make the "Switch"

These findings make switchgrass, which was already enjoying modest agricultural fanfare, especially attractive.

"Our findings show that switchgrass that's grown for biofuel," says Casler, "can also be grown for conservation and other uses without the fear of possible genetic contamination. We need to pay attention to the origin of switchgrass seed populations, but we've learned that seeds can be transferred widely within the hardiness zone in which they originated."

Switchgrass as a source of renewable energy still requires more research before its full potential is realized. Casler says that the plant's biofuel future probably lies in specially designed seed mixtures with supporting role-type plants—including beneficial legumes that fix their own nitrogen.

If Casler's right, fields of soft, willowy switchgrass growing alongside native legumes like pure prairie clover and Illinois bundleflower could someday provide us with a source of green energy as well as a window into our country's verdant past.—By **Erin Peabody,** ARS.

This research is part of Rangeland, Pasture, and Forages, an ARS national program (#205) described on the World Wide Web at www.nps.ars.usda.gov.

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STEPHEN AUSMUS (D758-1)

Using DNA markers to characterize differences among switchgrass plants related to geographic variation, technician Nick Baker (left) and geneticist Michael Casler analyze the DNA markers of switchgrass plants using capillary gel electrophoresis.

Proving Their Prowess—Insects Help Preserve Germplasm Collection

Entomologist Steve Hanlin often finds himself sharing his workspace with 5,000 honey bees.

"You need to approach them calmly," observes his colleague, technician Sharon McClurg. "And you have to be careful what you lean up against."

Hanlin and McClurg wrangle other pollinators as well. It's all part of a plan to protect the genetic purity of more than 49,000 plant accessions maintained at the North Central Regional Plant Introduction Station (NCRPIS) in Ames, Iowa. The station maintains numerous germplasm collections belonging to the National Plant Germplasm System, which is administered by the Agricultural Research Service.

Hanlin and McClurg manage colonies of several insects for pollinating certain plants in the germplasm collections during their controlled seed increase, or "grow-out." During this period, curators cultivate plants to obtain seeds that contain the same distinct genetic characteristics as their parent material.

Grow-outs sometimes take place within field cages and greenhouses designed to shelter the emerging plants. This ensures that seeds or clonal materials contained in the NCRPIS collections are capable of germinating, or are viable. NCRPIS provides this germplasm for research and educational purposes—in 2006, some 20,000 packets of seeds were sent to scientists around the world.

In most cases, seeds won't develop without pollination, and if just any insect were to pollinate these accessions in the open, genetic havoc could ensue. Enclosing the plants in a screened space with a selected insect pollinator keeps other insects out and guarantees that only the selected insect will carry out pollination—one that can do the job.

NCRPIS Pollinators—The A list

Honey bees *(Apis mellifera)* have been the heavy hitters since 1957, when NCRPIS began its controlled-pollination program. They are used in about 700 field and greenhouse cages year-round.

Hanlin ensures that there are enough honey bees to meet demand. In a building with a faint aroma of beeswax, he hand-assembles a "nuc"—short for nucleus—which will contain an assortment of worker bees and an introduced queen bee. After 2 weeks, if the workers and queen have set up shop—as indicated by the presence of eggs or larvae—he'll place the nuc in a field cage so that the bees can get to work.

Though colony collapse disorder (CCD) has resulted in heavy honey bee losses for some beekeepers in the United States, the honey bees at Ames have not been affected yet. But even before CCD emerged as a problem, NCRPIS staff always looked for opportunities to diversify the pollinators they used.

More Placid Pollinators

Under the direction of NCRPIS research leader Candice Gardner, McClurg has worked with Hanlin since 2004 to integrate another pollinator into the mix. The alfalfa leafcutting bee *(Megachile rotundata)* is already a favorite of alfalfa producers. Hanlin and McClurg buy 16 gallons of alfalfa leafcutting bee pupae—at 10,000 pupae per gallon—every year.

They have devised a staged system for nurturing the pupae—which arrive wrapped up in leaf cells created by adult bees the previous growing season—through their emergence as new adults ready for action. Nonstinging and more docile than honey bees, the solitary alfalfa leafcutting bees perform best in hot, dry weather.

At NCRPIS, two species of mason bees—the hornfaced bee *(Osmia cornifrons)* and the blue orchard bee *(Osmia lignaria)*—stay busy even when some other pollinators find fluctuating springtime temperatures too cool. Though mason bees are nonstinging and easy to rear in captivity, they are creatures of habit and will only pollinate from April until June.

To create a nesting domicile for the mason bees, Hanlin tucks about 40 paper

PEGGY GREB (D1063-1)

Entomologist Steve Hanlin checking honey bees in a "nucleus" hive, which is used to house bees that pollinate plants inside cages at the North Central Regional Plant Introduction Station.

straws inside a plastic pipe. In autumn, he checks to see whether the straws are capped with mud, which is a signal that larvae are protected within. Domiciles with larvae are put in cold incubation until they are needed in the spring. Then the domiciles are placed in field cages, where the adults emerge.

"Mason bees don't like being moved around, either," Hanlin says. "Once I've put their domicile in a field cage, it has to stay put—or else they won't work."

Bees That Like a Challenge

Sometimes only the big guys will do. Though bumble bees (*Bombus impatiens*) are adept at pollinating many plants, at NCRPIS they are usually paired with plants that are just plain difficult—for example, ornamentals with trumpet-shaped flowers.

Bumble bees are tricky to rear in captivity, so Hanlin procures them from an outside supplier. Mildly aggressive, they will sting if provoked. But the benefit from these bees outweighs the cost. They work year round, can be moved from cage to cage, put in long hours each day, and keep going even in cool, cloudy weather.

A Pest Becomes Productive

Flies don't mean to pollinate. It's just something they do while they're doing something else—flying from flower to flower to find the perfect resting spot.

In cages, these lazy flies are particularly good at partnering with honey bees to pollinate carrots and other plants in the carrot family. Two kinds of flies—blue bottle flies (*Calliphora* sp.) and house flies (*Musca domestica*)—have been put to work at NCRPIS.

Entomology and curatorial staff collaborated in establishing flies as pollinators, which are acquired as pupae from an outside vendor. "They're very successful," McClurg says. "They provide plant curators with a short-term pollinator that isn't expensive."

Because house flies have a limited life span, the lab replenishes around 80 cages

PEGGY GREB (D1059-1)

Steve Hanlin and biological science aid Kristen Peterson examine blue bottle fly pupae before placing them in a cage with carrot plants.

PEGGY GREB (D1060-1)

A cluster of blue bottle fly pupae.

PEGGY GREB (D1061-1)

A blue bottle fly pollinating florets.

SCOTT BAUER (K7791-11)

Blue orchard bee.

PEGGY GREB (D1062-1)

An adult worker honey bee collecting pollen.

PEGGY GREB (D1064-1)

Steve Hanlin moves a nucleus honey bee hive to a cage containing sunflowers.

with about 10,000 new flies every week in the summer. The pace slows in fall and winter to around 5,000 a week. Blue bottle flies last longer in the cages, but more complete pollination occurs when both species are present.

These insects earn their keep, but they can't escape their reputation. "Flies are flies," Hanlin concedes. "Some people do find them irritating."

Kathy Reitsma, one of the NCRPIS plant curators, is pleased with how well the pollination program has worked out. "There are pros and cons to each pollinator," she says. "We know we can always pollinate the plants by hand if insect pollinators are not available, but we prefer to use insects whenever possible."—By **Ann R. Perry,** ARS.

This research is part of Plant Genetic Resources, Genomics, and Genetic Improvement, an ARS national program (#301) described on the World Wide Web at www.nps.ars.usda.gov.

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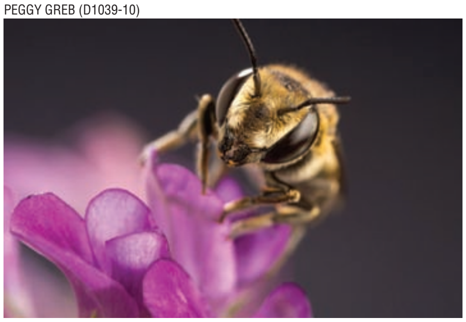
PEGGY GREB (D1039-10)

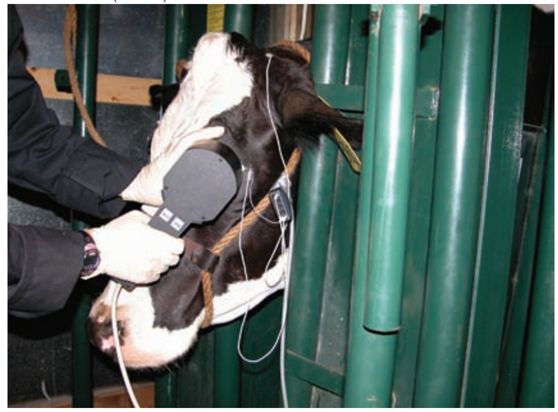


An alfalfa leafcutting bee on an alfalfa flower.

JUSTIN GREENLEE (D1609-2)

A recording electrode (a thin wire filament) is placed across the corneal surface of this steer's eye in preparation for a new method to test for signs of TSE (transmissible spongiform encephalopathies).

JUSTIN GREENLEE (D1609-3)



A Ganzfeld illuminator, held an inch from the eye, delivers a flash of light, causing a retinal response that is recorded by the electrode.

Retinal Scan Technology Identifies Early TSE Symptoms in Cattle

Diagnosing and preventing transmissible spongiform encephalopathies (TSEs) is a major priority for U.S. agriculture. Fortunately, ARS scientists have developed and tested a research tool that could be used to screen live cattle for these fatal neurological diseases.

TSEs affect many animals, attacking the central nervous system. The diseases are characterized by misshapen forms of naturally occurring, and usually harmless, proteins known as "prions." In comparison to their counterparts in healthy animals, prions in infected animals are irregularly folded.

Identifying improperly folded prions is one way to diagnose TSE-infected animals. Another is to examine brain tissue for the riddled, spongy appearance characteristic of TSE-induced brain deterioration. Unfortunately, these tests require that the animal be killed beforehand.

But scientists at the National Animal Disease Center in Ames, Iowa, have determined that retinal scanning technology can detect changes associated with TSE infection in live cattle.

Led by veterinary pathologist Justin Greenlee, the Ames scientists determined that TSE infection leads to observable changes in cattle retinas. In cattle, as in humans, sight occurs when light enters the eye, and the retina converts it into neuronal signals that the brain interprets as visual images. Within the retina is a type of cell—known as the "bipolar cell"—that plays an important role in this process.

"Bipolar cells take input from the photoreceptors at the back of the eye and hand it off to the ganglion cells that form the optic nerve," Greenlee explains. "Without the bipolar cells, the message that begins in the photoreceptors never reaches the brain."

Greenlee's team included postdoctoral research associate Jodi Smith and ARS technician Leisa Mandell, in collaboration with Iowa State University assistant professor M. Heather West Greenlee. By examining specially stained retinal samples under the microscope, they observed structural changes in bipolar cells in cattle that had been infected with a TSE.

In a later study, the scientists used electroretinography (ERG) to measure retinal response to a flash of light in healthy cattle and in TSE-infected cattle. The scientists observed significant changes in the component of the ERG that measures the response of the bipolar cells, supporting their earlier findings.

In addition, ERG detected these changes about 12.5 months after the cattle had been experimentally infected by intracranial inoculation, about 5.5 months before they generally began to show clinical symptoms.

Now the researchers are investigating how to speed up the testing process and make it more practical for use outside of a laboratory environment.

"Right now, the procedure takes 10 to 20 minutes," Greenlee says. "An ideal real-world test based on this technology would take just a few seconds."

Further research is also required to verify that the results can be replicated in animals infected with other TSEs.—By **Laura McGinnis,** ARS.

This research is part of Animal Health, an ARS national program (#103) described on the World Wide Web at www.nps.ars.usda.gov.

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Genomics, Phenomics Research Paves the Way for Improved Animal Health and Productivity

The Agricultural Research Service's genomics and phenomics research is laying the foundation for future livestock production improvements. Understanding how inherited characteristics relate to specific genomes will eventually allow researchers to develop tools that can be used to guide animal breeding, selection, and management decisions. Throughout the United States, ongoing ARS research projects are changing the way industry members breed, raise, and produce our nation's most valuable agricultural animals.

STEPHEN AUSMUS (D1149-8)

Geneticist Curt Van Tassell and biological technician Alecia Bertles select bull semen samples for DNA extraction and testing using the SNP50 BeadChip technology.

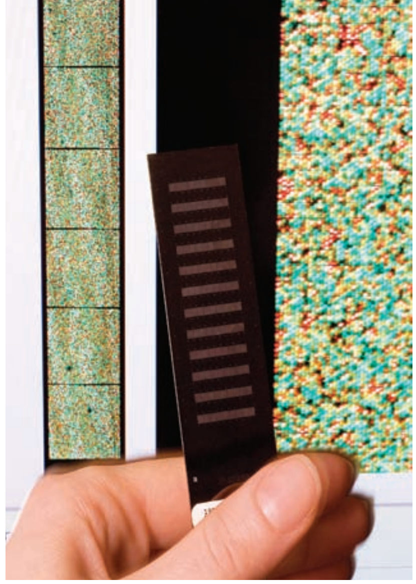
Identifying DNA Markers and Traits

ARS scientists at Clay Center, Nebraska, and Miles City, Montana, joined an international consortium in sequencing the bovine genome in 2002. Today, ARS scientists throughout the country are using this information to improve beef cattle management and production.

Some ARS researchers are using genomic research to improve animal health. This is particularly useful in situations with infected but asymptomatic cattle, says Mohammad Koohmaraie, former director of the Roman L. Hruska U.S. Meat Animal Research Center (USMARC) at Clay Center. For example, cattle can carry diseases like bovine respiratory disease (BRD) without having symptoms. This complicates attempts to assess their genetic resistance. Having ways to identify asymptomatic cattle or those at higher risk of illness would allow scientists to more accurately gauge how genes affect resistance.

To improve their assessments, USMARC researchers led by geneticist Larry Kuehn are working with scientists at the ARS National Animal Disease Center in Ames, Iowa, to develop large collections of cattle phenotypes, or observable traits. These

Definitions of Selected Genotypic Terms, Page 22



STEPHEN AUSMUS (D1158-1)

The SNP50 BeadChip. Each chip has the capability of testing just under 80 million DNA-coated glass beads, allowing ARS researchers to test 60,000 locations at once, with a substantial amount of intended redundancy to ensure accuracy.

include traits such as general immune-system functionality, body temperature, respiratory rate, and feeding behavior. The phenotypes will be drawn from populations representing prominent breeds in the U.S. beef industry.

"By examining a larger group of traits, we can more accurately classify animals into categories according to their potential disease risk or resilience," Koohmaraie says. This will enable researchers to identify traits that are most indicative of potential BRD risk and determine how those traits relate to genetic resistance to it.

One tool that could help scientists in this and other projects is the Illumina Bovine SNP50 BeadChip—a glass slide containing thousands of DNA markers called "single nucleotide polymorphisms," or SNPs, which are used to find relationships between DNA markers and traits of economic importance.

The BeadChip has research applications for both beef and dairy cattle. Design was led by ARS researchers at Beltsville, Maryland, in collaboration with scientists at Clay Center, the University of Missouri, and the University of Alberta in Canada. The chip is being used at all those locations and many others—a total of at least 23 locations in 11 countries.

A single chip generates about 53,000 genotypes for each of 12 individual animals. DNA samples from the animals are applied to the BeadChip, chemically labeled, and scanned to produce genotypes. Statistical analyses of genotypes can identify relationships between DNA markers and economically relevant production traits.

"Genomic tools like the 50K SNP chip will provide the greatest opportunity to transfer our genomic discoveries in a usable form to the industry," Koohmaraie says.

Beef Cattle: Fat and Feed Efficiency

One project using the BeadChip technology is a USMARC investigation into the influence of genetics on feed efficiency. Research leader Cal Ferrell, geneticist Mark Allan, and their colleagues are identifying phenotypes that relate to postweaning feed efficiency and lifetime productivity in beef cattle.

"One objective of the study is to determine the genetic variation in feed efficiency among individuals and breeds, using quantitative and genomic technologies," Ferrell says. The researchers are also using the genotypes generated from the chip to find relationships between DNA markers and phenotypes that can be used to enhance genetic selection in beef cattle.

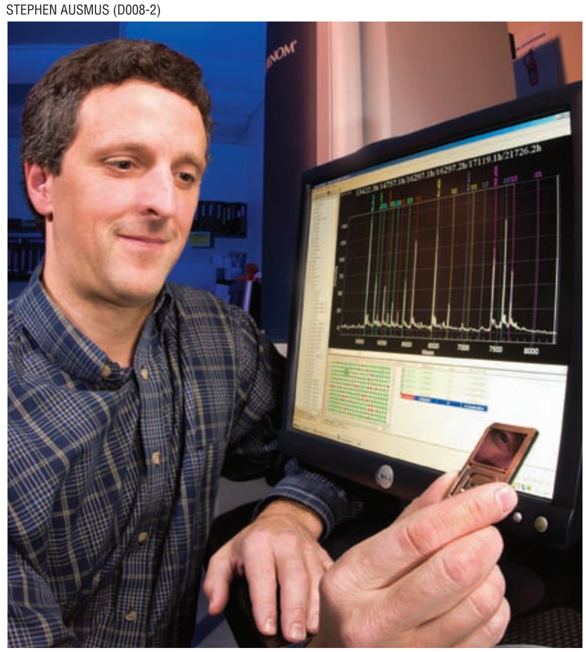
"These studies could lead to development of genomic tools that could enhance the accuracy of breeding and management decisions," Allan says. "Genetic markers provide opportunities to improve selection for traits that are difficult to measure in an industry setting."

ARS scientists are also using genomic research to improve beef cattle production at the Fort Keogh Livestock and Range Research Laboratory in Miles City, Montana. There, they have identified genetically significant areas called "quantitative trait loci" (QTLs) related to production traits such as beef quality and composition, feed efficiency, and reproductive success.

"Our work has led us to loci with significant effects on beef quality and composition, which have potential implications for human health," says geneticist Mike MacNeil. MacNeil, geneticist Lee Alexander, and physiologist Tom Geary have collaborated with USMARC geneticist Warren Snelling to analyze whole-genome scans of 328 cattle bred by crossing Wagyu and Limousin parents. Wagyu is a Japanese breed with substantially more marbling than the more muscular French breed, Limousin.

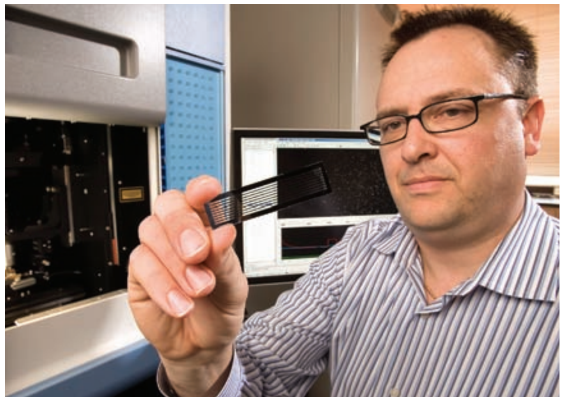
In all, the team has identified seven QTLs related to tenderness, palatability, and fat composition. They found a region on chromosome 2 that influences the concentration of monounsaturated fat—believed to be healthier than saturated fat—in beef. With further research, in collaboration with USMARC chemist Tim Smith, they hope to develop genetic markers associated with the variation in this trait. That could ultimately lead to identification of the gene or genes responsible and allow for marker-assisted selection in other cattle breeds to alter the fatty acid content of the meat.

STEPHEN AUSMUS (D008-2)



The chip that geneticist Gary Rohrer is holding allows him to evaluate more than 380 pigs for genetic variations at 6 different regions of the genome. This information will help determine which genes affect reproduction in pigs.

STEPHEN AUSMUS (D1150-1)



Geneticist Tad Sonstegard prepares to load a flow cell into a genome analyzer to generate a billion bases of DNA sequence for SNP discovery in cattle.

Cream of the Crop: Breeding Better Dairy Cattle

ARS scientists at Beltsville played a vital role in designing the BeadChip and are using it in genomics-based studies on dairy cattle. Beltsville geneticist Curt Van Tassell is leading development of a new genomic method to identify bulls that produce daughters with optimum milk production, calving ease, and other traits.

"Progeny testing," the method now used to determine a bull's genetic merit, is time-consuming and costly. At ARS's Bovine Functional Genomics Research Unit, Van Tassell, with ARS geneticists Tad Sonstegard and George Wiggans, is working to whittle down the cost of progeny testing to about $500 a bull.

Their approach is called "genome-enhanced improvement." It combines computer-aided statistical analysis with more than four decades' worth of records on dairy performance and conformation to help locate desirable genes.

Collaborating with the Beltsville team are professors Jerry Taylor and Robert Schnabel with the University of Missouri-Columbia; and Illumina, the San Diego firm that manufactures the BeadChip.

The researchers plan to examine a total of 53,000 SNPs from 12,000 cows and bulls representing several commercial dairy breeds and an ARS research population at Beltsville. Then they'll correlate SNP data to traits of interest, such as milk, fat, and protein production.

Eventually, information derived from the markers will help dairy producers streamline their identification and breeding efforts. And, Van Tassell says, cutting test costs while increasing the rate of genetic improvement in dairy cattle will help make the U.S. germplasm industry more competitive globally.

Healthy Chickens

Hans H. Cheng, a geneticist at the ARS Avian Disease and Oncology Laboratory in East Lansing, Michigan, worked with Michigan State University's Jerry Dodgson, a microbiologist, to build the chicken genome map. Now, Cheng and his colleagues are using the map to identify resistance genes to Marek's disease. These genes can be used to breed chickens with superior resistance. At the same time, the scientists have made molecular clones of the virus that causes the disease—a crucial first step to building more effective vaccines through biotechnology.

Since the initial sequencing of the chicken genome, many

T.A. OLSON (D1192-1)

Cool Cows

ARS genomics research is also helping beef cattle beat the heat, thanks to researchers at the Subtropical Agricultural Research Station (STARS) in Brooksville, Florida. They have identified molecular markers tightly linked to the Slick gene, which codes for short, sleek hair that helps keep cattle cool in subtropical heat.

The discovery is very important to the beef cattle industry, since it should greatly facilitate the Slick gene's introgression, or movement, into other economically important breeds, such as Holstein or Angus, to improve their heat tolerance. Many studies in Florida have shown slick-haired animals to have internal temperatures about 1°F lower during summer than contemporaries with normal hair coats.

Mapping the gene's locus is the first step towards identifying the mutation responsible for slick hair coat. STARS researchers have found a strong association between at least two closely positioned markers on chromosome 20 and the slick-haired phenotype. The markers were identified through DNA sequencing.

These results suggest a role for marker-assisted selection to identify homozygous Slick bulls—that is, sires with the same alleles—that will produce only slick-haired progeny. Some Senepol bulls were tested using these markers, and the results indicated excellent potential for identifying homozygous bulls. The same gene also appears to be responsible for the slick hair coat in Romosinuano cattle.—By Alfredo Flores, ARS.

chicken gene sequences have been entered into the National Center for Biotechnology Information's GenBank. This repository is one of the first places researchers look to see whether a gene sequence they are interested in has already been found and entered into the database.

Animal scientist Mark Richards, in the Animal Biosciences and Biotechnology Laboratory at Beltsville, is studying groups of genes involved in basic physiological functions, such as feed-intake regulation and nutrient use in poultry.

Of current interest is the gene that codes for the pancreatic hormone glucagon. Glucagon counterbalances insulin to regulate blood glucose levels in all vertebrates. It works to raise blood glucose that has been lowered by insufficient nutrition or stress.

The glucagon gene codes not only for glucagon, but also for other metabolic hormones, including the glucagon-like peptides 1 and 2 (GLP-1 and GLP-2). Each has unique physiological functions that are distinct from glucagon. Together, glucagon, GLP-1, and GLP-2 are contained in a precursor protein, called "proglucagon."

"The glucagon gene is an interesting one to study because it functions differently in chickens than in other vertebrate species," says Richards. For example, the glucagon gene is active in the pancreas of mammals, but in chickens, it's expressed in high

PEGGY GREB (K8765-5)

Geneticist Hans Cheng (left) and Michigan State University professor Jerry Dodgson examine genetic marker results. They are in the process of integrating genetic and physical maps, which helps verify the accuracy of both maps and enables more efficient isolation of important genes from the chicken genome.

STEPHEN AUSMUS (D1160-19)

Molecular biologists Caird Rexroad (left) and Gregory Wiens examine breeders in the selective breeding program at the National Center for Cool and Cold Water Aquaculture.

Fabulous, Fit Fish

ARS genomics research has been instrumental in identifying economically significant genes not only in livestock, but also in fish.

Scientists Caird Rexroad and Yniv Palti, at the National Center for Cool and Cold Water Aquaculture in Leetown, West Virginia, have developed tools for rainbow trout genome research. This includes construction of a genetic map that is being integrated with a physical map to facilitate alignment of the trout genome with the sequenced genomes of model organisms.

The scientists and their collaborators have developed functional genomic technologies to identify genes that affect disease resistance and stress tolerance. In addition, the entire genome of the bacterial pathogen *Flavobacterium psychrophilum*—which causes bacterial cold water disease—has been sequenced and annotated by molecular biologist Greg Wiens, microbiologist Tim Welch, and collaborators. This work should help identify virulence genes and vaccine targets. Bacterial cold water disease is a chronic and potentially fatal condition, especially in young fish.

How fish use nutrients is another important aspect of aquaculture genomics. For fish geneticist Ken Overturf and colleagues at Hagerman, Idaho, the ideal rainbow trout's genetic makeup would allow it to thrive well on feeds that provide nutritious proteins and oils from plants instead of from fish. That would give growers of plant crops a new market and reduce aquaculture's reliance on ocean-going fish for trout-feed ingredients. A study that involved testing about 1 million rainbow trout identified a genetic characteristic that distinguishes those that may grow well on plant-based feed.

ARS research has also promoted the use of genomics to identify fish breeds. At Stoneville, Mississippi, scientists in the Catfish Genetics Research Unit—led by research leader Kenneth Davis, molecular biologist Geoff Waldbieser, and geneticist Brian Bosworth—have developed a genotyping system to identify blue catfish, channel catfish, and their hybrids as early as 1 day after fertilization.

The Stoneville team has also developed genotyping systems to identify the parents of catfish spawn collected from communally stocked ponds, as well as individual catfish strains. Now the scientists are developing molecular methods to determine the sex genotype of hormonally feminized channel catfish. It's a big step toward developing a YY strain that will sire only faster growing males.—By Sharon Durham, Marcia Wood, and Alfredo Flores, ARS.

levels in the stomach too. Another difference is the number of precursor proteins produced by the glucagon gene. In chickens and mammals, the gene codes for a precursor protein that contains glucagon, GLP-1, and GLP-2. Richards's team found that in chickens, it codes for an additional protein that contains just glucagon and GLP-1.

"The differences may be linked to the high levels of circulating glucose found in birds, which are more than twice the levels that are found in humans," says Richards. "Such differences may help explain why chickens appear to be more resistant to insulin than mammals."

ARS research is also shedding light on poultry pathogens, such as the parasite *Eimeria*. *Eimeria* causes coccidiosis, a disease that costs U.S. poultry producers more than $700 million annually. ARS immunologist Hyun Lillehoj is using genomics to decipher the molecular interactions between poultry and several strains of *Eimeria* that commonly infect poultry.

STEPHEN AUSMUS (D1144-4)

Using a microarray gene scanner, (left to right) immunologist Hyun Lillehoj and molecular biologists Duk Kyung Kim and Hong Yeong Ho investigate differential immune gene expression of two different chicken lines after the chickens were vaccinated against coccidiosis.

Working with her colleagues at the Animal Parasitic Diseases Laboratory in Beltsville, Lillehoj has identified key genes of poultry immune cells that respond to the presence of *Eimeria*. Some genes encode important cytokines and chemokines, molecules produced by white blood cells to kick-start the immune response after infection. These genes exhibit a heightened response the first time a bird is infected with *Eimeria*. But in later infections, these same genes have a much more modulated response. Sometimes their activity levels stay the same or even decrease below the levels observed in uninfected birds.

"Understanding how these different intestinal immune cells and molecular signals function in the face of *Eimeria* infection is a key step in developing new, effective vaccines," Lillehoj says. "These findings will go a long way towards helping us find ways to control coccidiosis without using antibiotics."

The Next Pig Thing

A farmer in Iowa wants to breed pigs that will produce lean, tender meat. An animal health inspector in North Carolina wants to identify the origin of a contagious virus that has spread throughout the state. A consumer advocacy group in California wants to verify the labeling accuracy of name-brand pork products available at local stores. Recent advances in swine genomic research mean that such goals are easier to achieve than ever before.

USMARC scientists are collaborating with an international coalition of researchers to sequence the swine genome. This has led to expansion of publicly available DNA-based tools, which are facilitating improved production efficiency and animal health.

The coalition—the Swine Genome Sequencing Consortium (SGSC)—includes ARS; the USDA Cooperative State Research, Education, and Extension Service; the University of Illinois; the Alliance for Animal Genomics; England's Wellcome Trust Sanger Institute; Scotland's Roslin Institute; the Korean Livestock Institute; the Beijing Genome Institute; Iowa State University; North Carolina State University; the National Park Board; North Carolina Pork Producers; France's National Institute for Agricultural Research, and many other organizations. Since its inception in 2003, the SGSC has pieced together more than 267,000 segments across all pig chromosomes. This information can be viewed at http://pre.ensembl.org/Sus_scrofa/index.html.

"USMARC scientists have led the field in developing publicly available SNP markers," says animal geneticist Gary Rohrer, who's leading the ARS swine genome research efforts.

He and his colleagues at USMARC, the University of Illinois, Iowa State University, and the National Pork Board are developing a SNP chip similar to the one being developed for cattle. Though the swine genome hasn't been completely sequenced, the researchers have developed markers that enable them to identify individuals, determine parentage, and predict offspring performance.

ARS researchers have made many contributions to the international scientific community's understanding of livestock genomics. Their continuing input to this ever-expanding body of knowledge will have benefits for agriculture throughout the world. —By **Laura McGinnis, Sharon Durham, Ann Perry, Jan Suszkiw,** and **Don Comis,** ARS.

This research is part of Food Animal Production (#101) and Animal Health (#103), two ARS national programs described on the World Wide Web at www.nps.ars.usda.gov.

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JAMES MOODY (D1348-1)

Lab Saves Water While Researching New Ways To Fight Weeds

ARS ecologist Adam Davis inspects one of the sample tubes of a seed-bank elutriator. This elutriator can process hundreds of soil seed-bank samples a day, washing away soil particles and leaving behind weed seeds for further study.

Some weed species have a nifty survival strategy. In a given field, less than half of their total store of seeds will germinate, while the other portion remains dormant in soil. These so-called seed banks are waiting to sprout another day—long after the effects of herbicides have worn off, for example.

"Reducing seed banks of annual weeds such as common lambsquarters, giant foxtail, and common waterhemp is among the most important steps that farmers can take in managing weed populations in crop fields," says ARS ecologist Adam S. Davis. "But how to do that in a sustainable manner isn't known."

One possibility being explored by Davis and others in ARS's Invasive Weed Management Research Unit at Urbana, Illinois, is to encourage predation of weed seeds by birds, rodents, insects, and even bacteria. (See "Unearthing New Clues to Controlling Weeds," *Agricultural Research,* May 2006.)

But matching the right predator to its host weed requires considerable field data. Fortunately, the scientists can rely on a device known as an "elutriator." About the size of a gas grill, it fires high-powered jets of water into soil-core samples containing weed seeds taken 10 to 30 centimeters below the surface of crop fields. Scientists then collect the seeds to determine their species, number, health, and ability to germinate.

Up until a few years ago, says Davis, this seed sorting was done by hand over a sink with the faucet running. Now, that tedious and time-consuming process is carried out with high efficiency by the elutriator, which can handle 48 soil-core tubes. It blasts them with water at the rate of nearly 6 gallons a minute, or 360 gallons an hour, to extract the seeds inside. ARS plant physiologist Lori Wiles, in 1996, designed the current model Davis's lab uses.

"The elutriator is really important to our research. It gives us the capability to do seed-bank studies that few other laboratories have," says Davis. "But we were feeling guilty about using all that water."

Enter James L. Moody, the lab's biological science technician. After listening to the scientists' concerns, Moody took a closer look at the machine and saw room for improvement. By making a few modifications, including hooking up a submersible pump to the elutriator's settling tank and installing a water meter and a flow-limiting valve in the water line, Moody successfully put those worries to rest.

Indeed, in later trial runs and research, his modified elutriator consistently reduced the machine's water use by nearly 13 percent, or 50 gallons per hour, mainly by recycling the water. More tweaking is planned. In fact, says Davis, "We're aiming for a 50-percent reduction."—By **Jan Suszkiw,** ARS.

This research is part of Plant Biological and Molecular Processes (#302) and Crop Protection and Quarantine (#304), two ARS national programs described on the World Wide Web at www.nps.ars.usda.gov.

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STEPHEN AUSMUS (D1189-2)

Shotgun Proteomics

Detecting the Dairy Cow Immune Response Is a High-Tech Blast

In a milking parlor at the ARS National Animal Disease Center in Ames, Iowa, molecular biologist John Lippolis collects milk samples from a Jersey cow in an effort to answer basic questions about infection mechanisms in dairy cattle.

The term "shotgun proteomics" may sound like research conducted by scientists operating outside the law. But molecular biologist John Lippolis is using it to close in on the dynamics of the dairy cow immune system.

"I want to be able to find something that dairy farmers will incorporate into their management practices," Lippolis says. "They tend to be very cautious about adopting new practices unless there's a clear benefit."

Lippolis works at the Periparturient Diseases of Cattle Research Unit, which is part of the ARS National Animal Disease Center in Ames, Iowa. He is using proteomics—identifying the proteins that make up a cell—to identify and study neutrophils, the white blood cells that are a key part of the immune system.

He estimates that the neutrophil proteome—the entire collection of proteins produced by neutrophils—may have some 100,000 different types of proteins, so this is no small task. But it could provide critical information to use in the battle against mastitis, a bacterial infection costing dairy producers some $2 billion in lost milk production and related costs each year.

When dairy cows develop mastitis, neutrophils are among the first and most important cells of the immune response to fight the infection. Unfortunately, neutrophils are suppressed around the time that cows give birth, making the cow more susceptible to mastitis then.

Shotgun proteomics is a cutting-edge tool for conducting a scattershot and detailed search for key immune system proteins. Using this approach, Lippolis systematically surveyed the circulating bovine neutrophil to identify its neutrophil proteins and track how they change during infection. The alternative to shotgun proteomics is to study one protein at a time, which takes much more labor and time.

Pinpointing the Proteins

For his initial study, Lippolis obtained blood samples from 6 dairy cows, which yielded around 60 million neutrophils. After breaking the protein portion of the neutrophils down into individual peptides—small fragments of proteins—he set out to identify the most plentiful peptide groups in his samples.

Mass spectrometry can be used to identify low concentrations of different compounds in chemically complex mixtures. Peptides are transported into the mass spectrometer via fluid that passes through a tube—just the diameter of a tiny thread—at the rate of a millionth of a liter every minute.

Using mass spectrometry in combination with liquid chromatography, Lippolis was able to identify thousands of different types of peptides and use that information to identify hundreds of proteins. Nearly 35 percent were associated with basic cellular metabolic pathways, including most of the proteins involved in the production of cellular energy. Another 30 percent were involved in cell structure and mobility or in immune functions.

"We needed to do a lot of runs to make sure we found as many different proteins as possible," Lippolis says. "But we knew we'd miss a lot of the proteins that aren't present in significant numbers."

Still, Lippolis was confident he had found many of the major neutrophil protein players, and he proceeded to the next step—identifying significant changes in neutrophil proteins when infections like mastitis develop.

Change Is Not Always Good

Lippolis then compared neutrophil proteins in pregnant cows with those in cows that were in an immunosuppressive interlude after calving. Compared to the pregnant cows, he observed that the immunosuppressed cows had 40 proteins with notable changes in their expression. As a result of these changes, some cellular functions were stepped up, while others were diminished.

Lippolis also compared neutrophils in periparturient cows—those in an immunosuppressive interlude—with cows that had been given the steroid dexamethasone to artificially suppress immune function. Though he found some similar changes in protein expression in both groups, there were also some significant differences between them.

"This suggests that neutrophils don't have a one-size-fits-all response to immunosuppressive events," Lippolis notes. "We need to keep these differences in mind when we develop models for studying dairy cow immunosuppression."

Know Your Enemy

Lippolis then approached his research from another angle—examining neutrophils and the bacterial strain of *Escherichia coli* responsible for mastitis infections. He cultured the pathogen in either milk or a standard laboratory broth and found more than 1,000 bacterial proteins, 30 percent of which exhibited expression changes.

One finding focused on the molecular back-and-forth between *E. coli* and lactoferrin, a protein found in both milk and neutrophils. An adequate supply of iron—which acts as a catalyst for bacterial growth—is needed to support *E. coli's* survival in any environment. Lactoferrin responds to infection by sequestering the free iron in milk, which limits the iron available to *E. coli*. But the up-regulation of some of the *E. coli* proteins Lippolis observed tripled the pathogen's ability to bind to iron, which significantly upped its chances for survival.

A protein involved in *E. coli's* osmotic regulation was also up-regulated when the bacterium was grown in milk. Researchers believe osmotic regulation may play a role in the expression of genes regulating virulence and may also affect bacterial growth in some way.

These findings about *E. coli* proteins may give researchers new paths to explore in developing antibiotic therapies to treat mastitis. Dairy farmers would welcome the new tools—and Lippolis would be able to fulfill his wish of helping improve their dairy production practices. He's pleased with his progress, but he's hoping for more in the future.

"The biggest challenge we face in studying the dairy cow proteome is that we don't have comprehensive data for the bovine genome," he says. "Forty percent of the proteins we identified in the first study were bovine proteins, but the rest were homologous to proteins found in humans, mice, or rats. When we have a complete bovine genome and understand the function of all the proteins, we'll be able to fully use proteomic tools to improve dairy cow health."—By **Ann Perry,** ARS.

STEPHEN AUSMUS (D1188-18)

John Lippolis uses a tandem mass spectrometer, which collects the data necessary for proteomics.

This research is part of Animal Health, an ARS national program (#103) described on the World Wide Web at www.nps.ars.usda.gov.

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Definitions of Selected Genotypic Terms, Page 22

Multiplying *Metarhizium*

Scientists tap sturdy new form of fungus for better biopesticides.

Metarhizium anisopliae is one mold you might not mind having around. Instead of growing on bread or shower curtains, it prefers the bodies of ticks and insect pests, such as termites, locusts, tsetse flies, and others.

Indeed, a *Metarhizium* strain dubbed "F52" is the chief active ingredient in four federally registered mycoinsecticide products for controlling soft-bodied ticks and certain beetles and weevils.

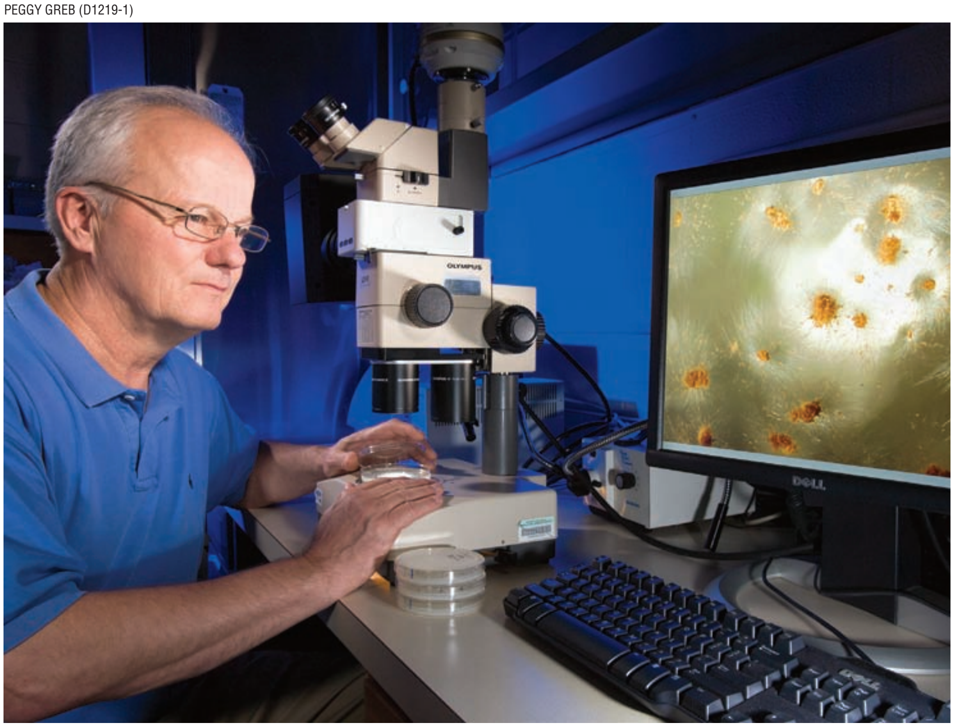
Now, even better mycoinsecticides—targeting soil-dwelling insect pests—could be on tap, thanks to ARS scientists' discovery that *Metarhizium* can produce specialized clumps of fungal cells called "microsclerotia."

Agricultural Research Service microbiologist Mark A. Jackson and ARS entomologist Stefan Jaronski made the discovery in 2004 and have since developed a patent-pending method of churning out billions of microsclerotia inside vats called "fermentors."

STEFAN JARONSKI (D591-1)

The fungus *Metarhizium anisopliae* sporulating on a sugarbeet root maggot.

PEGGY GREB (D1219-1)



Microbiologist Mark Jackson evaluates spore production by air-dried microsclerotia (rewetted and incubated on water agar here) produced by *Metarhizium anisopliae.*

Making More and Tougher Fungi

Before the duo's finding, only plant-disease-causing fungi like *Sclerotinia sclerotiorum* were reported to produce microsclerotia—not their insect-infecting brethren.

"We found with *Metarhizium* that we could produce these sclerotial bodies in liquid culture under certain conditions," says Jackson, with ARS's National Center for Agricultural Utilization Research in Peoria, Illinois. "The advantage of this is that we can now make a form of this fungus that can survive drying and storage for easy application by farmers to the soil to kill insects."

Traditionally, the form of choice for making mycoinsecticides has been the conidia, or spore, which forms thin tubes that penetrate an insect host's outer shell, or cuticle. The fungus only infects certain insect hosts, however, and never people, pets, or livestock.

"*Metarhizium's* conidia are like little time bombs," explains Jaronski, with ARS's Northern Plains Agricultural Research Laboratory in Sidney, Montana. "They don't germinate until they contact the insect cuticle. Then, they use a combination of mechanical pressure and a cocktail of enzymes to breach the cuticle and invade the insect's circulatory system. The infected insect invariably dies within a few days."

In a standard production approach, *Metarhizium* is grown on nutritious cakes called "solid substrate." The fungus produces abundant conidia, which are then collected, dried, and coated onto granules made of corn grits or other granular carriers or mixed directly into the soil. But the solid-substrate approach is time consuming and labor intensive for this purpose, notes Jackson.

Microsclerotia—tight bundles of pigmented fibers that resemble pepper flakes—are a far tougher form of fungus. Moreover, they serve as a safe haven on which *Metarhizium's* conidia can be readily produced to infect insects that get too close while crawling about in the soil.

Other researchers have produced granules from air-dried, regular mycelium (the fungus's main body) or mycelium encapsulated in a polymer, says Jaronski. But these forms suffer from poor shelf life or cost too much for most farmers.

Cheaper and Faster, Too

In studies at Sidney using *Metarhizium* strain F52, conidia-only granules germinated 7-10 days after being applied to soil. Microsclerotia-based formulations germinated within 4 days and produced greater numbers of spores.

Jackson partly credits the microsclerotia's increased germination rate to their ability to tolerate lower soil moisture. Another factor may be the sheer number of microsclerotia that can be produced and applied to the soil using the liquid-culture technique he and Jaronski developed.

The researchers' current microsclerotia production rate is 30 grams of wet fungal biomass (fermentation material containing fungal cells) per liter in about 4 days. Solid-substrate systems, by comparison, take 2 weeks to produce commercial quantities of conidia, and more time is needed to prepare the granules. "The solid-substrate system has its place in the production of some fungi, but it's more costly," says Jackson.

Microsclerotia can also be formulated into granules and sized more easily than conidia-based formulations. This makes microsclerotia more compatible with farmers' seed planters and pesticide granule applicators. Biopesticide makers stand to benefit, too: "Using microsclerotia should allow companies that make mycoinsecticides to get into markets where, ostensibly, the size and shape of their products have kept them out," Jaronski says. Microsclerotial granules should also readily qualify for the organic crop market, whereas the binders used with conventional granular carriers disqualify those granules, he adds.

A Moldy Match for Maggots

Since 2004, Jaronski has teamed with North Dakota State University scientists in Fargo to test conidia-coated corn-grit granules of F52 against the fly species *Tetanops myopaeformis,* whose maggot stage is a top pest of sugar beets nationwide.

The results of their tests have been encouraging, especially when *Metarhizium* is combined with oat or rye cover crops as part of an integrated pest management approach. (See "Beeting Back the Enemy," *Agricultural Research,* Sept. 2006, pp. 16-17.)

"Under low insect pressure, these fungi work as well as the insecticide terbufos," Jaronski reports. "For high insect pressure, we're looking at integrating the fungus with a live cover crop. So far, the two are giving us significant protection with no loss of yield."

In 2006, Jaronski began comparing conidia-only corn-grit granules with microsclerotia-based ones derived from the liquid-culture method for which ARS filed a patent in September 2007.

In laboratory assays, around 25 percent of sugarbeet root maggots exposed to the spores produced on corn-grit granules in clay soils had died by 3 weeks. In microsclerotia-treated soil, 100 percent were dead in the first week. These observations reflect the faster and greater conidia production by microsclerotia in soil. During 2007 field trials, beets in microsclerotia-treated plots also suffered fewer scars from maggot feeding.

Oddly enough, the researchers have been unsuccessful in using their liquid-culture technique to obtain microsclerotia from other insect-killing fungi used as biological control agents—notably *Beauveria bassiana* and *Paecilomyces fumosoroseus.* But they *were* able to produce microsclerotia with several different strains of the *Metarhizium* fungus.

"That's one of the strange things about these microsclerotia," says Jaronski. "The process for producing them only works with *Metarhizium.*" That hasn't deterred a major biopesticide maker from taking notice, though. "The technique is applicable not just to sugarbeet root maggots, but to any soil-dwelling pest that's attacked by this fungus," Jaronski says.—By **Jan Suszkiw,** ARS.

This research is part of Crop Protection and Quarantine (#304) and Quality and Utilization of Agricultural Products (#306), two ARS national programs described on the World Wide Web at www.nps.ars.usda.gov.

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STEFAN JARONSKI (D1221-1)

Healthy sugarbeet root maggots (about 1/4 inch long), fat from feeding on and severely damaging a sugar beet root. In this stage, they will overwinter to pupate and emerge the following spring.

"Tunnel Vision" Tracks Emissions

In the basement of the ARS National Soil Tilth Laboratory (NSTL) in Ames, Iowa, a tunnel resting on a waist-high platform—and large enough for a person to fit in comfortably—stretches down a side hallway.

"We got it secondhand from an out-of-state university," soil scientist Tom Sauer says. "It had been used for heat-transfer studies, and it was scheduled for disposal."

Sauer and his colleagues are using the tunnel to model how air emissions from animal-production facilities travel across the landscape and to develop strategies for minimizing the impact of these emissions.

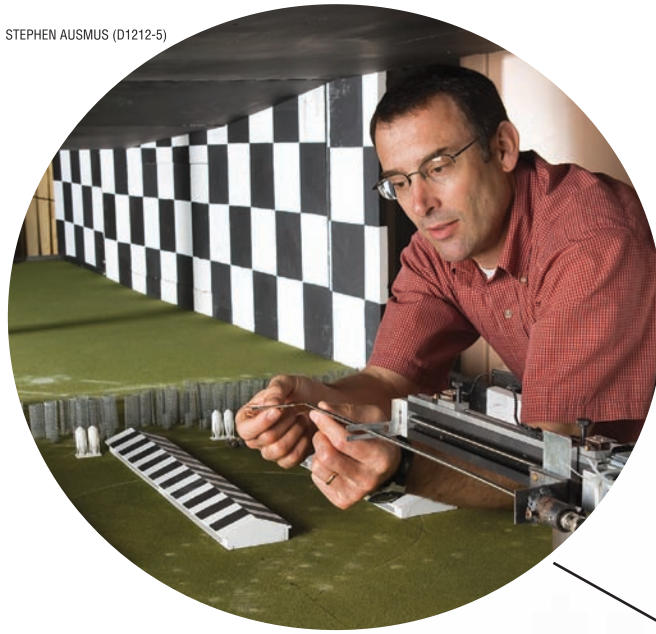
Air flows across the land the way water flows over and around rocks and other barriers in a stream. As winds approach a building, storage tank, or other structure, the air currents accelerate around the sides and over the top of the structures. The shift in speed creates new disturbances in the downwind airflow.

These dynamics dictate the effectiveness with which air travelling over an animal-production facility can pick up and transport problematic emissions from manure, dust, and other sources. Some of the prime offenders are ammonia and hydrogen sulfide, which have noxious odors, and methane and nitrous oxide, which are both greenhouse gases. Emission of tiny particulate matter—now known as a hazard to human health—is also a concern.

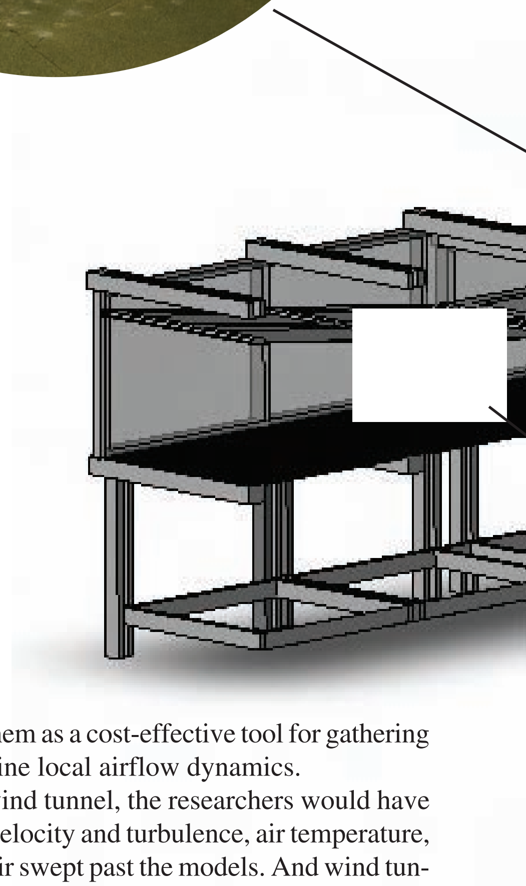
These emissions can come from buildings, fields, liquid-waste lagoons, or manure pits. Wind speed and direction, topography, structures, facility management, climate, and vegetative cover all affect airflow—and affect where these emissions end up. Even the smallest facility will have a range of variables that significantly affect the amount of emissions that are transported, the strength of those emissions, and their final destination.

"Carrying out air emissions testing in an actual animal-production environment is expensive, and practically speaking, it's just difficult," Sauer says. "And if we do conduct studies at an actual facility, our results can only be applied to that facility and to the conditions that were present during the tests. Any data we collect will have limited value for developing general models of emission transport."

STEPHEN AUSMUS (D1212-5)



With most of his upper body inside the wind tunnel, soil scientist Tom Sauer exchanges one of the highly sensitive sensor tips in the hot film anemometer before one of many experiments measuring airflow and turbulence.



Studies on a New Scale

So Sauer and plant physiologist Jerry Hatfield—who is also the NSTL director—embarked on a 3-year study that involved a series of wind tunnel tests. They wanted to see how the location and placement of buildings and waste-storage facilities affect the transport of odor constituents like ammonia and hydrogen sulfide. The National Pork Board contributed financial support for this research.

Previous tests with enclosed wind tunnels—where the variables of dynamic airflow can be monitored on scaled-down versions of landscapes—have shown that these scale model studies provide accurate and reproducible assessments of field conditions. Wind tunnels had rarely been used to study agricultural buildings, but Sauer and Hatfield saw them as a cost-effective tool for gathering data that would help define local airflow dynamics.

With a low-velocity wind tunnel, the researchers would have the ability to change air velocity and turbulence, air temperature, and the angles at which air swept past the models. And wind tunnel studies could complement data gathered at animal-production facilities by recreating similar conditions in a physical model and then repeating measurements with changing variables.

"We wanted to find out whether aboveground manure-storage facilities and lagoons that are located downwind of buildings or other structures are exposed to increased wind speed and turbulence—or if the upwind structures actually protect them," Sauer explains.

Sauer arranged for an existing wind tunnel to be rescued and brought to Ames, where it took a year to reassemble and modify. When the 40-foot tunnel was ready for action, a 6-inch-square checkerboard pattern was painted along one wall to provide scale for photographs and flow-visualization tests.

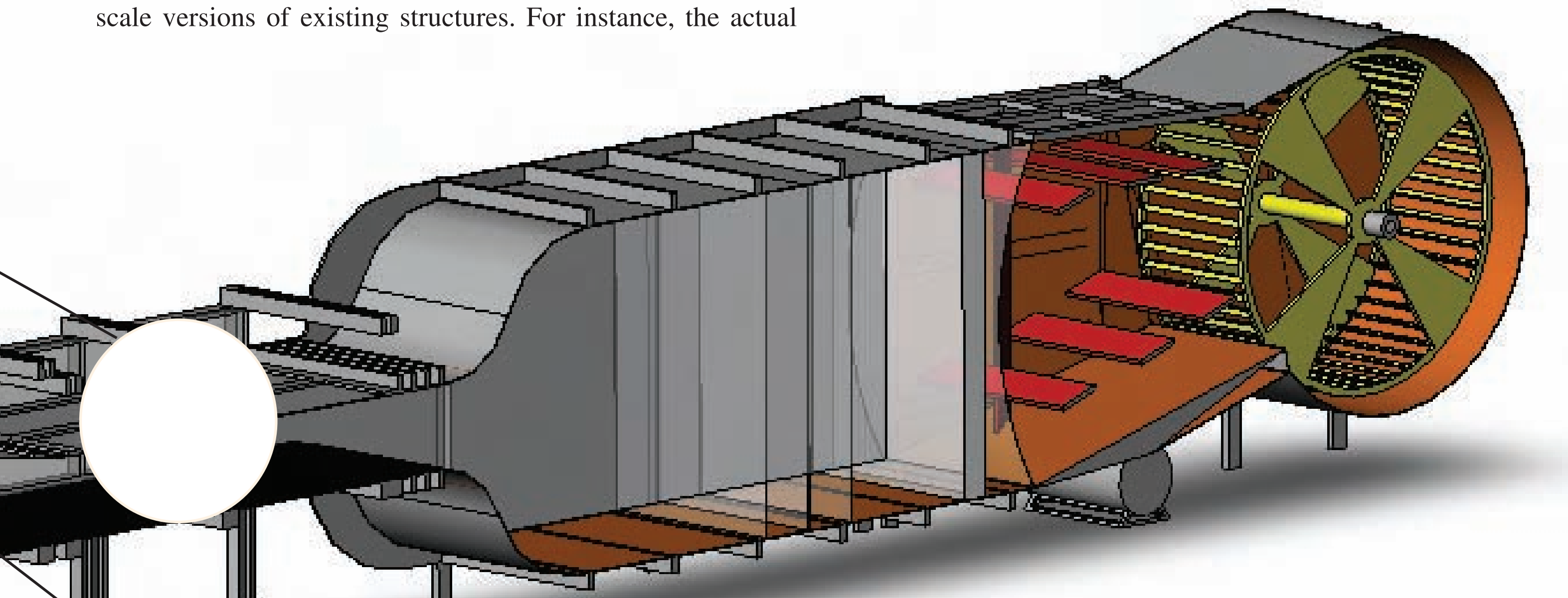
The heart of the wind tunnel is a 1950s Army surplus blower with a 15 HP electric motor located at one end to generate maximum windspeeds of 30 miles per hour over the scale models.

A Model Farm

Sauer and Hatfield then constructed their test farm, complete with scale replications of swine-finishing units, above-ground slurry tanks, and lagoons. Their balsa wood models were 1:300 scale versions of existing structures. For instance, the actual pig-finisher units modeled were about 40 feet wide and 200 feet long, with maximum 17-foot peak heights.

The scientists arranged four of these model buildings on their "farm" in several different configurations with the model storage tanks. All the structures had magnets on the bottom, which allowed the scientists to easily reposition the models—and kept them from being tossed around by the variable breezes.

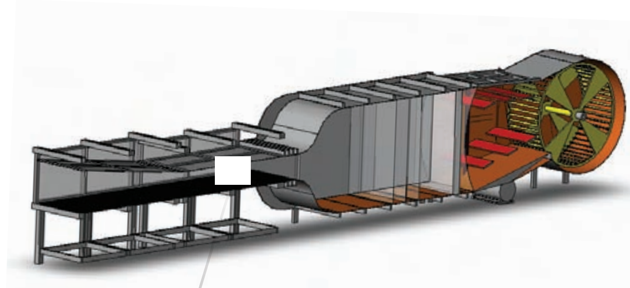
The floor of the tunnel near the models was covered with a vinyl mat—the kind used by model railroaders—which mimicked a groundcover of cut grass. In some tests, a windbreak, created from eight rows of 2-inch-high wire-mesh model trees, was positioned upwind of the building models and downwind of the manure-storage models.



At the National Soil Tilth Laboratory in Ames, Iowa, a low-speed wind tunnel was designed and built to determine the effects of farm buildings and surrounding landscapes on agricultural emissions. Drawing by Jadon Kool.

STEPHEN AUSMUS (D1213-10)

To better interpret airflow patterns around physical structures, plant physiologist Jerry Hatfield (left) and Tom Sauer use smoke to help them observe airflow patterns inside the wind tunnel.



Sauer and Hatfield also set up obstacles to create a surface boundary layer of air that would mimic the effects of Earth's atmospheric boundary layer. An array of triangular spires and a short fence upwind of the model facility helped to generate air turbulence of the correct scale and intensity, which then flowed through and around the obstacles.

Models of the lagoons and storage tanks presented a different challenge. The scientists did not want to use actual odor-generating vapors in their studies.

"We're in a laboratory facility," Sauer notes. "We figured the other people here wouldn't appreciate the authenticity." Instead, they used water vapor or smoke from dry ice to stand in for ammonia and hydrogen sulfate emissions.

When the miniature stage was finally set, they turned on the fan and held onto their hats.

A Mighty Wind

Air flow velocities and turbulence intensities were measured with a sensor attached to a robotic arm that could move in all directions. The sensor—which consisted of a very fine quartz-coated wire—could be heated to more than 900°F, and it measured how quickly the winds carried heat away at 83 points behind the building models.

STEPHEN AUSMUS (D1214-20)

Using model farm buildings, silos, and trees (wire mesh coils serve as trees), agronomist Guillermo Hernandez (left) and Tom Sauer evaluate the effect of model arrangements on airflow. Hernandez makes an adjustment to one of the highly sensitive probes as Sauer monitors the real-time data signal.

Sauer and Hatfield took pictures of the smoke patterns generated by the dry ice to capture airflow patterns around the model structures. They also measured evaporation rates from the model storage tanks and lagoons. For some of their trials, they added a "hill" and repositioned some of the other structures to assess the effects of surface roughness and topography on airflow and emissions transport.

After the winds died down, a computer program sorted out the data generated by the range of velocity patterns and turbulence intensities. The researchers found that when livestock buildings were situated parallel with airflow, small and discrete turbulent wake zones resulted. These zones converged at higher velocities, and partially dissipated by the time they reached the equivalent of 170 feet downwind.

But buildings situated perpendicular to airflow created a larger—and taller—turbulent wake zone and had a significant downwind flow that persisted out to the same distance.

Sauer and Hatfield also found that structures on average slowed air velocity by around 67 percent. Not surprisingly, buildings placed perpendicular to the airflow had the greatest effect, while buildings at a 30-degree angle only slowed air velocity by around 38 percent.

"These studies show how much the placement of animal housing units and manure-storage facilities can work in combination with prevailing winds and site conditions to affect the distance that potential agricultural air emissions can travel," says Sauer. "They strongly indicate that we should be able to reduce the downwind air-quality impacts from animal production by modifying the layout of a production facility."

"Now that we have wind tunnel observations on airflow velocity patterns, we'll also be able to suggest an optimal placement for field sensors when we carry out studies on actual facilities," Hatfield adds.

These findings also show that producers could derive a direct—and permanent—benefit of improved air quality with just the one-time cost of figuring out the best building placement.

"In our air-emissions research, we're dealing with the same type of questions that water-quality scientists work with," Hatfield notes. "How do agricultural practices fit into the landscape? How do they help producers and benefit the environment? We're trying to find the best ways to reduce the environmental footprint of agriculture and to enhance our natural resources."—By **Ann Perry,** ARS.

This research is part of Air Quality, an ARS national program (#203) described on the World Wide Web at www.nps.ars.usda.gov.

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Milkweed: From Floss to Fun in the Sun

Milkweed is popularly known as a favorite food of the monarch butterfly caterpillar. Historically, though, farmers considered milkweed a noxious weed. Today, some farmers actually cultivate milkweed for its soft, silky floss, which is used commercially as a hypoallergenic filler in high-end pillows, comforters, and jacket linings.

But floss isn't the only useable portion of this native American plant, which grows throughout the country. In studies at ARS's New Crops and Processing Technology Research Unit in Peoria, Illinois, chemist Rogers E. Harry-O'kuru is experimenting with new, value-added uses derived from unsaturated oil in the seed of common milkweed, *Asclepias syriaca.*

Harry-O'kuru's analysis of the waxes and different fatty acids in the oil shows it has potential use as a base material in sunscreen, cosmetics, and skin- and hair-care products, including moisturizers and conditioners.

Many of today's sunscreens use chemical filters or blocks to protect skin from two types of ultraviolet radiation, UV-A and UV-B, at wavelengths of 290 to 400 nanometers (nm). The effects of UV-B exposure are usually temporary—an example being the sunburn a careless beachgoer must endure for a few days. Repeated or prolonged exposure to UV-A radiation—such as that experienced by lifeguards or road crews—can cause premature aging and skin cancer. The filters and blocks work by absorbing or scattering such radiation before it penetrates and damages skin.

Recently, interest has grown in sunscreen and cosmetic products that not only protect skin, but nourish it. Harry-O'kuru's milkweed-oil-based sunscreen aims to fill the bill on both counts. It contains natural antioxidants, such as tocopherols, and cinnamic acid derivatives like ferulic acid, which occurs naturally in many plants and is highly absorbent of UV rays.

A key step in the process, which ARS has patented, is using zinc chloride to catalyze the conversion of milkweed oil's triglycerides into the UV-absorbing cinnamic acid derivatives.

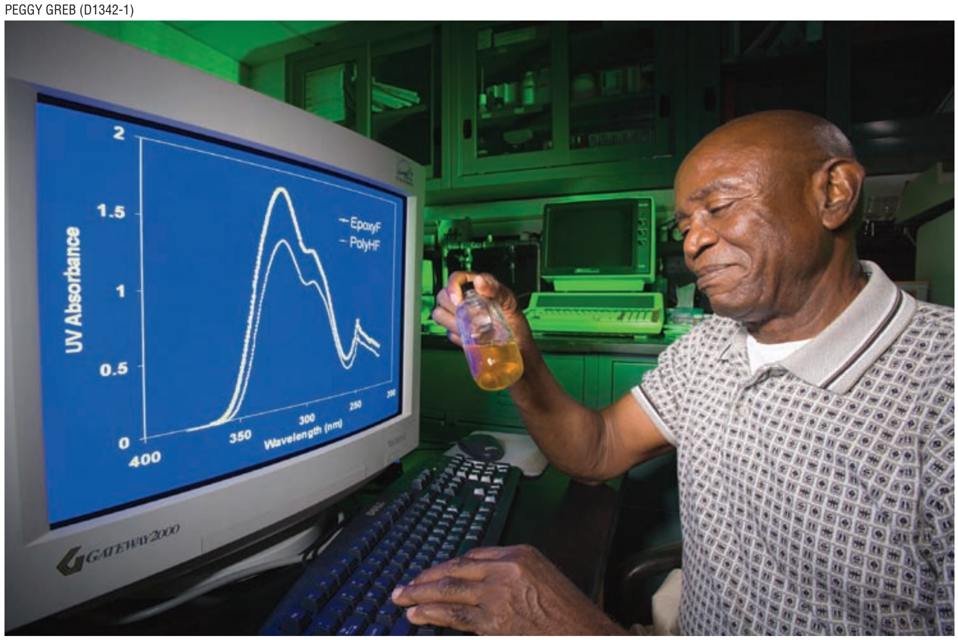
In laboratory tests, the derivatives strongly absorbed UV rays in the range of 260 to 360 nm, wavelengths that can damage skin. The milkweed-oil product accomplished this at very low concentrations (1 to 5 percent by weight)—a range far below that approved for today's topical skin formulations, says Harry-O'kuru.

Harry-O'kuru says his current sunscreen compounds are clear liquids, but gels, creams, sticks, and aerosol sprays are also possible. The sunscreen's unique combination of fats and waxes may qualify it as biodegradable and may keep it from washing off during a swim.

Besides skin- and hair-care products, the UV-absorbent formulation could also be tailored for use in epoxies, paints, or other industrial applications.

Says Harry-O'kuru, "We're hoping a stakeholder will become interested in developing the technology further."—By **Jan Suszkiw,** ARS.

PEGGY GREB (D1342-1)



Milkweed oil has potential use in sunscreens because it can protect skin from two types of ultraviolet (UV) radiation. Chemist Rogers E. Harry-O'kuru studies UV radiation absorbance spectra of modified milkweed oils.

This research is part of Quality and Utilization of Agricultural Products, an ARS national program (#306) described on the World Wide Web at www.nps.ars.usda.gov.

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Brachypodium

A Little-Known Grass Gains Research Fame

David Garvin may be the first U.S. scientist to pursue research using a wild grass known as "purple false brome," *Brachypodium distachyon,* as a model plant.

Garvin, a plant geneticist with the ARS Plant Science Research Unit in St. Paul, Minnesota, learned of the short-statured plant's possibilities from researchers working in Europe. Early in 2002, he started his own investigations to explore its potential to serve as a model, or surrogate, for its close relatives—including wheat, rye, and barley.

Unlike these familiar crops, *Brachypodium* has a small genome—about one-fiftieth that of wheat, for instance.

Garvin's goal—then and now—is to use *Brachypodium* to speed discovery of genes that will improve wheat's resistance to diseases such as rusts, *Fusarium* head blight, and others that can cause dramatic yield losses.

"Since *Brachypodium* is closely related to wheat, barley, and other small grains," Garvin says, "pinpointing genes for important traits such as disease resistance in *Brachypodium* should help us quickly find those same genes in these major crops. We expect their functions and relative locations on *Brachypodium* and wheat chromosomes to be about the same."

Garvin's work attracted the attention of plant molecular biologist John Vogel at the ARS Western Regional Research Center in Albany, California, near San Francisco. Vogel saw the value of using this new model plant to improve energy crops such as switchgrass—a *Brachypodium* cousin.

Vogel's intent: To discover how to more quickly and inexpensively break down the plant cell walls that make cellulosic ethanol costly to produce.

With other ARS colleagues, Garvin and Vogel have made ARS a world leader in getting *Brachypodium* adopted as an international model for grass-crop research. Seeds of *Brachypodium* plants that Garvin developed specifically for research have been sent freely to scientists in 25 states and 20 nations. According to Garvin, these genetic stocks are now being used for innovative genetic, genomic, physiological, and molecular biological research for grass-crop improvement.

And, thanks to his lab and greenhouse experiments, some of these research-ready plants now have an impressive seed-to-seed turnaround time of less than 8 weeks.

One of Garvin's genetic stocks, for example, was chosen for use in an international venture to sequence the *Brachypodium* genome. Based at the U.S. Department of Energy's Joint Genome Institute in Walnut Creek, California, the project will reveal the structure of all the plant's genes and other genetic material. Garvin, Vogel, and Michael Bevan of the John Innes Center in the United Kingdom are co-directors of this genome journey, which has already yielded a rough draft and is expected to produce an improved version sometime this year.

PEGGY GREB (D1217-1)

Geneticist Yong Gu and molecular biologist John Vogel examine transgenic *Brachypodium* plants in a growth chamber. The plant in Gu's hand is setting seed.

Brachypodium—Transformed!

In their California laboratories and greenhouses, Vogel, geneticist Yong Gu, and research leader Olin Anderson—all with the Genomics and Gene Discovery Research Unit—have used Garvin's plants in groundbreaking experiments.

Vogel, for instance, was the first to report the successful shuttling of new genes into *Brachypodium* via a bacterium, *Agrobacterium tumefaciens.* This biotech approach offers more precision than another popular method—one that relies on a gene gun to blast genes into plant tissue.

A good model plant accepts, and activates, genes that scientists move into it. The process, called "transformation," allows researchers to learn more about the genes—what they do inside the plant, and how they might be enhanced or perhaps silenced.

"An efficient transformation system is an absolute requirement for a model system," says Vogel, who recently hit a transformation rate of about 60 percent. It's among the highest reported for any grass.

For the most part, grasses remain very resistant to accepting genes that scientists—using techniques of modern biotechnology—would like to give them. "Grasses are notoriously difficult to transform," says Vogel. That makes the hard-won transformation rate for *Brachypodium* even more noteworthy.

A Map Takes Shape

Garvin's genetic stocks were just as essential to experiments that have yielded the first-ever physical map for *Brachypodium.* The map, developed by Yong Gu in collaboration with Ming-Cheng Luo at the University of California-Davis, depicts the location of neighboring, or contiguous, stretches of *Brachypodium* DNA.

The California team has pried yet more genetic secrets out of Garvin's plants. The researchers analyzed a portion of the plants' activated genetic material, or RNA, contained in bits of roots, stems, leaves, and other tissue snipped from seedling and fully grown *Brachypodium.* Their experiment identified indicators of active genes, or ESTs (short for expressed sequence tags). These ESTs act like signposts to guide scientists to genes that were actively working inside the tissue.

ESTs quicken the hunt for genes of interest. The Albany scientists identified more than 20,400 ESTs and posted them on the publicly available GenBank website, an encyclopedia of genomic information from the world's plants, insects, worms, humans, and other forms of life.

"This was the first significant genomic resource for *Brachypodium,"* says Vogel. "Until then, GenBank had only nine *Brachypodium* ESTs."

"Ready access to genetic and genomic research materials such as genetic stocks, maps, genes, and the genome sequence is required to make a stand-out model plant," Garvin says. "We're giving scientists worldwide that type of access. For the past 6 years, we've shared *Brachypodium* seeds, provided practical information and research data, and worked cooperatively with a large community of interested plant researchers here and abroad.

"All this has helped transform *Brachypodium* from a relative unknown to a worldwide favorite for grass-crop research."—By **Don Comis** and **Marcia Wood,** ARS.

This research is part of Plant Genetic Resources, Genomics, and Genetic Improvement (#301) and Bioenergy and Energy Alternatives (#307), two ARS national programs described on the World Wide Web at www.nps.ars.usda.gov.

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DAVID GARVIN (D1211-1)

Plant geneticist David Garvin harvests tissue of *Brachypodium* for DNA extraction in his laboratory on the campus of the University of Minnesota.

Banking on Biobutanol

New Method Revisits Fermenting This Fuel From Crops Instead of Petroleum

A modified method of producing biobutanol is in the works to make the fuel more competitive with ethanol as a clean-burning alternative to gasoline and other fossil fuels.

Biobutanol offers several advantages over ethanol. It can be transported in existing pipelines, is less corrosive and less prone to water contamination, can be mixed with gasoline or used alone in internal combustion engines, and packs more energy per gallon than ethanol.

Up until the mid-20th century, biobutanol was produced from fermented sugars, such as glucose in corn or molasses. But low yields, coupled with high recovery costs and the increased availability of petroleum feedstocks after World War II, sidelined fermentation-based systems for biobutanol production.

Today, petrochemicals still reign supreme as the feedstock of choice for making butanol, a four-carbon alcohol that's mainly used as an industrial solvent. But jumps in the price of oil have rekindled interest in tapping butanol as biobased fuel, notes Nasib Qureshi. He's a chemical engineer with the ARS National Center for Agricultural Utilization Research at Peoria, Illinois. Indeed, in June 2006, DuPont, Inc., of Wilmington, Delaware, and the British energy company BP announced joint plans to operate a United Kingdom-based production plant dedicated to producing biobutanol from sugar beets.

Turning Straw Into Biofuel Gold

Up until late 2003, Qureshi's own biobutanol studies dealt with new ways of fermenting glucose and other sugars from corn. But then he switched to wheat straw, drawn by its abundance and promise as a lower-cost alternative to glucose-based feedstocks.

"There's only so much you can do with sugar because of the competing uses for it," says Qureshi, who's in the Peoria center's Fermentation Biotechnology Research Unit. Wheat straw, by comparison, is typically left on crop fields after harvest to forestall soil erosion or used as feed or bedding for livestock—though new, value-added uses are being explored. The team is also working with barley straw, corn stover, and switchgrass.

Like other biobutanol processes, Qureshi's approach calls on species of *Clostridium* bacteria to carry out the critical task of fermentation. But before the bacteria can perform such work, the straw must be pretreated and hydrolyzed. The hydrolysis step uses enzymes to break apart the straw's cellulose and hemicellulose components. This liberates the simple sugars within so that the bacteria can ferment them into three products: acetone, butanol, and ethanol. Butanol is produced in the greatest concentration, but all three are valuable chemicals.

PEGGY GREB (D1240-2)

Chemical engineer Nasib Qureshi adds enzymes into a bioreactor to simultaneously hydrolyze wheat straw into simple sugars and produce butanol.

PEGGY GREB (D1241-1)

Technician John Michael Henderson combines dilute sulfuric acid with ground wheat straw as a pretreatment. The wheat straw is then autoclaved and transferred to a bioreactor for butanol production.

Three Steps in One

Normally, four preparatory steps (pretreatment, hydrolysis, fermentation, and recovery) are carried out separately and sequentially. But in his studies, Qureshi—together with ARS Peoria colleagues Michael A. Cotta and Badal C. Saha—devised a way to combine three of the four steps.

After the wheat straw has been pretreated with dilute sulfuric acid or other chemicals, the material is fermented in a bioreactor containing three different types of commercial enzymes and a culture of *C. beijerinckii* P260, a strain Qureshi obtained from Professor David Jones of the University of Otago in Dunedin, New Zealand.

Qureshi's approach allows the enzymes and bacteria to do their jobs simultaneously. As soon as the enzymes hydrolyze the straw and release its simple sugars, the bacteria set to work fermenting them. Throughout, a procedure known as "gas stripping" (the fourth step) is used to remove the acetone, biobutanol, and ethanol as they're produced. Gas stripping also protects the bacteria by keeping biobutanol levels from reaching levels harmful to them.

In early trial runs, the method increased biobutanol productivity by twofold above traditional glucose-based fermentation—but the bacteria fermented the sugars faster than the sugars became available. So, to ensure optimum performance, Qureshi found it necessary to feed small batches of additional sugar to the bioreactor.

Later studies showed that the adjustment, called "fed-batch feeding," significantly increased biobutanol production. During a 22-day fed-batch operating period, a culture of *C. beijerinckii* P260 converted nearly 430 grams of sugar (glucose, xylose, arabinose, galactose, and mannose) into 192 combined grams of acetone, biobutanol, and ethanol.

If scaled up further, the process could yield 307 combined kilograms, or 99 gallons, of acetone, biobutanol, and ethanol from 1 ton of wheat straw. The P260 strain produces a specific ratio of the three chemicals, but efforts are now under way at Peoria to develop genetically modified bacteria that will make only biobutanol.

Qureshi says he is planning to scale up production levels in 2009. "Then, we'll look at the economics of using hydrolyzed wheat straw to see how we're doing and move this process forward."—By **Jan Suszkiw,** ARS.

This research is part of Bioenergy and Energy Alternatives, an ARS national program (#307) described on the World Wide Web at www.nps.ars.usda.gov.

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Making Microbes Do More Work

ARS Fermentation Biotechnology Research Unit scientists Nasib Qureshi, Bruce Dien, and Loren Iten have another trick up their sleeves for making biobutanol. Together with Lars Angenent, a Cornell University professor in Ithaca, New York, and graduate student Matthew Agler, the team is developing a two-stage fermentation process for producing biobutanol from corn fiber.

The fibers are byproducts of corn wet-milling, but any plant material containing cellulose and hemicellulose will do. The first step involves pretreating the fiber with chemicals or hot water. Dien and Iten then ship the softened fibers to Angenent, who feeds the pretreated fibers to mixed cultures of different microbial species. By adjusting pH levels and other environmental factors, he coaxes the microbes to convert the fiber's sugars into butyrate, a butanol precursor. Angenent then ships the butyrate back to Peoria, where Qureshi uses his own special strain to make biobutanol.

"The enzymes usually used to convert pretreated biomass to fermentable sugars are very expensive. But the mixture of microbes used in this case to make butyrate produce their own enzymes. This means a huge potential cost savings," says Dien.

Unwelcome microbes pose a contaminant problem in traditional fermentation systems, he adds, but "we expect that the mixed microbial culture applied here will be more resistant to contaminants."

Dien and Qureshi's butanol research at Peoria falls under a competitive USDA grant.—By **Jan Suszkiw,** ARS.

PEGGY GREB (D1238-1)

Biochemical engineer Bruce Dien (left) loads reactor vessels with corn fiber while microbiologist Loren Iten lowers samples into a sand bath heater to pretreat for fermentation.

STEPHEN AUSMUS (D1235-10)

Soil scientist Doug Karlen instructs technician Tanya Ferguson (accompanied by her hearing guide dog) on how to visually assess soil quality impacts of harvesting crop residue as feedstock for bioenergy production. The foreground shows signs of severe soil erosion where about 90 percent of the stover was harvested.

Cellulosic Ethanol From Corn Stover

Calculating—and Improving—the Bottom Line

In the Midwest, 100 to 150 million tons of corn stover—crop residue—is now left on fields to prevent erosion and return nutrients to soil. Now corn stover is being eyed as a possible source of cellulose for biofuel production. But the costs and benefits of harvesting stover need to be determined.

"Crop residue is not just trash," says soil scientist Doug Karlen, who works at the ARS National Soil Tilth Laboratory in Ames, Iowa. "We need to find ways to develop site-specific practices for managing corn stover removal—not a 'big-box' approach to soil management. With the right approach, corn stover can have bioenergy benefits for U.S. consumers and producers alike."

Karlen, who is the research leader in the Soil and Water Quality Research Unit, is part of a national team conducting multiyear evaluations of the environmental and economic costs and benefits that might accrue from large-scale corn stover removal to produce ethanol. This project—the Renewable Energy Assessment Project, or REAP—is under way at sites in Alabama, Colorado, Indiana, Iowa, Minnesota, Nebraska, Oregon, Pennsylvania, and South Dakota.

Karlen recently finished a round of research that looked at how harvest practices affect fertilizer costs and the quality of the harvested stover for biofuel feedstock. His research team included Iowa State University engineer Stuart J. Birrell, Idaho National Laboratory (INL) scientist Corey W. Radtke, and ARS plant physiologist Wally Wilhelm. Wilhelm is in the Agroecosystem Management Research Unit in Lincoln, Nebraska.

It's All in the Cut

In 2005, this group—along with INL scientist Reed L. Hoskinson, who has since retired—established experiments in cornfields near Ames and then harvested the cornstalks at four different heights to measure the amount and quality of stover that could be harvested using different removal strategies.

The scientists varied the amount of biomass removed by changing the type and cutting height of the combine head.

Their "high-cut top" harvest was obtained using a row-crop head and cutting the plants just below the cob so that only the cob and plant parts above it entered the combine. This left a 30-inch stubble behind on the field.

The "normal cut"—which used a standard harvester head with snapping rollers—left only 16 inches of stubble. But this cut did not increase the amount of harvested biomass, because more plant material was pulled through the rollers and left on the ground.

"Low-cut" harvests—which reaped almost the entire cornstalk and the cob—were also made with the row-crop head and left only about 4 inches of stubble. A second low-cut harvest, called "high-cut bottom," took place after the high-cut top harvest. The collected biomass from this cut consisted only of the lower 30 inches of cornstalks and any remaining leaves. In all of the harvest scenarios, the grain was separated from the cob before the researchers started their assessments.

After the harvests were complete, the scientists evaluated factors such as how stover removal could potentially affect future crop production and soil quality, how potential ethanol production might vary with harvest protocol, and how to deal with engineering challenges associated with harvesting.

The researchers found that the base of the high-cut bottom feedstock was around 64 percent water, which decreased its value as a feedstock. Any biomass with high water content is generally more expensive than dry biomass to harvest, store, and transport to an ethanol conversion facility.

The team also found that stover removal resulted in per-acre losses of up to 45 pounds of nitrogen, 2 to 4 pounds of phosphorus, and 23 to 38 pounds of potassium. In some soil types, these losses could result in long-term potassium deficiencies that would reduce crop productivity unless the fields were amended with fertilizers.

Translated into dollars, the low-cut harvest scenario could cost producers $25 to $30 per acre, depending on their fertilizer costs. Compensating for loss of other soil nutrients—including calcium, magnesium, iron, zinc, copper, and manganese—would increase producer costs even more.

Conversion Calculations—Cobs and All

Stover from the four harvest groups was then converted to fuels via thermochemical processing. Karlen's team measured the resulting energy yield and decided that the most likely factor driving conversion efficiency was the level of moisture in the feedstock.

The team also used a screening method to estimate how the four groups of stover responded to chemical pretreatment. These pretreatments partially break down feedstock, making the plant sugars more easily accessible for fermentation.

STEPHEN AUSMUS (D1236-13)

Soil scientist Doug Karlen (left) discusses commercial combine modifications that Stuart Birrell, associate professor at Iowa State University, designed to collect corn stover as a bioenergy feedstock for the REAP (Renewable Energy Assessment Project) cooperative research project.

They found that using a common pretreatment with the high-cut top stover resulted in production of significantly more ethanol than the high-cut bottom stover. These results indicate that the high-cut top stover would be less expensive to prepare for ethanol conversion.

After pretreatment and fermentation screening, they found that the resulting ethanol yields between the normal-cut and high-cut top harvests were indistinguishable. This suggests that normal-cut stover harvest—characterized by convenience and speed, acceptable stover water content, and potentially lower processing costs—appears to give producers their best stover harvest option for biofuels.

"Our results indicate that the cob and upper portion of the corn stover have the best characteristics for being made into ethanol. And if we harvest just this part of the plant for biofuel, we will probably leave enough crop residue on the field for soil conservation," Karlen says.

The team plans to continue its research on how harvest height affects stover quality. They will also vary agronomic practices—such as crop spacing, fertilization rates, and use of annual and perennial cover crops—to assess how these factors affect stover quality.

These long-term studies support regional corn producers in their search for optimal combinations of sustainable practices that maximize production, reduce costs, and protect natural resources.—By **Ann Perry,** ARS.

This research is part of Soil Resource Management, an ARS national program (#202) described on the World Wide Web at www.nps.ars.usda.gov.

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Zein—A Corn Compound With Diverse Valuable Uses

Zein (pronounced "ZEE-in"), the chief protein in corn, has been commercially available for more than 60 years. In fact, until petroleum nudged it aside, zein was a chief ingredient for making adhesives, varnishes, binders, and films. Today, zein is mainly used in specialty coatings.

But now, thanks to renewed interest in biobased products stemming from rising crude oil prices, zein is getting a second look. At the ARS National Center for Agricultural Utilization Research (NCAUR) in Peoria, Illinois, for example, chemist David J. Sessa has developed new methods of physically and chemically modifying purified zein to create two polymer products: hydrogels and microspheres.

PEGGY GREB (D1276-1)

David Sessa, a chemist in the ARS Plant Polymer Research Unit, is checking for air bubbles in a chromatographic column to be used for decolorizing and deodorizing yellow zein solutions. Sessa is co-designer of the multicolumn apparatus for pilot-scale operation.

Cosmetic, Biomedical Uses—and Beyond

Sessa's purification step overcomes a hurdle that's slowed the corn protein's wider commercial use—elimination of its yellowish color and distinct odor. His method of modifying the purified zein into either hydrogels or microspheres has set the stage for new potential uses in biomedicine. These include drug-delivery applications, drug capsules that dissolve in the body, and scaffolding for growing and shaping bone or other tissues.

Purified zein has food uses, too. In chewing gum, it could serve as an alternative to petroleum-based resins, which are slow to degrade, and natural ones, like chicle, which must be imported.

Finally, zein could also see action on the industrial front in the production of bioplastics, paper coatings, and packaging materials.

By drying zein-based hydrogel and compressing it, Sessa adds, "We've made films with good tensile strength and elasticity. Normally, protein-based films are brittle. But the films from these compressed hydrogels have excellent properties—and you don't have to add plasticizers."

What's more, the films degrade into harmless byproducts—amino acids, the building blocks of protein.

Another trait that could bode well for zein's biomedical future is its apparent resistance to bacterial growth. Sessa and colleagues tried to establish bacterial growth with zein using various growth media. But, he says, "We couldn't even get any visible growth to count."

Rethinking Zein Production

Different methods exist for extracting zein from feed streams containing whole corn kernels or corn gluten meal, a coproduct of ethanol production. These zein products are odorous and yellow.

Sessa sought to improve on methods that use activated carbons (AC). These porous, charcoal-like substances work by binding

PEGGY GREB (D1278-1)

Using an Instron universal testing machine, technician Gary Kuzniar evaluates the mechanical properties of a tensile bar from chemically modified zein.

to, and trapping, the compounds that cause zein's color and odor. The problem is that 37 percent to 95 percent of the zein is lost in the process. This, in turn, ratchets up production costs and restricts zein's broader commercial use.

Sessa decided to examine the problem from the ground up. First, he isolated and identified the chemical structures of the compounds responsible for zein's color—namely the xanthophylls lutein and zeaxanthin. "Those xanthophylls are imbedded in the zein helix structure, which makes them very difficult to remove," he says.

Sessa then looked at what causes the off-odor. He isolated and identified the major contributor to be diferuloylputrescine, a compound from the bran fraction that tightly binds to protein.

Next, Sessa evaluated how well various AC products adsorbed the compounds from zein. This evaluation included monitoring the effects of temperature and AC pore size and depth. His consideration of AC alternatives eventually led to his use of zeolites, which are silicate- or clay-based particles whose pores act as molecular sieves in which zein's color and odor compounds are trapped during purification.

Of about 150 native and synthetic zeolites, Sessa narrowed his search down to 2 synthetic types that best suited his needs: type "A" and type "X." He was able to increase his zein yields by reducing the protein-component adsorptions to about 25 percent.

"I found zeolite 5A did a particularly good job," he reports.

In keeping with his bottom-up approach to improving zein purification, Sessa also devised a novel method of using ultraviolet light spectroscopy to monitor zein deodorization during purification. And by collaborating with an engineer at a Salt Lake City, Utah-based company, Sessa's ideas were used to construct column-based, pilot-scale equipment for purifying zein.

With the multicolumn system, an algorithm can be devised to selectively switch the flow of zein in aqueous ethanol through each of the columns. Normally, zein is pumped from the top down, but lab-scale studies Sessa conducted show it's best to reverse the flow. "Otherwise, you get compaction of the column medium bed," he says, which lessens the recovery of zein.

In March 2007, Sessa filed a patent (U.S. SN 11/728,700) on the system.

Sessa's work and industry collaborations couldn't have come at a better time, especially with the push towards biobased products and renewable fuels like ethanol. The 2007 ethanol production of 7.8 billion gallons produced 14.6 million metric tons of dried distiller's grains (DDGs), now used in livestock feed, and about 8.2 million metric tons of corn gluten meal. The dry-milling operation represents 64 percent and the wet-milling operation is 36 percent of total ethanol production.

Traditionally, corn gluten meal, the more expensive byproduct, has been the chief source of zein. But, says Sessa, "The issue now is how to extract zein from DDGs. If you can accomplish that cheaply, then you open up all sorts of doors."—By **Jan Suszkiw,** ARS.

This research is part of Quality and Utilization of Agricultural Products, an ARS national program (#306) described on the World Wide Web at www.nps.ars.usda.gov.

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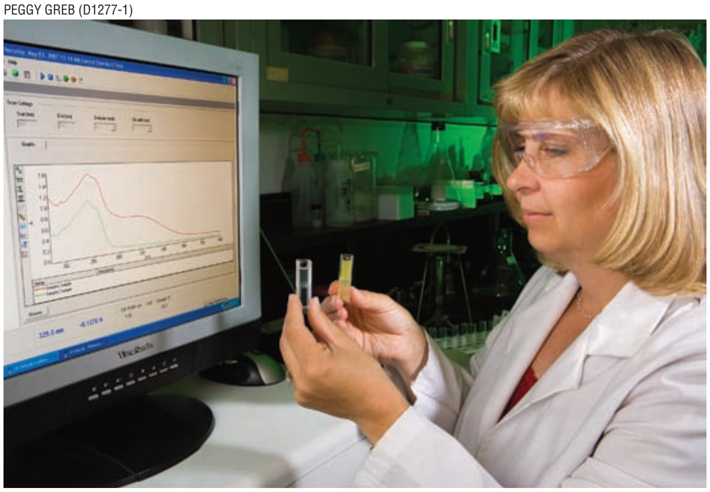
PEGGY GREB (D1277-1)



Technician Mardell Schaer performs spectrophotometric analysis of a yellow zein solution (right) and decolorized and deodorized zein (left). The yellow zein solution is represented by red on the screen, and the decolorized and deodorized zein is represented by green.

From Golf to Gulf, Drainage Industry Greens

Scientists at six Agricultural Research Service (ARS) laboratories in five states are collaborating to bring three industries together with agribusiness to clean drainage water before it contributes to water-quality problems in the Gulf of Mexico and elsewhere.

The idea is to join forces for mutual benefit—using industrial products/byproducts from wallboard production, electric power plants, steel mills, water-treatment plants, foundries, and other industries to clean drainage water. The industries benefit from having an alternative to landfilling their byproducts. In turn, they benefit the drainage, farming, and turf industries by making sure the water they discharge downstream is clean for the next user as well as for the environment.

The focus for the turf industry is on golf courses, and one of the associations involved in this research is the U.S. Golf Association. Other collaborators include the Agricultural Drainage Management Coalition and the Industrial Resource Council.

Of 50 industrial products/byproducts used as potential filter materials, 5 are "quite promising."

Using Filters To Clean Golf Course Drainage Waters

A new idea being explored for cleansing drainage water from golf courses is to attach a filter cartridge to drainage-pipe outlets to neutralize nutrients and pesticides before they reach streams.

Kevin King, an agricultural engineer with the ARS Soil Drainage Research Unit (SDRU) in Columbus, Ohio, has spent the past 2 years testing commercial cartridges filled with the typical water cleansers used in drinking-watertreatment plants and home aquariums.

As part of a national agreement between ARS and the U.S. Golf Association, King is testing the filters on a golf course in Waco, Texas. Water samples are tested for pesticides 2,4-D, chlorothalonil, and metalaxyl and for phosphorus and nitrogen.

In cooperation with Agri Drain Corporation, near Adair, Iowa, KriStar Enterprises, Inc., in Santa Rosa, California, and Spectrum Research, Inc., in Duluth, Minnesota, King and colleagues at the ARS Pasture Systems and Watershed Management Research Unit in University Park, Pennsylvania, and Pennsylvania State University are also experimenting with the cartridges and other filter designs. Preliminary results are promising.

James C. Balogh, with Spectrum, and King installed the cartridge filters in a golf course tile drain in Duluth in August 2008. This is part of a larger golf course water-quality study. They're evaluating the efficacy of the filter cartridges for reducing overall nutrients and chlorothalonil in the entire watershed surrounding the golf course.

King and colleagues are also testing wastewater-treatment

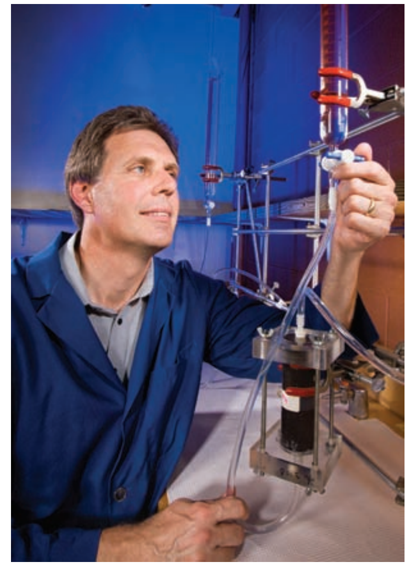
SPECTRUM RESEARCH, INC. (D1327-1)

ARS agricultural engineer Kevin King (right) and soil scientist Jim Balogh, of Spectrum Research, Inc., inspect a filter cartridge system being evaluated at a golf course tile drainage site. The cartridge system was donated by cooperating industry Kristar Enterprises, Inc.

Golf course water features, like this pond, provide an opportunity to manage discharge waters for filtration.

PEGGY GREB (D162-9)

PEGGY GREB (D1323-1)



Agricultural engineer Barry Allred conducts an experiment to measure permeability (water flow capacity) of a filter material.

residues, such as alum, to improve the ability of fairway vegetative buffer areas to filter out pollutants before they reach drainage-pipe outlets.

It Takes a Trench To Filter Farm Drainage

King's colleague at Columbus, agricultural engineer Barry Allred, is testing industrial products/byproducts as filtering materials for water draining off farm fields, where filter cartridges aren't practical. Instead, Allred would place filtering materials, such as coal-combustion byproducts, into an in-line treatment trench, so the water flowing from drainage pipes into the trench is cleansed before it reaches a stream.

Since 2007, Allred has batch tested 50 industrial products/byproducts as potential filter materials and has identified 5 of them as "quite promising." He plans further testing of these materials in the laboratory.

"We also hope to do broader feasibility tests to address a variety of issues involved in using industrial products or byproducts," he says. "These issues include the safety of the materials used—making sure we're not substituting a worse contaminant for a lesser one, for example. We also have to make sure that the filtering doesn't slow the drainage flow."

Allred's idea is to use industrial products/byproducts that are locally available in each state. "We have a lot of coal-burning power plants in Ohio, so a coal-combustion product such as high-carbon fly ash would be an inexpensive choice," he says.

"Surfactant-treated zeolite, a water-purification compound, and sulfur-modified iron, a sulfur-iron mix made with a patented process, also show promise for removing nutrients and pesticides from agricultural and turf drainage waters," Allred says.

At the University Park unit, scientists have been experimenting for nearly a decade with coal-combustion byproducts and other materials applied to soils or placed in ditches to treat runoff water. For the past 2 years, Ray Bryant, soil scientist and research leader, has tested gypsum byproduct, residual material from treating acid drainage from coal mines, and steel slag. The ditches in his studies drain fields that have been heavily manured and are key conduits of nutrients, particularly phosphorus. Bryant's approach is to filter water moving through the ditches to capture phosphorus at critical points before it reaches the Chesapeake Bay.

"We've trapped up to 50 percent of the phosphorus in some drainage waters," says Bryant.

In Beltsville, Maryland, agronomist Eton Codling, at the ARS Environmental Management and Byproduct Utilization Laboratory, found that drinking-water-treatment residuals and coal-bed ash reduced soluble phosphorus in two high-phosphorus soils by at least 50 to 90 percent. The residuals contained high aluminum and iron, and the coal-bed ash contained high calcium. These minerals bind to phosphorus, reducing its movement in the environment.

To determine the potential environmental impact of long-term use of these byproducts and the solubility of bound phosphorus under acidic conditions, Codling acidified both soils and grew several crops of bermudagrass. He found that phosphorus bound to the coal-bed ash became soluble over time, whereas phosphorus bound to the drinking-water-treatment residual did not.

Putting It All Together: Ditches, Wetlands, and Industry

Norm Fausey, SDRU soil scientist and research leader, and colleagues are working with faculty at Ohio State University in Columbus to plan the next phase of their research. They want to combine the best materials for all applications, including filter trenches for farm fields and cartridges for golf courses.

In Coshocton, Ohio, Martin Shipitalo, a soil scientist with the ARS North Appalachian Experimental Watershed Unit, is testing compost-filled filter socks placed in grassed waterways. The waterways convey water from fields to streams, filtering out soil and other solids but doing little for dissolved solids like phosphorus. Last year Shipitalo installed two filter socks in each of four grassed waterways to capture the phosphorus.

MARTIN SHIPITALO (D1326-1)

Runoff from a field planted with corn is directed into a grassed waterway where it passes through and over a series of compost-filled filter socks that help reduce levels of nutrients and herbicides.

PHYLLIS DIETER (D1325-1)

Soil scientist Martin Shipitalo checks the placement of a compost-filled filter sock in a grassed waterway. When runoff occurs from the cornfield (in background), it must pass through the sock. Automated samplers upstream and downstream from the sock determine its effect on nutrient and herbicide concentrations in the runoff.

"After seeing how compost alone does for a year, I will try adding iron residue from wastewater-treatment facilities to bind even more phosphorus to the compost," he says.

King's, Allred's, and Bryant's filtering systems would blend in well with Shipitalo's filter socks and the SDRU's Wetland Reservoir Subirrigation Systems (WRSIS), developed by Fausey, Allred, and colleagues at Ohio State University. The team constructs wetlands near farm fields to cleanse drainage water of nitrate and eroded soil. The water is then stored in a reservoir and used for irrigation later in the summer. The irrigation is done by reversing the flow of water in underground plastic corrugated pipes used to drain fields in early spring. WRSIS could easily be used with golf courses, since they already have reservoirs to store water for irrigation.

Results from 2003 through 2007 at three sites in northern Ohio show that two of the three wetlands were very effective in removing sediment, nitrate, and total nitrogen. "Besides providing new wildlife habitat and flood control, the wetlands and reservoirs also have water-quality benefits and help increase crop yields," Fausey says.

By further filtering the drainage water through industrial products/byproducts or other materials, "we could polish off the wetland's filtering process, taking care of anything that may have slipped through," Fausey says.

Pelletizing PAM

ARS soil scientist Darrell Norton's research should also cleanse and conserve water on farm fields and golf courses. Norton, from the USDA National Soil Erosion Research Laboratory at West Lafayette, Indiana, has found a way to pelletize polyacrylamide

(PAM) and gypsum byproducts together so farmers can apply them more easily. PAM is typically dissolved in water and applied to soil, and gypsum is applied as a powder. Not only were Norton's pellets easier to apply, they also reduced erosion just as well as the typical treatments—25 to 50 percent compared to untreated soil—and increased water infiltration, thus saving more rain for crop use.

Gypsum-PAM pellets can also be useful for temporary erosion control while establishing permanent vegetative cover on highway embankments and landfill caps and in earthen channels like those found in crop fields or around ponds or dams.

The recommendation from the study is to apply gypsum-PAM pellets at a rate of about half a ton per acre.

Norton and colleagues began ARS's PAM research in the early 1990s. PAM has been very successful for erosion control in irrigated areas because it does best in a very dilute solution of water, which fits irrigation perfectly. In rainy areas, however, "farmers would not irrigate just to apply PAM, because too much rain is why they need erosion control in the first place," Norton says.

The large volume of gypsum coming out of power plants encourages Norton that it will be used increasingly on farms. There are already places nationwide that grind up used wallboard and sell the gypsum powder to farmers and landscapers.

The gypsum-PAM pellets cost about $120 a ton, making them much more affordable than traditional erosion-control materials used by highway departments and construction crews.

PEGGY GREB (D1320-1)

Junior Cesar Avanzi, visiting scientist from the Federal University of Lavras, Brazil, collects sediment to assess the level of erosion control from the use of gypsum-polyacrylamide (PAM) pellets. In the background, Darrell Norton controls the rainfall simulator.

PEGGY GREB (D1319-1)

Soil scientist Darrell Norton applies pelletized gypsum and polyacrylamide (PAM) to a soil surface to control erosion prior to a rainfall-simulation test.

Commercial erosion-control practices, such as land grading and application of mulches and fiber mats, usually cost about $100 to treat just 1 square yard of land.

Farmers are increasingly realizing that helping their crops capture more rainwater can help their yields as much as extra fertilizer can. Gypsum-PAM pellets cost less per acre of land treated than commercial fertilizers do. Pellets could also be used to compensate for the loss of some crop residue removed for ethanol production.

Pam Rice, a chemist with the ARS Soil and Water Management Research Unit at St. Paul, Minnesota, and colleagues at the University of Minnesota-St. Paul, will study chemical changes caused by gypsum in waters draining from turfgrass field plots.—By **Don Comis** and **Jan Suszkiw,** ARS.

This research is part of Water Availability and Watershed Management (#211), Agricultural System Competitiveness and Sustainability (#216), and Crop Production (#305), three ARS national programs described on the World Wide Web at www.nps.ars.usda.gov.

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Ethanol Byproducts Offer Savings for Farmers and Industry

PEGGY GREB (D1344-1)

Technician Jennifer Cook and research leader Brian Kerr sample corn-based feed ingredients for nutrient-composition testing prior to feeding trials.

Ethanol production has profoundly changed the scene in corn and livestock country. The high cost of field corn and its diversion from livestock feed to ethanol production have created the need to substitute ethanol production coproducts for some corn feed.

Distiller's grains are the residual material from converting grain, such as corn or sorghum, into ethanol. Distiller's grains are either wet (WDG) or dried (DDG). Adding back liquid solubles to distiller's grains makes a coproduct known as "wet distillers grains with solubles" (WDGS). If dried, that mixture is known as "dried distiller's grains with solubles" (DDGS). There are more than 20 forms of these coproducts, most of which are suitable as feed supplements for cattle, swine, and poultry.

ARS scientists across the country are testing the coproducts of ethanol and biodiesel production on hundreds of livestock, including beef cattle, dairy cows, pigs, poultry, and fish. They want to see whether and how distiller's grains fit into feed rations. They're testing the effects on every aspect of raising animals, from economics, animal growth and health, digestibility, meat and milk production, and carcass characteristics to environmental issues, such as the nitrogen and phosphorus content of manure.

PEGGY GREB (K10522-1)

Animal scientist Andy Cole examines cattle feed containing wet distiller's grains.

Blending distiller's grains into feed could lower costs for livestock producers and provide a market for recycling these ethanol coproducts, which are growing in supply as more and more ethanol is produced. In the Midwest alone, ethanol producers generate 10 million tons of DDG annually.

Recycling distiller's grains is good for distillers, grain farmers, livestock producers, and the environment. It lowers the costs of ethanol production and helps offset some of the losses of field corn diverted to make ethanol.

The goal is to recycle distiller's grains into as many products as possible, not only nutritious and low-cost livestock and aquaculture feed, but also human food supplements and nutraceuticals as well as nonfood products.

Andy Cole, an animal scientist at the ARS Renewable Energy and Manure Management Research Unit in Bushland, Texas, says as new ethanol plants of various sizes open and existing plants change their processing techniques, this affects the type of coproducts produced.

"We've found that coproducts change from plant to plant and within plants," Cole says.

Other Great Grains

The researchers are also testing barley and sorghum, which could be used for biofuel production. Use of these and other alternative grain crops to produce ethanol is just one of many factors that make ethanol and byproducts research so dynamic.

Cole explains that distiller's grains are likely to come from sorghum and corn in the Southern Plains. "This is one of the differences between Southern Plains and Northern Plains states," Cole says. "There are more ethanol plants in the Northern Plains, and they have a head start on us. But we have larger feedlots, so management techniques are different here. Also, we feed our cattle steam-flaked corn rather

Collaborators in This Research

- The Consortium for Cattle Feeding and Environmental Science, which includes West Texas A&M University, Texas Tech University, New Mexico State University/Clayton Livestock Research Center, Texas AgriLife Research, Texas AgriLife Extension Service, Texas Veterinary Medical Diagnostic Laboratory, the Texas Cattle Feeders Association, and the USDA Agricultural Research Service.
- The National Sorghum Producers and the Texas Corn Producers Board.
- Kansas State University, Oklahoma State University, and the University of Minnesota at St. Paul.

PEGGY GREB (D1345-1)

Brian Kerr evaluates piglets' ability to use nutrients in corn coproducts for growth and development. After 4 weeks of a diet supplemented with dried distiller's grains with solubles (DDGS), the immune response of piglets increased.

than dry-rolled corn, which is common in the Northern Plains. Steam-flaked corn has more net energy than dry-rolled corn, so adding distiller's grains tends to lower the feed-energy value of steam-flaked-corn-based diets. But with dry-rolled-corn-based diets, distiller's grains probably add to the feed-energy value."

With cooperators (see box above), Cole is testing WDGS from sorghum and corn and from blends of both. They're combining sorghum-based distiller's grains with steam-flaked corn in beef cattle finishing diets. They have also done some preliminary experiments at a large commercial dairy to study the feeding value of WDGS for lactating dairy cows.

Cole and his colleagues have tentatively found that WDGS are best used at 10 to 20 percent of the diet for beef cattle that are fed steam-flaked corn-based diets in the Southern Plains.

They are currently studying safety concerns, such as too much dietary sulfur, which can be fatal to cattle.

PEGGY GREB (D1343-1)

Physiologist Tom Weber prepares samples for real-time PCR analysis to see whether genes that regulate growth and immune function are expressed, or turned on, when pigs consume biofuel coproduct supplements in their diet.

DDGS Fuel Pig Growth—and Health

Tom Weber, an ARS physiologist at the Swine Odor and Manure Management Research Unit in Ames, Iowa, says that rising feed costs and increasing use of corn for biofuel production have pushed swine producers to find new feed supplements for younger swine.

"Producers had already started using DDGS for older pigs, based on ARS studies of the benefits of supplementing swine feed with high-fiber byproducts such as DDGS. Now we're looking at piglets."

That is why Weber, research leader Brian J. Kerr, and ARS microbiologist Cherie Ziemer studied the effects of feeding fibrous coproducts to young pigs. The team divided weanling pigs into four groups and fed them either a standard control diet or a diet supplemented with DDGS, soybean hulls, or citrus pulp.

After 4 weeks, the researchers found that DDGS increased the immune response of piglets, possibly making them more resistant to illness. The response observed was an increase in cytokine expression in the pigs' small intestines.

"This response could partially explain earlier findings that pigs with DDGS in their diets exhibited reduced levels of ileitis, a common inflammation of the small intestine," Weber says.

KURT ROSENTRATER (D1353-1)

Agricultural engineer Kurt Rosentrater and technician Sharon Nichols examine the quality of pelleted animal feed made from DDGS.

"Our study shows that we can feed DDGS successfully to young pigs without altering their growth. And it's clear that these DDGS may result in improved health."

Over the past few years, Kerr, Weber, and Ziemer have conducted about a dozen studies with a swine herd at Iowa State University. Kerr is now studying energy and amino acid availability in 13 different coproducts of the corn-milling industry.

Weber agrees with Cole about the ever-changing nature of ethanol coproduct research: "It's quite dynamic. As the processes change, you keep getting different forms of DDGS and other coproducts," Weber says. "We have no lack of coproducts to study."

Weber found that, for adult pigs, they can use up to 40 percent DDGS mixed with corn and soy-meal feed. For piglets, they use 7.5 percent, because the piglets tend to grow less with too much fiber.

Kurt Rosentrater, an agricultural and bioprocess engineer with the ARS North Central Agricultural Research Laboratory at Brookings, South Dakota, says the swine and poultry industries have been consuming more DDGS in recent years.

Rosentrater leads one of the largest DDGS utilization efforts in ARS, outside of the ARS regional research centers at Peoria, Illinois, and Wyndmoor, Pennsylvania. He works with faculty at South Dakota University, Northern Illinois University, and North Dakota State University, as well as several commercial entities. His research program is investigating methods of using DDGS as ingredients in cattle feed and human food and as fillers in biodegradable plastics. He is also examining the role of manufacturing conditions on the products' chemical and physical properties and how these relate to storability and material-handling characteristics.

For example, Rosentrater sampled distiller's grains coproducts at commercial ethanol plants in South Dakota. He found that they had nearly 30 percent protein and between 30 and 40 percent fiber, making them suitable as feed not only for livestock, but also for aquatic animals and pets.—By **Don Comis** and **Ann Perry,** ARS.

This research is part of Manure and By-product Utilization (#206), Bioenergy and Energy Alternatives (#307), and Quality and Utilization of Agricultural Products (#306), three ARS national programs described on the World Wide Web at www.nps.ars.usda.gov.

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Other Uses for Distiller's Grains

Here are some examples of other ARS research to recycle distiller's grains into as many products as possible:

Human Food Supplements and Nutraceuticals

Phytosterols, lecithin, as well as carotenoid antioxidants, such as lycopene, are some of the ingredients that could be mass produced from distiller's grains and sold as human food supplements. Some research along these lines is being done with Syngenta Corporation in Wilmington, Delaware.

ARS scientists are also searching many barley types for these kinds of healthy ingredients that can be gleaned from distiller's grains, including from barley lines being developed at the ARS Small Grains and Potato Germplasm Research Unit in Aberdeen, Idaho. *Research of the ARS National Center for Agricultural Utilization Research (NCAUR), Peoria, Illinois, and the ARS Eastern Regional Research Center, Wyndmoor, Pennsylvania.*

Better Fish Feed

Barley DDGS with improved nutritional value can be used as a new feed for rainbow trout. *Research of the ARS Small Grains and Potato Germplasm Research Unit, Hagerman Fish Culture Experiment Station, Hagerman, Idaho.*

Adding lysine allowed tilapia to thrive on feed with 40 percent DDGS or more. Catfish fed DDGS-containing diets showed more resistance to enteric septicemia, a major fish disease. *Research of the ARS Aquatic Animal Health Research Unit, Auburn, Alabama.*

Mulch

Distiller's grains can make a mulch that is both an organic fertilizer and a weed-inhibitor. ARS has a cooperative research and development agreement with Summit Seed Incorporated of Manteno, Illinois, on this research. *Research of NCAUR, Peoria, Illinois.*

CROP BIOPROTECTION RESEARCH (D1054-1)

Plant pathologist David Schisler scores greenhouse wheat treated with microbial antagonists for symptoms of *Fusarium* head blight.

Flower Power—Bacteria Style—Checks Fungal Foe of Wheat

Flower-dwelling bacteria could soon join the fight against *Fusarium graminearum,* the fungus that causes *Fusarium* head blight disease, or scab, in wheat, barley, and other cereal crops.

According to David A. Schisler, a plant pathologist in ARS's Crop Bioprotection Research Unit at Peoria, Illinois, the bacteria colonize the flower's anthers, or pollen-making structures, which naturally exude a smorgasbord of nutrients. One of these, choline, is rich in carbon that the bacteria need for growth.

But what does the wheat plant get in return? Protection, it turns out.

In greenhouse studies and field tests conducted in Peoria and Wooster, Ohio, starting in 2002, Schisler and colleagues have shown that inoculating wheat anthers with specific choline-metabolizing bacteria helps keep scab to a minimum. Unchecked, the disease causes economic losses in wheat by crippling the growth of kernels and turning them chalky white.

The researchers aren't sure exactly how the bacteria protect the crop, but one possibility may be tied to the fungus's reliance on choline as a chemical signal that cues it to form a specialized tube for breaching anther tissues. By gobbling up the anthers' choline stores, the bacteria leave less around for the fungus, depriving it of that chemical cue. "The bacteria may keep that signal from being as 'visible' as it would be otherwise," says Schisler. That observation got him and colleagues thinking: "If we could cover wheat heads and anthers with organisms that use choline, could we 'blind' the scab fungus to this cue?"

Of 123 choline-metabolizing bacteria they isolated from wheat-field soils and tested in the greenhouse, the researchers chose 10 strains whose scab-suppression warranted further study under field conditions.

Spraying formulations of the bacteria onto plots of two commercial wheat cultivars, at the time of flowering, reduced severity of scab by as much as 63 percent. That was close to the level of disease control attained with Folicur 3.6F, the only fungicide that can currently be considered for use against scab on wheat.

A *Pseudomonas* species dubbed "AS 64.4" proved to be the best all-around performer. But its future as the magic bullet against scab is unlikely. More realistic would be its combination with other scab-fighting microbes, including yeasts and antibiotic-secreting strains of bacteria that Schisler's group previously identified and patented.

So why bother with the choline eaters, then? "Because they have a different mode of action. Combining them with the other antagonists could result in a more uniform and higher level of activity against scab," explains Schisler. Mindful of that prospect, ARS has applied for a patent on the choline eaters.

Meanwhile, Schisler's group is collaborating with a company seeking to develop one of the yeasts as a biopesticide. That could give growers another measure of insurance against scab outbreaks, which have cost billions of dollars in losses to U.S. wheat and barley.—By **Jan Suszkiw,** ARS.

This research was partly supported by the U.S. Wheat and Barley Scab Initiative, managed by ARS, and is part of Plant Diseases, an ARS national program (#303) described on the World Wide Web at www.nps.ars.usda.gov.

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CROP BIOPROTECTION RESEARCH (D1052-1)

Typical premature whitening of a wheat head infected with the fungus that causes *Fusarium* head blight.

Federal-Wide Partnering for a Better Life

The Agricultural Research Service continually looks for opportunities to partner with other federal agencies. These partnerships are designed to augment research programs, expedite research results, exchange information and knowledge, enhance U.S. trade and the nation's economy, and improve the quality of life for all Americans.

The improvement of quality of life includes cooperative research for clean water; safe, plentiful, and high-quality food; and the health of our people, our livestock, and the environment.

Since its founding in 1953, ARS has worked closely with other federal agencies, both within and outside the U.S. Department of Agriculture. Examples include giving science-based recommendations to USDA Natural Resources Conservation Service (NRCS) personnel for use with farmers and ranchers and providing data to guide U.S. Environmental Protection Agency (EPA) regulations. ARS has worked with almost every other federal agency at one time or another, including the U.S. Departments of Defense and Homeland Security.

ARS has worked with almost every other federal agency at one time or another.

ARS often shares the same land or buildings with other federal agencies or with universities. "In fact, many ARS scientists do their work in university facilities alongside university researchers," says Steven R. Shafer, ARS deputy administrator for Natural Resources and Sustainable Agricultural Systems. "This is a long-standing partnership with a highly distinguished tradition of productivity, arising through a leveraging of federal and state resources."

ARS takes a lead role nationally in a "greening" initiative designed to encourage offices in all federal departments to set an example by doing things such as using fuel-efficient vehicles and biobased fuels and buying biobased versions of products such as cleaners and carpet backing.

(NRCSIA00063)

Natural Resources Conservation Service hydrologist Laurel Foreman uses a global positioning system in Iowa to record the status of a filter strip planting, one of the practices being evaluated by her agency in conjunction with ARS and the Cooperative State Research, Education, and Extension Service.

A new collaboration between ARS and the USDA Agricultural Marketing Service has been established to coordinate market-driven research strategies across agencies. An ARS-developed computer crop-growth model for potatoes is an example of a research product being shared among agencies for financial and marketing guidance: The USDA Farm Services Agency wants to use it to assess loan risk and to guide loan applicants in the selection of profitable rotation crops.

Partnering for Clean Water

Making sure that taxpayers are getting their money's worth from publicly funded conservation measures is the goal of the Conservation Effects Assessment Project (CEAP). Most of the funds for agricultural conservation come from USDA through the Farm Security and Rural Investment Act, known as the "Farm Bill."

KEITH WELLER (K8721-4)

All CEAP watersheds use the SWAT computer model to predict effects of farm practices on water quality. Here, technician Jeff Nichols collects a sample from a watershed in Ames, Iowa, during studies to refine the model.

CEAP involves multiple partnerships among federal, state, and local government agencies; universities; and private organizations, including environmental groups such as the Sierra Club. The project now has 38 watersheds throughout the country, from the New York City area to California.

The lead USDA agencies in CEAP include ARS, NRCS, and the Cooperative State Research, Education, and Extension Service.

Conservation practices being assessed include conservation buffers; streambank fencing; erosion control; wetlands conservation and restoration; establishment of wildlife habitat; and management of grazing land, tillage, irrigation water, nutrients, and pests.

EPA cooperates with various aspects of the project. Additionally, many CEAP watersheds are in areas where EPA and states are investing Clean Water Act funds to improve water quality and protect drinking water.

The Cannonsville, New York, watershed is a good example of the type of cost/benefit analysis being done through CEAP.

JAMES FOSSE (D1375-2)

At the National Centers for Animal Health in Ames, Iowa, this state-of-the-art Biosecurity Level 3 animal-containment building is part of the new, larger facility shared by ARS and APHIS.

Its nearly 355 square miles drain into a reservoir that is part of a system of reservoirs and aqueducts supplying about 94 percent of New York City's drinking water. Excess phosphorus, probably from farm waste, stimulates algal blooms that interfere with chlorination. New York City's water authority decided that underwriting the costs of conservation practices installed by farmers was a better buy than the technological fix of changing treatment methods for its drinking water.

Partnering for Healthy Animals

This year, a new facility housing the National Centers for Animal Health (NCAH) will open for business in Ames, Iowa. Around 1 million square feet of laboratories, animal housing, and other facilities will be occupied by hundreds of scientists and support personnel—and hundreds of animals.

USDA scientists from ARS and the Animal and Plant Health Inspection Service (APHIS) already work side-by-side in Ames. They support the nation's $100 billion livestock industry with animal disease research, diagnostics, and training. They also test vaccines and evaluate veterinary biological products.

ARS conducts much of USDA's scientific research. APHIS, which is USDA's regulatory arm for diagnosing animal diseases and licensing vaccines and diagnostics, will have two centers within the NCAH: the National Veterinary Services Laboratories (NVSL) and the Center for Veterinary Biologics (CVB).

PEGGY GREB (D1389-1)

Clifford Johnson, director NHANES Program, Centers for Disease Control and Prevention, DHHS, and Alanna Moshfegh, ARS research leader at the Beltsville Human Nutrition Research Center, review recent sodium intake data from *What We Eat in America,* the dietary interview part of the National Health and Nutrition Examination Survey.

Improving Aquaculture Feed

Alternative feeds are making a splash in the aquaculture industry.

Most feed for farm-raised fish includes lots of fishmeal. This places pressure on the source—wild ocean-dwelling fish—so the aquaculture industry is investigating alternatives.

The U.S. Department of Agriculture is collaborating with the National Oceanic and Atmospheric Administration to promote development of nontraditional aquaculture feeds. The initiative involves two USDA agencies: the Agricultural Research Service and the Cooperative State Research, Education, and Extension Service.

"The agencies have made great progress, meeting with representatives from universities, industry, interest organizations, and the general public to identify research needs and outline plans," says Jeff Silverstein, leader of ARS's national program for Aquaculture.

Ongoing research for this initiative has helped shed light on nutritional profiles of potential feed alternatives and methods for increasing the level of nutrients available in novel feed ingredients such as plants, biofuel coproducts, and fish-processing wastes.—By **Laura McGinnis,** ARS.

TROUTLODGE, INC. (D1376-1)

Atlantic salmon is one of the major species grown in aquaculture and is an important target for alternative feeds development.

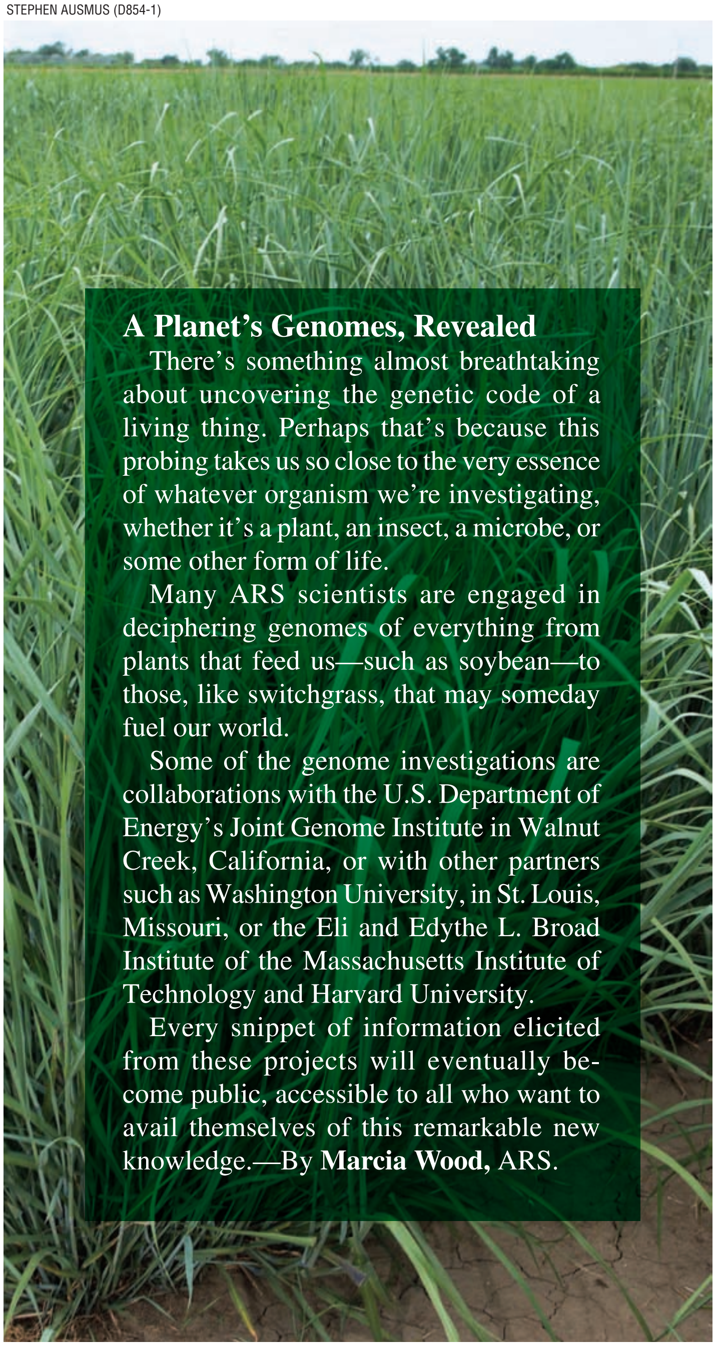
"Sharing a physical workspace will allow ARS scientists to fine-tune the research that supports APHIS regulatory activities," says Steven Kappes, who is the ARS Deputy Administrator for Animal Production and Protection.

NVSL director Beth Lautner agrees. "The new NCAH quarters will facilitate even stronger partnerships between ARS and APHIS," she says.

Scientists will have access to state-of-the-art Biosecurity Level 3 (BSL-3) animal-containment facilities at NCAH to study a range of endemic, zoonotic, and foreign animal diseases. BSL-3 structures are designed to contain infectious pathogens that may cause serious or potentially lethal diseases.

"These world-class facilities will streamline and enhance not only the partnership between APHIS and ARS, but also the capabilities of the laboratories," says CVB director Rick Hill.

STEPHEN AUSMUS (D854-1)



A Planet's Genomes, Revealed

There's something almost breathtaking about uncovering the genetic code of a living thing. Perhaps that's because this probing takes us so close to the very essence of whatever organism we're investigating, whether it's a plant, an insect, a microbe, or some other form of life.

Many ARS scientists are engaged in deciphering genomes of everything from plants that feed us—such as soybean—to those, like switchgrass, that may someday fuel our world.

Some of the genome investigations are collaborations with the U.S. Department of Energy's Joint Genome Institute in Walnut Creek, California, or with other partners such as Washington University, in St. Louis, Missouri, or the Eli and Edythe L. Broad Institute of the Massachusetts Institute of Technology and Harvard University.

Every snippet of information elicited from these projects will eventually become public, accessible to all who want to avail themselves of this remarkable new knowledge.—By **Marcia Wood,** ARS.

Elite bioenergy switchgrass growing in eastern Nebraska.

Partnering for Healthy People

Nearly 50 years ago, Congress mandated that a national survey be conducted periodically to collect statistical data about the current dietary intake and health of people in the United States. USDA conducted a series of nationwide food-consumption surveys to assess U.S. dietary intakes. In addition, the U.S. Department of Health and Human Services (DHHS) provided dietary-survey data as part of the National Health and Nutrition Examination Survey (NHANES).

In the last decade, the leadership of USDA-ARS and DHHS completed a major integration of these two dietary surveys. The resulting national food and nutrient survey is called *What We Eat In America*.

The current national survey is based on a highly efficient, customized dietary data-collection method called the Automated Multiple Pass Method, or AMPM. The AMPM was developed by researchers at the ARS Food Surveys Research Group (FSRG), part of the Beltsville [Maryland] Human Nutrition Research Center (BHNRC). AMPM is carried out during the NHANES and involves both in-person and telephone interviews.

BHNRC researchers also maintain large databases of the nutrient values of foods that are key to accurately estimating the amount of nutrients in foods that survey respondents report eating. FSRG researchers use those nutrient data profiles to process survey results. They produce data tables that show how much fat, protein, vitamins, and minerals people consume on average, by gender, age, and income categories. They also determine how U.S. diets measure up to dietary standards designed to maintain health and prevent chronic diseases.

The new survey's data are released jointly by USDA-ARS and DHHS in 2-year cycles and so far include 2001-2002, 2003-2004, and 2005-2006.

The dietary survey data, nutrient intake tables, and related analytical studies are used by various private and public groups to assess the effects of diet and health policies and programs.—By **Don Comis, Ann Perry,** and **Rosalie Marion Bliss,** ARS.

This research is part of Animal Health (#103), Human Nutrition (#107), Aquaculture (#106), and Water Resource Management (#201), four ARS national programs described on the World Wide Web at www.nps.ars.usda.gov.

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Partnering With Industry Pays Off

"ARS is an agency that is profoundly good at partnering," says ARS Office of Technology Transfer (OTT) assistant administrator Richard J. Brenner.

Brenner offers up the following numbers as proof: In 2008, ARS participated in 230 active cooperative research and development agreements (CRADAs) with external partners, including universities and private industry. In the same year, ARS partnered in 5,466 other collaborative agreements and contributed research that led to 116 new invention disclosures and 29 issued patents.

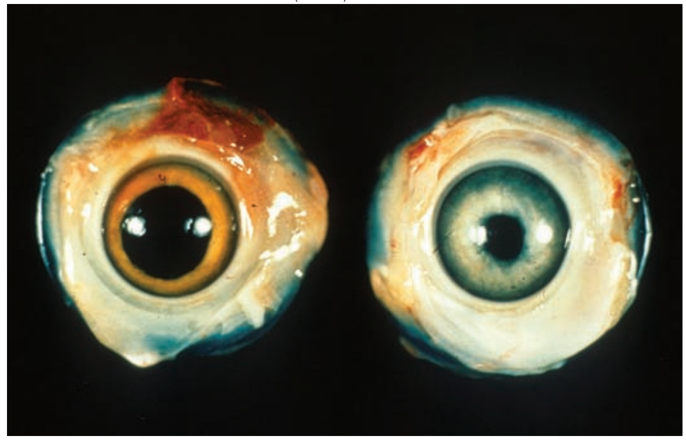
OTT representatives routinely attend trade shows and provide prospective industry partners with information on the advantages of working with ARS scientists.

The staff also develops strategic partnerships with outside organizations and pursues patents and licensing that facilitates technology transfer to the marketplace.

In September 2007, OTT closed the deal on its first partnership intermediary agreement (PIA). The inaugural agreement was signed with the Maryland Technology Development Corporation to focus on technology-based business development that will boost the economic sustainability of privately held companies in Maryland. PIAs are another way that ARS can establish agreements with nonprofit organizations that then serve as intermediaries to assist ARS with its technology transfer. A second PIA was signed with the Mississippi Technology Alliance in December 2008.

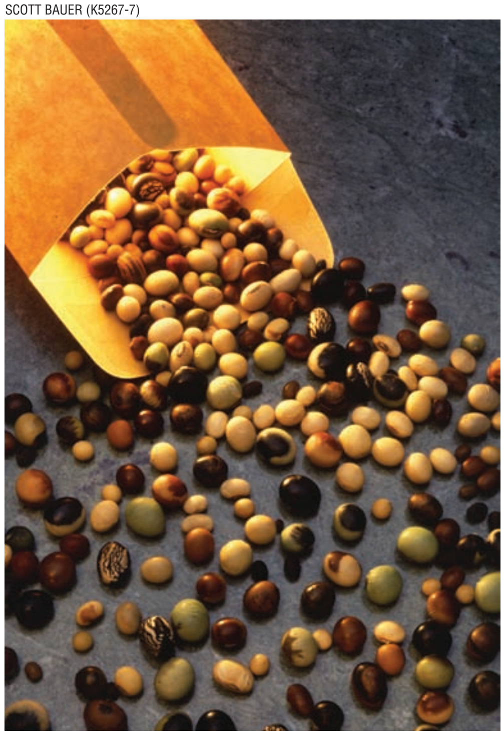
"Partnering brings research dollars to the bench and increases the likelihood of rapid adoption by the private sector," Brenner says. "Our role at OTT is to facilitate and nurture these external partnerships for our scientists. The bottom line is that a CRADA lasts for up to 5 years—but a good research partnership and its impact will go on for much longer."

ARS AVIAN DISEASE AND ONCOLOGY LABORATORY (K9494-1)



Left: Normal chicken eye. Right: Eye with lesions and irregular pupil caused by Marek's disease.

SCOTT BAUER (K5267-7)

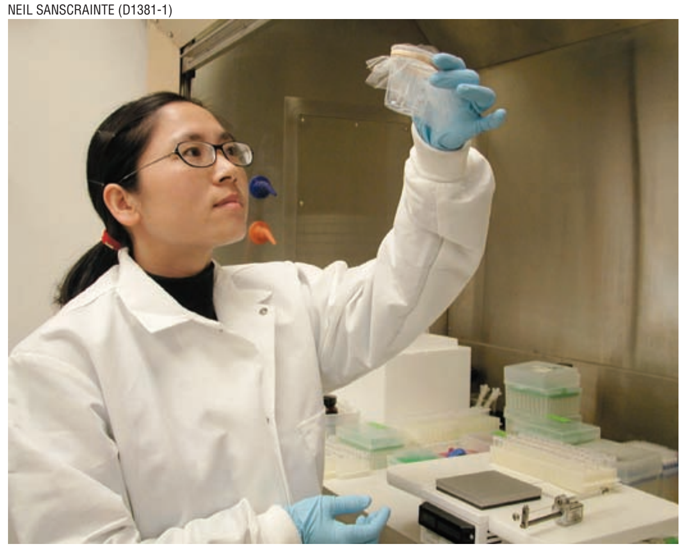


Soybeans from the National Soybean Germplasm Collection housed at Urbana, Illinois, show a wide range of colors, sizes, and shapes.

PEGGY GREB (D952-1)

Geneticist David Hyten harvests leaf tissue from one of many plant progenies derived from crossing the soybean cultivar Williams 82 with a wild soybean.

NEIL SANSCRAINTE (D1381-1)



Research associate Julia Pridgeon examines adult mosquitoes following topical application of an experimental pesticide.

Private Partnering Premieres

In 1987, the ARS Avian Disease and Oncology Laboratory (ADOL) in East Lansing, Michigan, was the first government lab to sign a CRADA with a private company. That CRADA was with Embrex, Inc., now part of the Pfizer Poultry Health Division in Durham, North Carolina. It was for an in-the-shell vaccination technique against Marek's disease. Since then, ARS has had six more CRADAs with Embrex to develop the same technique for avian coccidiosis, salmonella, and other viral diseases.

Through industry grants, the East Lansing lab is currently working with the following companies to protect poultry against viral diseases: Aviagen (Huntsville, Alabama), Cobb-Vantress, Inc. (Siloam Springs, Arkansas), Hy-Line International (Des Moines, Iowa), and Hendrix Genetics (The Netherlands).

The lab began in 1939 to respond to an epidemic of avian leukosis. In 1977, in concert with industry, ARS developed a quick test for the disease, putting industry on a secure path until a more virulent form appeared in 1989.

In response, ARS immediately redirected its research to address this threat, working with industry to bring the new strain under control with another quick test and an eradication program.

"This lab continues its historical role as a leader in poultry tumor research, namely on Marek's disease, avian leukosis, and reticuloendotheliosis. Without ARS, control of viral tumors wouldn't be what it is today," says ADOL research leader and veterinary medical officer Aly Fadly.

The lab is always trying to keep ahead of viruses that are constantly evolving into more virulent forms that evade current controls. ADOL continues to work with private and public researchers around the world through grants and other agreements.

Field Crop Fellowship

Only two races of maize were used to develop the Corn Belt Dent variety of field corn grown by U.S. producers. These two races contained less than 1 percent of the genetic characteristics found in maize varieties worldwide.

The Germplasm Enhancement of Maize (GEM) Project is an ARS-led partnership of public and private organizations that encourages scientists and breeders to explore the remaining 99-plus percent of maize genetic diversity. The goal is to adapt exotic maize races for U.S. production and develop new lines that can be used by U.S. maize-breeding programs.

Mike Blanco, an ARS geneticist who works at the North Central Regional Plant Introduction Station in Ames, Iowa, is in charge of managing GEM.

"There are between 250 and 300 maize races in total," Blanco observes. "We are coordinating a multisite cooperative program for germplasm evaluation, development, and information sharing. We want to find traits that can improve maize yield and stress resistance and enhance other value-added characteristics."

NUO SHEN (D818-1)

Geneticist Mike Blanco pollinates tropical exotic maize as a first step in breeding corn with improved disease resistance, nutritional quality, and bioenergy potential.

More than 60 collaborators in private industry, universities, and other groups around the world currently contribute to GEM activities, which to date have resulted in the release of 202 maize lines. These lines, used in public and private research and breeding programs, were developed from 24 races collected in 14 countries.

Some of the released germplasm has provided new genetic sources of resistance to excessive heat, drought, and other environmental stresses. Other lines are resistant to insect pests and mycotoxins. Varieties have also been developed with improved protein, oil, and starch content and traits that could enhance ethanol production.

Should a new threat—like an invasive insect or emerging disease—appear, these diverse maize lines might also contain a reservoir of resistance traits that current commercial varieties, with their narrow genetic base, do not possess.

"GEM is off to a great start," says Blanco. "Now we'd like to find new ways to breed exotic maize germplasm and identify favorable traits. We also want to develop new lines from GEM germplasm to explore how genetic diversity and its structure affect trait expression."

Shared Stakes for Soybean Partners

The St. Louis, Missouri-based United Soybean Board (USB) consists of 68 farmer-directors who oversee investments of the soybean checkoff fund on behalf of all U.S. soybean farmers.

ARS and USB have a long, fruitful history of collaboration aimed at furnishing America's growers with the best varieties that science and plant breeding have to offer. "This collaboration with USB allows us to pool resources in areas of common interest—with economic and other benefits to multiple industries and consumer groups," says Roy Scott, ARS national program leader for crop production and protection.

Many ARS- and USB-funded projects involve multiple ARS laboratories and cooperators. One long-term project involves seed composition—oil improvement for human consumption and protein-meal improvement for animal consumption. Led by Joseph W. Burton at the ARS Soybean and Nitrogen Fixation Research Laboratory in Raleigh, North Carolina, project researchers have developed new information about the genetics of oil and protein-meal traits, discovered DNA markers associated with these traits, and made several breeding lines available to other soybean breeders in both public and private industry.

From another long-term project, five slow-wilting soybean breeding lines were developed that yield 4 to 6 bushels more than conventional varieties under various drought conditions. These new breeding lines are being used in commercial breeding as parent material. Thomas Carter, also with the Raleigh laboratory, leads the project.

On the industrial front, a Peoria, Illinois-based ARS project led to a soy-flour-based glue that's been used since late 2001 in wood composites—a contribution to green construction materials.

Recent projects include efforts to:

• create a library to genetically characterize more than 19,000 soybean accessions in the USDA Soybean Germplasm Collection using DNA markers known as "single nucleotide polymorphisms" (SNPs). Each accession's 50,000 SNP markers will provide soybean researchers and plant breeders with a valuable resource for improving soybeans. David L. Hyten and Perry B. Cregan at ARS's Soybean Genomics and Improvement Laboratory (SGIL) in Beltsville, Maryland, are coordinating the multilocation project.

• test low-phytate soybean meal that, when fed to farm-raised rainbow trout, will reduce the fish's excretion of phosphorous pollutants in water—from hatch to harvest. Frederic T. Barrows leads the project at ARS's Hagerman Fish Culture Experiment Station in Hagerman, Idaho.

• genetically engineer soybeans for resistance to soybean cyst nematodes. In preliminary tests by Benjamin Matthews at SGIL, 80 to 90 percent of juvenile female nematodes that fed on roots of the resistant soybeans died or failed to mature by 30 days.

ARS PHOTO (K4705-9)

ARS scientists are evaluating compounds to identify active ingredients that demonstrate efficacy for killing mosquitoes. Shown here is an *Aedes (Ochlerotatus)* sp. mosquito on human skin.

Ganging Up Against Insect Pests

Scientists at the ARS Center for Medical, Agricultural, and Veterinary Entomology's Mosquito and Fly Research Unit in Gainesville, Florida, are evaluating compounds from a large chemical library to identify active ingredients that demonstrate efficacy for killing mosquitoes and other disease carriers.

Led by entomologist James Becnel and research associate Julia Pridgeon, the team uses high-throughput systems to screen large numbers of compounds for toxicity to mosquitoes. This research is conducted under a CRADA with a chemical company.

"High-throughput systems allow us to use a combination of bioassays with mosquito larvae, data processing, and structural analyses to quickly screen and test each chemical's potential as an insecticide," says Becnel.

Different bioassay methods are used to test various activities of candidate insecticides. After being tested against immature mosquitoes, the pesticides are tested against adult mosquitoes to select new active ingredients that might be registered and used for their control.

"In our laboratory tests, several compounds have already been found to be more toxic to mosquitoes than currently registered insecticide products," says research leader Gary Clark. "We have conducted tests with many different compounds in an effort to determine which might best be developed by chemical companies and meet the needs of our mosquito-control stakeholders."

The search for new, environmentally friendly insecticides for public health use is being done in partnership with the military as well as industry. The Deployed War-Fighter Protection Research Program has provided funds and promoted industry collaborations and partnerships to advance this important initiative.

This U.S. Department of Defense program has just begun its second 5-year funding period, which has encouraged several ARS laboratories to apply their scientific skills to the practical problem of protecting U.S. military personnel in the field from vector-borne diseases.—By **Ann Perry, Rosalie Marion Bliss, Don Comis, Sharon Durham,** and **Jan Suszkiw,** ARS.

This research is part of Animal Health (#103), Plant Genetic Resources, Genomics, and Genetic Improvement (#301), Plant Biological and Molecular Processes (#302), Aquaculture (#106), Plant Diseases (#303), and Veterinary, Medical, and Urban Entomology (#104), six ARS national programs described on the World Wide Web at www.nps.ars.usda.gov.

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ARS Research From Lab to Market

Hydrogen is often touted as a fuel of the future, and it's produced by some of the world's tiniest organisms. Now a patent-pending "green" technology from the Agricultural Research Service holds promise as a source of hydrogen for use in fuel cells that power pollution-free cars and more.

Probiotics—supplements that contain beneficial microorganisms—aid the immune and digestive systems of humans and other animals. ARS scientists are now developing probiotic bacteria that may lower blood pressure and protect dairy foods from harmful microbes. And some other ARS-tested probiotics are now being used in pet products.

These are just a few examples of how ARS research discoveries move from the laboratory to the marketplace and ultimately to consumers.

Technology transfer is the process by which successful government research, often conducted over long periods of time, is moved to private companies for further development and commercialization. Often, business owners protect their development investment by licensing patent-protected research. Other times, patent protection is not desirable, such as when ready access to research benefits the most end users quickly.

Either way, ARS has been credited with more than 800 new patents and 1,300 cooperative research and development agreements, which enable ARS scientists to work with commercial businesses on research projects of mutual importance. ARS has entered into more than 300 license agreements with business and nonprofit organizations. ARS has also entered into an average of 3,000 new partnership agreements per year with a variety of universities, nonprofit organizations, government agencies, and other industry groups in the past decade.

Some recent partnership developments are described here.

Clean Energy From Tiny Organisms

Renewable sources of energy—such as hydrogen—that don't produce greenhouse gases are needed to solve global energy shortages. Fossil fuels—coal, oil, and natural gas—are nonrenewable energy sources implicated in global climate change.

A new green technology developed cooperatively by ARS and North Carolina State University (NC State) scientists could lead to production of hydrogen from nitrogen-fixing bacteria.

The patent-pending invention could provide a source of hydrogen for use in fuel-cell technology. Fuel-cell devices combine hydrogen and oxygen to produce electricity and water and are considered efficient, quiet, and pollution free. Fuel cells are now being tested in a range of products, including automobiles that release no emissions other than water vapor.

ARS scientists Paul Bishop and Telisa Loveless and NC State scientists Jonathan Olson and José Bruno-Bárcena developed the technology, which is now available for licensing.

Purée Opens Markets for Sweetpotato

Sweetpotato is often called a nutritional powerhouse. Now, a patent-pending process has widened the market for using sweetpotatoes as an ingredient in food products. In 2007, the U.S. sweetpotato crop was estimated at a value of $374 million.

All shapes and sizes of sweetpotatoes can be used in a new purée that, after processing, can be stored unrefrigerated and is shelf stable for more than a year. The purée is now being used by manufacturers and food-service distributors as an ingredient in baked goods and baby foods.

The purée is made by rapid microwave heating. The unique process was developed, tested, and jointly patented by collaborators with ARS and NC State, both in Raleigh, North Carolina; and Industrial Microwave Systems, L.L.C., in Morrisville, North Carolina.

VAN-DEN TRUONG (D1382-1)

In a pilot plant at North Carolina State University, plant coordinator Gary Cartwright oversees the testing of aseptic packaging of sweetpotato puree using rapid microwave processing.

ARS food scientist Van-Den Truong, with the Raleigh-based Food Science Research Unit, and his collaborators tested the product extensively at an NC State pilot plant.

Snow Hill, North Carolina-based Yamco LLC licensed the process for exclusive commercial production of the purée. The company is solely owned by seven North Carolina sweetpotato growers.

Whey-Protein Muscle Puffs

A variety of starch-based snack products now boast up to 35 percent more protein, thanks to value-added, specially treated whey proteins. Whey, a byproduct of cheese manufacturing, normally would not combine easily with starches because of differences in molecular shape and structure.

But ARS scientists found that blended corn and whey-protein products could be puffed, or extruded, under high-pressure and low-moisture conditions. The resulting products are expanded and crunchy, rather than shrunken and tough.

ARS food technologist Charles Onwulata worked with

PAUL PIERLOT (D1372-1)

At the Eastern Regional Research Center in Wyndmoor, Pennsylvania, food technologist Charles Onwulata processes texturized whey proteins with a twin screw extruder to make healthy snack products.

Philadelphia, Pennsylvania-based Jerome Harden with Harden Foods, Inc., under a cooperative agreement to commercialize the ARS-patented ingredient. Onwulata is with the ARS Eastern Regional Research Center in Wyndmoor, Pennsylvania.

The company later licensed the technology and has introduced a new branded line of healthy snack foods, including cheese curls, tortilla chips, and corn chips. Harden Foods has also entered into agreements with other businesses to develop an array of nutritional products for the sports nutrition market, including fitness centers and retail stores. One such product, called "Muscle Puffs," contains 33 grams of protein per serving.

New Sweetener on the Block

Energy drinks, snack bars, shakes, and breakfast cereals that can help people with diabetes manage their diet are boasting a new slow-release carbohydrate-based sweetener. The carbohydrate syrup, called sucromalt, was developed under a trust-fund agreement between ARS chemist Greg Côté and geneticist Tim Leathers and scientists at Minneapolis, Minnesota-based Cargill, Inc.

Carbohydrates that are quickly digested and absorbed result in a rapid rise in blood glucose levels and can be considered high-glycemic-index foods. But sucromalt, having 70 percent of sugar's sweetness, is considered a low-glycemic-index sweetener that provides a blunted glycemic response in the body.

The ARS team's biocatalysis technology was licensed to Cargill, which has filed three patent applications covering the process of making sucromalt—or its use in various food products.

Metalworking Fluids Go Green

Alcoa, Inc., executives couldn't wait to replace mineral-oil-derived lubricating fluids with new cost-effective soybean-oil based fluids. Alcoa is the world's leading producer of primary and fabricated aluminum and alumina.

To help reduce its dependency on nonrenewable petroleum-based products, Alcoa contacted the ARS National Center for Agricultural Utilization Research (NCAUR) in Peoria, Illinois, to discuss developing biobased lubricating and metalworking fluids. These fluids keep aluminum metal from welding to steel rollers used to produce the aluminum sheets for everything from beverage cans to aircraft-wing panels.

A team of ARS scientists, led by chemist Sevim Erhan in NCAUR's Food and Industrial Oil Research Unit, worked with Alcoa on new technologies under a cooperative agreement. The resulting biobased fluids perform well and conform to industrial standards. And when vegetable oil is used instead of petroleum, Alcoa earns carbon credits and reduces worker exposure to chemicals and fumes emitted by petroleum-based fluids.

Biobased metalworking fluids have now been tested or used at Alcoa facilities in Reno, Nevada; Lancaster and Pittsburgh, Pennsylvania; Texarkana, Texas; Davenport, Iowa; Cleveland, Ohio; Point Henry, Australia; and Fusina, Italy.—By **Rosalie Marion Bliss,** ARS.

This research is part of Quality and Utilization of Agricultural Products, an ARS national program (#306) described on the World Wide Web at www.nps.ars.usda.gov.

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SCOTT BAUER (D1380-1)

Visiting chemist B.K. Sharma measures friction and wear of soy-based oil for use in Alcoa's metalworking fluids.

Fuel for Cars and Leftovers for Livestock

Biofuel research isn't just a matter of finding the right type of biomass—corn grain, soybean oil, or other material—and converting it into fuel. Scientists must also find environmentally and economically sound uses for the coproducts from biofuel production.

For example, producing a gallon of biodiesel from soybean oil also yields around two-thirds of a pound of crude glycerin. When this glycerin is refined to 99 percent purity, it can be used in many products, including pharmaceuticals, foods, drinks, cosmetics, and toiletries.

Livestock and poultry producers are looking for new sources of feed supplements to save costs and boost nutrition. "Several scientists have shown that it is possible to supplement pig diets with dried distiller's grains, which remain after ethanol production. Though this can result in equivalent animal productivity, it can also result in increased manure production—and higher levels of volatile organic compounds, which may increase odor emissions," says animal nutritionist Brian Kerr, who works at the ARS Swine Odor and Manure Management Research Unit at Ames, Iowa. "We decided to look at using the coproducts from biodiesel production as feed supplements because no such data was available to the livestock industry."

STEPHEN AUSMUS (D1340-2)

Loading samples into a real-time PCR instrument, physiologist Thomas Weber measures gene expression in tissues collected from piglets fed diets containing coproducts from the biofuels industry.

Kerr partnered with animal scientist William Dozier—formerly in the ARS Poultry Research Unit at Mississippi State and now with Auburn University—and Iowa State University colleague Kristjan Bregendahl to see whether crude glycerin could be used to supplement livestock feed.

Glycerin Supplements Are a Success

Kerr led studies that examined how crude glycerin feed supplements affected swine energy use. In five different experiments, he supplemented the diets of starter pigs and finisher pigs with different levels of crude glycerin. Overall, these studies showed that the sample of crude glycerin contained an apparent metabolizable energy (AME) concentration of 3,207 calories per kilogram (kcal/kg). AME is a standard measure of energy used in nutritional studies.

Pigs fed the crude glycerin were able to digest it efficiently, and it provided them with a supply of caloric energy that basically equaled that of corn grain. A followup study showed no effects on weight, carcass composition, and meat quality in pigs fed diets containing 5 percent or 10 percent crude glycerin from weaning to market weight.

Meanwhile, Dozier and Bregendahl evaluated the use of glycerin supplements in poultry feed. They used 48 egg-laying hens and 1,392 broilers in 4 research studies.

After feeding four levels of crude glycerin to laying hens, Bregendahl determined the AME in the crude glycerin to be 3,805 kcal/kg. He also compared feed consumption, egg production, egg weight, and egg mass (calculated by multiplying egg production and egg weight) and found no significant differences among the four groups.

"Glycerin supplements were well utilized for egg production by the hens," he says.

Dozier, meanwhile, conducted three broiler studies. In his first study, young broilers consumed either a control diet with no glycerin supplementation or feed with a 6-percent glycerin content. His results indicated that glycerin provided the 7- to 11-day-old broilers with an AME of 3,621 kcal/kg. Later research resulted in similar findings for older broilers. Glycerin supplements at varying levels provided 21- to 24-day-old broilers with an AME of 3,331 kcal/kg and 42- to 45-day-old broilers with an AME of 3,349 kcal/kg.

Overall, the data indicates that crude glycerin is an excellent source of energy in swine and poultry rations and can be used without harming animal performance, carcass composition, or meat quality.

"This research project has been a success so far," says Dozier. "We will have a total of six peer-reviewed papers from this research, and we've been invited to present the results at national and international nutrition conferences. But we still need additional research on how to handle glycerin as an alternative feedstuff for swine and poultry in integrated feed mills."

He also notes that from a nutritional standpoint, this technology can serve as an alternative dietary energy source that could result in lower feed costs.

STEPHEN AUSMUS (D1339-3)

Animal nutritionist Brian Kerr feeds piglets crude glycerin (the dark liquid), which he has shown to be an excellent source of energy for the animals.

Crude glycerin does contain small amounts of methanol and salt, which could potentially limit its use as a feed supplement. Additional studies might be needed to assess how much methanol livestock can safely ingest in glycerin supplements, which would help regulators refine U.S. standards for using crude glycerin in livestock feed.

But as U.S. biodiesel production continues to boom, crude glycerin supplements could be a win-win situation for biodiesel producers and farmers alike.

"Swine and poultry producers are very interested in supplementing livestock feed with glycerin," Kerr notes. "This way, crops can be used for both biofuels and for livestock at the same time."—By **Alfredo Flores** and **Ann Perry,** ARS.

This research is part of Food Animal Production (#101) and Manure and Byproduct Utilization (#206), two ARS national programs described on the World Wide Web at www.nps.ars.usda.gov.

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SEVIM ERHAN (D1120-1)

Soy-Based Hydrogel

Ready for Biomedical Exploration

Soy-based hydrogel mixed with water (left) and in powder form (right). The polymer has great potential as a drug-delivery agent.

Agricultural Research Service (ARS) scientists in Peoria, Illinois, have done it again.

They've added yet another invention to an already long list of oleochemical accomplishments that includes petroleum-free newspaper ink, industrial lubricants, hydraulic fluids, and aircraft deicers.

Their latest addition is a "hydrogel." Made from soybean oil, it's a squishy but durable polymer that expands and contracts in response to changes in temperature or acidity levels. These characteristics make it "suitable for use in the hair-care and drug-delivery areas," says ARS chemist Sevim Z. Erhan. Another potential use is in wound dressings.

Erhan and ARS chemist Zengshe Liu developed the hydrogel in studies at ARS's National Center for Agricultural Utilization Research in Peoria. Their invention dovetails with the center's mission of developing new, value-added uses for corn, soybeans, and other Midwest crops, which will benefit farmers, processors, and consumers. A key focus of the center is to explore options to reduce the myriad uses of petroleum, which include making fuel and polymers like plastic.

"Today's hydrogels are mainly made of synthetic polymers, like polyacrylic acid, polyacrylamide, and so on," notes Erhan, who leads the center's Food and Industrial Oil Research Unit. Soybean oil offers the advantage of being a homegrown polymer resource—one that need not be imported or mined from the Earth. Indeed, in 2006, U.S. farmers planted 76 million acres of soybeans, equal to about 38 percent of the world's total oilseed production.

There are environmental benefits, too. Vegetable-oil-based polymers like the soy hydrogels are biodegradable, notes Erhan. "The only disadvantage," she adds, "is that their water-absorbing capacity is lower than that of petroleum-based hydrogels."

One area where this may not pose a problem is drug delivery. In collaboration with Erhan and Liu, University of Toronto professor Xiao Yu Wu has formulated the new hydrogel into minuscule particles that effectively deliver controlled doses of the breast-cancer drug doxorubicin.

Wu's team encapsulated the doxorubicin in stearic acid, a waxy lipid that, together with the particles, releases the drug at prescribed temperatures and pH values.

In drug-release experiments Wu's team published in the *Journal of Pharmaceutical Research,* nanoparticle-delivered doxorubicin proved eight times more toxic to cancerous cell lines than when it was delivered in a lipid-water solution.

Erhan and Liu first developed the soy-based hydrogels in 1999. Their method uses a two-step process—ring-opening polymerization and hydrolysis—to create a crosslinked polymer backbone with carboxylic groups that gives the hydrogel its unique properties.

Soy proteins are known allergens. But Erhan doesn't anticipate this being an impediment to the hydrogel's potential use as a drug-delivery agent, because soybean oil's chemical structure is completely changed by the two-step manufacturing process.—By **Jan Suszkiw,** ARS.

This research is part of Quality and Utilization of Agricultural Products, an ARS national program (#306) described on the World Wide Web at www.nps.ars.usda.gov.

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Back to the Past With New TSE Testing

Transmissible spongiform encephalopathies (TSEs) are rare—but lethal—neurodegenerative disorders that affect a range of mammals, including humans. Bovine spongiform encephalopathy (BSE)—or "mad cow disease"—is one TSE that has had significant economic and public health impact. The TSEs in the United States are found in sheep, goats, elk, and deer.

TSE epidemiology is complicated by the fact that a definitive diagnosis cannot be made until after an animal has died. ARS chemist Eric Nicholson and veterinary medical officer Robert Kunkle have found a way to facilitate postmortem TSE diagnoses—even when tissue samples are in short supply.

Proteins called "prions" are produced in all animals. But the development of abnormal prions is believed to prompt the onset of TSE-related damage to brain tissue. If an animal dies from a TSE infection, both abnormal and healthy prions can be found in its body tissues.

Researchers check tissues for abnormal prions with either Western blotting (WB) or immunohistochemistry (IHC). WB is used for fresh or frozen tissues, and IHC is used to test formalin-fixed tissue that has never been frozen. Sometimes only formalin-fixed tissues are available for testing—a situation the BSE surveillance program faced in 2005, when an entire tissue sample was inadvertently preserved in formalin. In that instance, initial IHC results were not conclusive, but there were no other fresh or frozen tissue samples available for WB testing.

Nicholson and Kunkle, who work at the National Animal Disease Center in Ames, Iowa, wanted to help scientists avoid similar situations in the future. They found a way to extract and identify abnormal prions in formalin-fixed tissue by using a combination of mild detergent, a series of freeze-boil cycles, and enzyme digestion. Initial results indicate that the accuracy of this method begins to decline 2 years after the tissue is first preserved, and is completely lost at the end of 6 years.

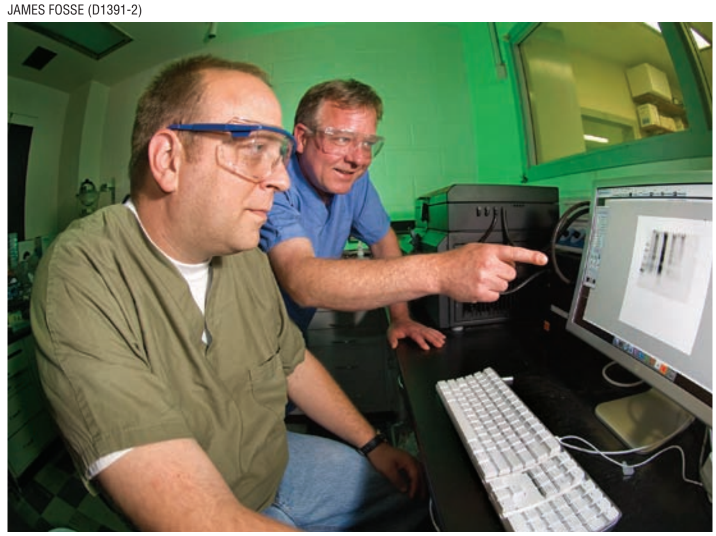
"With this technique, we can easily distinguish between tissues from TSE-positive and TSE-negative animals," Nicholson says. "And it requires only a minimal adaptation of existing Western blotting procedures."

Nicholson and Kunkle also devised a way to use WB to test for TSE in tissues that had been fixed in formalin and preserved in paraffin. Their results equaled—and at times even exceeded—the effectiveness of WB analysis for tissues that had only been fixed in formalin.

These combined results add to the tools animal scientists can use to study the development and spread of TSEs. Their findings will facilitate WB testing of tissue samples that were originally archived for microscopy examination and should simplify preservation of samples collected in the field.

WB and IHC analyses can also be conducted on the same preserved sample—a breakthrough that could significantly enhance ongoing TSE research in the field and in the lab.—By **Ann Perry,** ARS.

JAMES FOSSE (D1391-2)



Chemist Eric Nicholson (left) and veterinary medical officer Robert Kunkle discuss a Western blot image from a TSE test of formalin-fixed tissues.

This research is part of Animal Health, an ARS national program (#103) described on the World Wide Web at www.nps.ars.usda.gov.

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Animal Studies Safeguard Food Supplies —and Human Health

PEGGY GREB (D1441-1)

Hundreds of different species of *Mycobacterium* are known to exist, and many of them have been infecting animals and humans—sometimes with deadly results—for thousands of years. Scientists at the Agricultural Research Service's National Animal Disease Center (NADC) in Ames, Iowa, are fighting back against two of its most damaging variants—Johne's disease and bovine tuberculosis (bovine TB).

M. avium subspecies *paratuberculosis* (MAP) is responsible for the onset of Johne's disease, which results in losses exceeding $200 million every year to the U.S. cattle industry. Experts believe that almost 70 percent of U.S. cattle herds are infected with MAP, and an animal can spread the disease soon after it is infected. But noticeable symptoms—including severe weight loss and diarrhea—can take up to 5 years to develop.

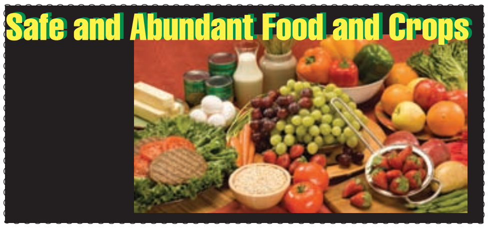
"MAP is like a stealth organism," says NADC microbiologist Judy Stabel. "It shelters in the host's white blood cells and stays at low levels until stress makes the disease apparent. And it's one of the hardest organisms to work with in the field."

"We need a better way to detect infected animals early on," adds NADC microbiologist John Bannantine.

Protein Prospecting

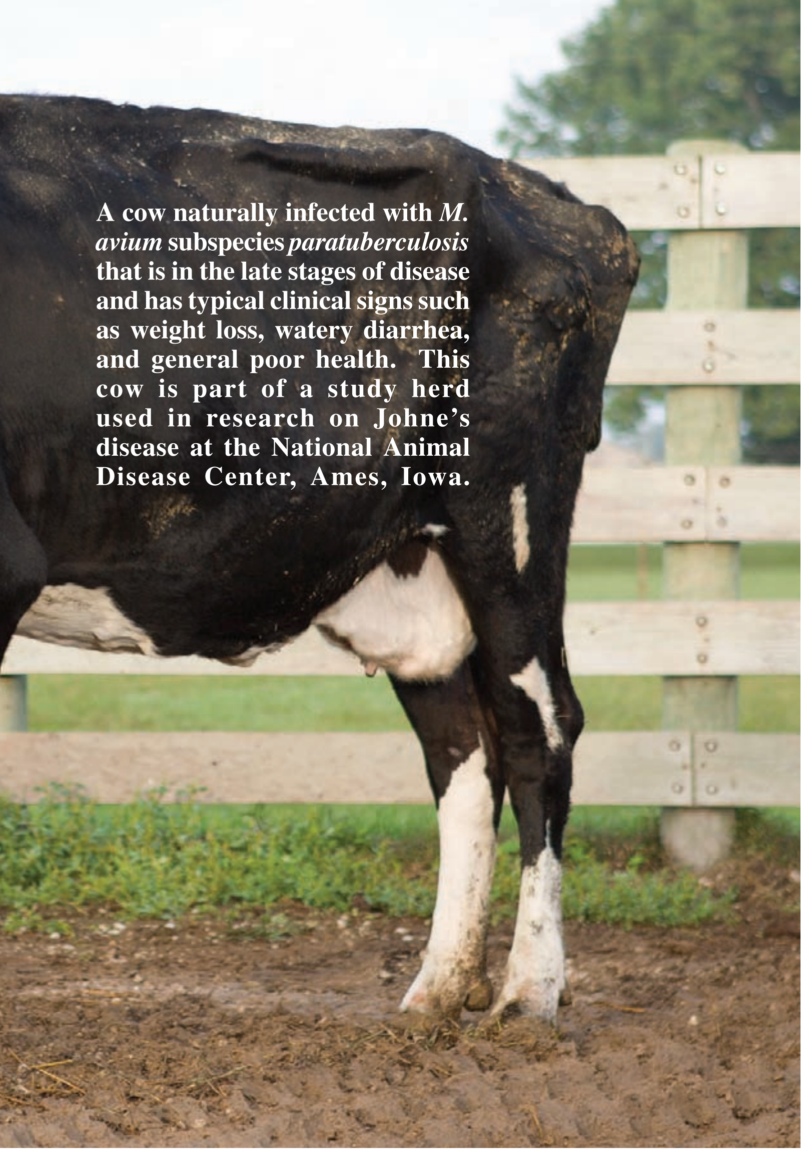
The MAP genome—which contains all its genes—has been sequenced, so researchers now have information about the different proteins that are made from those genes. Bannantine and colleagues took some of this genetic data and assembled an array of 96 proteins. They wanted to identify proteins that might be useful in confirming a diagnosis of Johne's disease or that might be targeted for therapeutic intervention.

The researchers used the 96 proteins in the array to identify which ones prompted the most robust immune response from antibodies in the serum of infected cattle. They found three proteins that consistently drew the strongest attacks from serum antibodies—a level of immune response that clearly linked the three proteins with the onset of the disease. Bannantine believes that with additional work, these segments might provide crucial building blocks for development of a diagnostic tool for Johne's disease.



PEGGY GREB (D1442-1)

Microbiologist Judy Stabel bottle feeds young calves involved in a project to evaluate immune responses to a vaccine that protects against Johne's disease.



A cow naturally infected with *M. avium* subspecies *paratuberculosis* that is in the late stages of disease and has typical clinical signs such as weight loss, watery diarrhea, and general poor health. This cow is part of a study herd used in research on Johne's disease at the National Animal Disease Center, Ames, Iowa.

"This protein array is the only one like it in the world," Bannantine says. "Because we've been so careful in selecting the MAP proteins for our array, we're confident that the antibodies are responding to MAP proteins—not similar proteins produced by other *Mycobacterium* species."

Bannantine is also very pleased that their studies have cleared up another aspect of MAP infection.

"When an animal is first infected, there is a cell-mediated response to the bacterium," he says. "We thought that another type of immune response—the one that produces antibodies—developed much later. But in experimentally infected animals, we can use this array to detect exposure to MAP as early as 70 days after the animal is infected, much earlier than previously reported in field studies."

The next step is to determine whether these early-detected antigens are recognized by infected cattle in real-world dairy herds. "We also need to determine the extent of cross-reactivity these proteins have with other environmental mycobacteria, because one problem with current Johne's disease tests is the lack of specificity," Bannantine says.

Stabel has been studying more about the early stages of the cell-mediated response to MAP and finding ways to diagnose the disease in young animals. "We've found a way to use information about the cell-mediated response to detect MAP in naturally infected calves that are only 6 months old," she says. "When animals this young are diagnosed, then the producer can decide how best to respond—either by removing the animal from the herd or looking at other options."

Stabel has also helped evaluate animal models for MAP research and has concluded that a smaller ruminant model—like goats or sheep—shows promise. "These animals are slightly quicker to reach a clinical disease state," she says.

New Approaches to an Ancient Disease: Tuberculosis

The USDA Animal and Plant Health Inspection Service (APHIS) tests more than 1 million animals every year for bovine TB. A lot can ride on the outcome. For instance, from May 2002 through June 2004, 875,616 cows from 687 California herds were tested for bovine TB. As a result, 13,000 cows were culled—a decision that necessitated federal payments as high as $4,000 per animal.

During that time, dairy herd quarantines were enforced to ensure that contaminated milk from infected cows did not enter the human food supply and transmit the disease to people. This additional enforcement cost individual producers as much as $70,000 per month in lost income.

Finding effective diagnostics and vaccines for bovine TB is crucial for livestock and human health and producer profits. At NADC, around 80 white-tailed deer—some so tame that they try to steal food from the pockets of their caretakers—are key to this effort.

"White-tailed deer are a significant reservoir of bovine TB," says NADC veterinary medical officer Mitch Palmer. "When the deer jump fences and share livestock feed, they can contaminate the feed with TB bacteria. That's one way cattle herds become exposed to the disease and become infected."

Vetting Vaccines

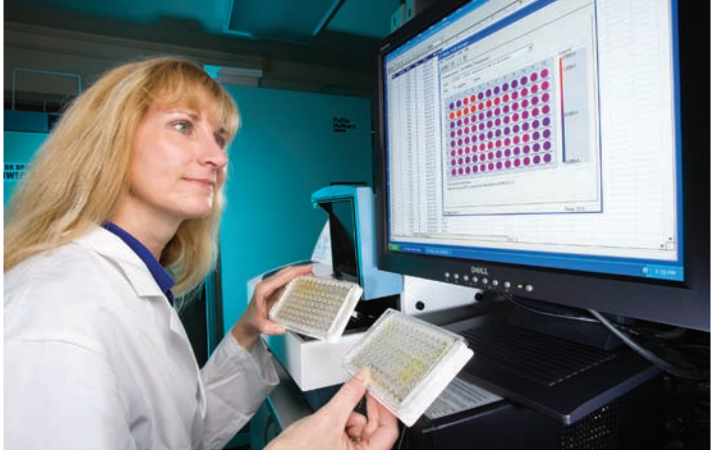
Because of the threat wild deer pose to domestic cattle, Palmer and his colleagues have been vaccinating white-tailed deer with the human TB vaccine *M. bovis* BCG. They want to see whether vaccination could be part of a successful effort to control the disease in wildlife.

"What we've found is that the vaccine is effective in decreasing the severity of the disease and that oral vaccination appears to be as effective as subcutaneous vaccination," Palmer says. "But we have some safety concerns. Because it's a live vaccine that stays in body tissues for up to 250 days, bacteria in the vaccine that prompt the protective immune response might also infect humans who consume venison from vaccinated deer. And vaccinated deer can shed the vaccine and expose other animals to the disease."

The vaccine research has yielded other significant findings as well—including results that could affect human health.

NADC molecular biologist Tyler Thacker led studies

PEGGY GREB (D1440-1)



Judy Stabel evaluates the results of the interferon-gamma blood test to diagnose cows infected with *M. avium* subspecies *paratuberculosis*.

indicating that in white-tailed deer, levels of interferon gamma (IFN-gamma)—a protein that is critical in the immune response to bovine TB infection—increase as the severity of the infection increases. This finding countered previous studies suggesting that levels of IFN-gamma rose when the immune system of a vaccinated animal was successfully fighting the pathogen.

Other results in the study suggested that elevated levels of IL-4, another immune protein, could be found in animals with lower infection levels. This also contradicted research with other animal species suggesting that IL-4 levels increase with disease severity.

“These results give us more accurate information about whether a trial vaccine is effective,” Thacker says. “Vaccine testing is expensive, so it’s helpful to have screening tools for interpreting results. Now that we know IFN-gamma is not a good predictor of vaccine efficacy, we can start looking for other indicators.”

The NADC scientists have also been using neonatal calves to test human TB vaccines. This approach is cheaper and safer to use than testing in nonhuman primates.

“We’ve devised a very effective method for inoculating neonatal calves with TB by using an aerosol challenge,” says NADC veterinary medical officer Ray Waters. “This gives us new options for testing human TB vaccines on animals with immune responses that closely resemble the human immune response.”

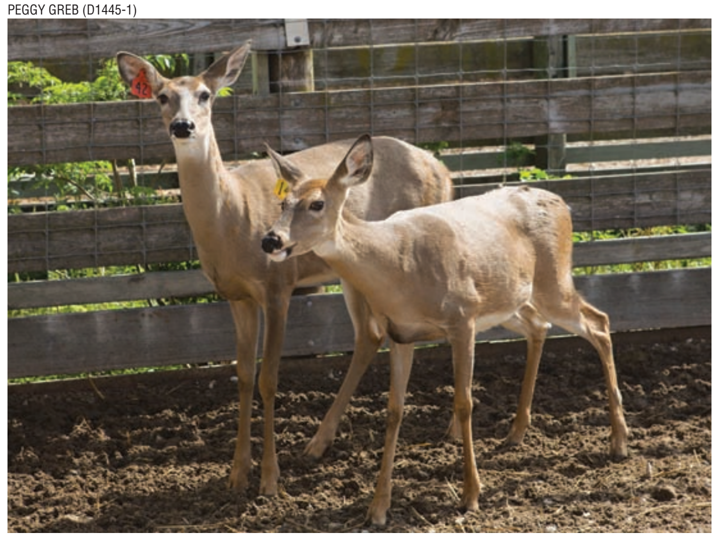
In addition, the most common time for administering the vaccine to humans is soon after birth—timing that is mimicked when neonatal calves are used for tests. The neonatal calf tests are currently the most effective bridge between testing in mice and nonhuman primates. Results have so far been very similar to the findings in nonhuman primates.

Vaccines or Diagnostics—Which Comes First?

“Vaccine development and diagnostic development are intertwined,” Thacker observes. “If we find indicators that predict immunity, then maybe we can refine those indicators and use them for diagnostic testing as well.”

Thacker, Palmer, and Waters are working on improving bovine TB tests for cattle. “We have serum-based tests that are not approved for use in the TB eradication program yet,” Palmer says. “But they are showing some potential for testing samples in the field, where time or temperature could affect the stability of the blood samples.”

PEGGY GREB (D1445-1)



Two of about 60 deer maintained at NADC in Ames, Iowa. The unique research herd is used to study vaccines for prevention of tuberculosis in white-tailed deer.

PEGGY GREB (D1447-1)

Microbiologist John Bannantine loads amylose resin affinity columns to purify recombinant *M. avium* subspecies *paratuberculosis* proteins produced in *Escherichia coli.*

PEGGY GREB (D1446-1)

Veterinary medical officers Ray Waters (left) and Mitch Palmer (right) prepare to collect blood to be used in developing improved tests for tuberculosis in cattle.

Meanwhile, producers and APHIS staff often use a commercial blood-based test called "Bovigam" to confirm a preliminary positive skin test diagnosis of bovine TB.

"Standardizing the protocol for using Bovigam is still a work in progress," says Waters. "Labs have been running different test protocols, so we're working on establishing standard measures for processing blood samples. For instance, we've found that using vessel plates to test samples is just as effective as using tubes, and with vessel plates we don't need to use as much of the reagent to obtain accurate results. We also need to determine optimal schedules for collecting and processing samples—and we need to determine a safe temperature range for maintaining those samples before they reach the lab."

Into the Wild

Animal health depends as much on meticulous fieldwork as it does on painstaking lab studies. So Waters, Palmer, and Thacker were part of a team that obtained blood samples from 760 wild deer and surveyed them for bovine TB, the largest such sampling to date. The team found that the blood tests accurately identified an infected animal about 70 percent of the time. On the other hand, the tests correctly identified a noninfected animal about 90 percent of the time.

Palmer explains, "With most tests of this type, there's typically a tradeoff between the accuracy of positive and negative results. We're continuing to work on this because we'd like to get both numbers in the high 80s or low 90s.

"We're approaching bovine TB research from a range of perspectives, but all of it helps us understand the immune response," Palmer continues. "When an animal is infected with TB, tissue destruction is caused by the host reacting to the TB pathogen. We want to understand what is causing the tissue destruction, which will help us find ways to treat the disease and intervene in the process of tissue destruction."—By **Ann Perry,** ARS.

This research is part of Animal Health, an ARS national program (#103) described on the World Wide Web at www.nps.ars.usda.gov.

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Rift Valley Fever

Rift Valley fever (RVF) was once confined to Africa—until cases were confirmed in Saudi Arabia and Yemen in 2000. These cases sparked concerns about the disease's ability to spread further. RVF has never reached the United States, but if it were to do so, researchers at the Arthropod-Borne Animal Diseases Research Laboratory (ABADRL) in Laramie, Wyoming, would be ready.

RVF is a potentially devastating zoonotic disease. Fortunately, ABADRL scientists are collaborating with colleagues in the United States and abroad to ensure that the nation will be prepared in the improbable event of a U.S. outbreak.

Under the leadership of microbiologist William Wilson, ABADRL scientists are pursuing RVF research with three main objectives: to determine which North American mosquito species are competent to transmit the RVF virus; to develop sensitive and specific diagnostic tools; and to develop and evaluate vaccines.

To meet the first objective, ABADRL scientists are investigating whether there is a vector genetic component to RVF transmission. Preliminary studies have examined which mosquito and midge species are more likely to act as virus vectors. The results suggest regional differences in the ability of mosquito populations to become infected and transmit the virus. To expand research in this area, ABADRL is increasing the size and scope of its existing mosquito colonies to include additional species.

The ABADRL scientists are working with the Canadian Food Inspection Agency and USDA's Animal and Plant Health Inspection Service to develop operator-safe, sensitive diagnostic tests for early detection. They are also cooperating with the U.S. Department of Homeland Security to evaluate the safety of potential commercial vaccines. ABADRL is collaborating with the Canadian agency to develop livestock challenge models to evaluate the efficacy of new vaccines as they are developed.

Such research ensures that the United States won't be caught off guard should an outbreak of RVF occur. The work also has immediate practical applications in countries currently affected by the disease.—By **Laura McGinnis,** ARS.

Munching on Garlic Mustard

A New Weevil in the Works

Garlic and mustard are common ingredients that can be found in American households. But garlic mustard? Well, that's a different story.

Garlic mustard, *Alliaria petiolata,* is considered one of the most problematic invaders of temperate forests in North America. According to legend, it was brought here from Europe in the 1860s as a culinary herb, but unfortunately, it doesn't taste very good. Since then, garlic mustard has spread to 34 U.S. states and 4 Canadian provinces.

"Garlic mustard is an invasive plant that gets a lot of attention," says ecologist Adam Davis, who has been studying the weed for years. "It's very noticeable and hard to eradicate because of its seed bank."

The term "seed bank" refers to seeds in the soil that are dormant but capable of germinating. Garlic mustard seeds can remain viable for more than 10 years. A single plant can produce hundreds of seeds, which scatter as far as several meters from the parent.

"You can spend a lot of time and money pulling garlic mustard up or spraying it with pesticides, but it'll just come back the next year," says Davis. "That's why it's such a problem. It's very resilient."

A member of the mustard family Brassicaceae, garlic mustard got its name because its leaves, when crushed, smell like garlic. Garlic mustard is a biennial plant, meaning it takes 2 years to complete its life cycle. During its first year, the plant is in the form of a rosette with kidney-shaped leaves that remain green throughout the winter. In its second year, the plant matures and produces small, white flowers, each with four petals in the shape of a cross. The mature plants either self-pollinate or are pollinated by insects, producing seeds that fall to the ground and enter the soil.

Garlic mustard is cold hardy and shade tolerant, enabling it to grow early in spring when most plants are not able to grow. It also secretes allelochemicals into the soil. Allelochemicals are chemical compounds a plant introduces into the growing environment to suppress growth of another plant. "It's kind of like chemical warfare against the native plants," says Davis.

The insects and fungi that feed on garlic mustard in its native habitat are not present in North America, increasing the weed's seed productivity and allowing it to outcompete native plants.

A Model Solution

To better understand garlic mustard and find a suitable biocontrol, Davis—in collaboration with colleagues at Michigan State University, Cornell University, the University of Illinois, and the Centre for Agricultural Biosciences International (CABI) in Switzerland—created a computer model that simulates the weed's life cycle.

PEGGY GREB (D1496-1)

Flowers of garlic mustard produce up to several thousand seeds per plant, making it difficult to control.

"In part, we wanted to answer ecologists' criticisms that biocontrol can potentially cause as many problems as it solves because of unintended consequences," says Davis. "We were looking for a way to choose agents that are most likely to succeed while reducing their potential for harm to native plants and environments. Ideally, we want to try to release only one organism, if possible."

TIM HAYE, CABI EUROPE-SWITZERLAND (D1498-1)

The weevil *Ceutorhynchus scrobicollis* is being evaluated for release as a biocontrol for garlic mustard. It feeds on the plant's root, root crown, and shoot base, reducing rosette survival and seed production.

Through this model, Davis was able to predict the type and severity of damage that would be needed to reduce garlic mustard's population growth rates. Davis performed an analysis using computer code that enabled him to change one variable at a time while keeping all the others constant, allowing him to probe the life cycle for the plant's weak point. He found that in order to make an impact, a biocontrol agent has to reduce garlic mustard's survival in the rosette stage and its ability to reproduce in the adult stage.

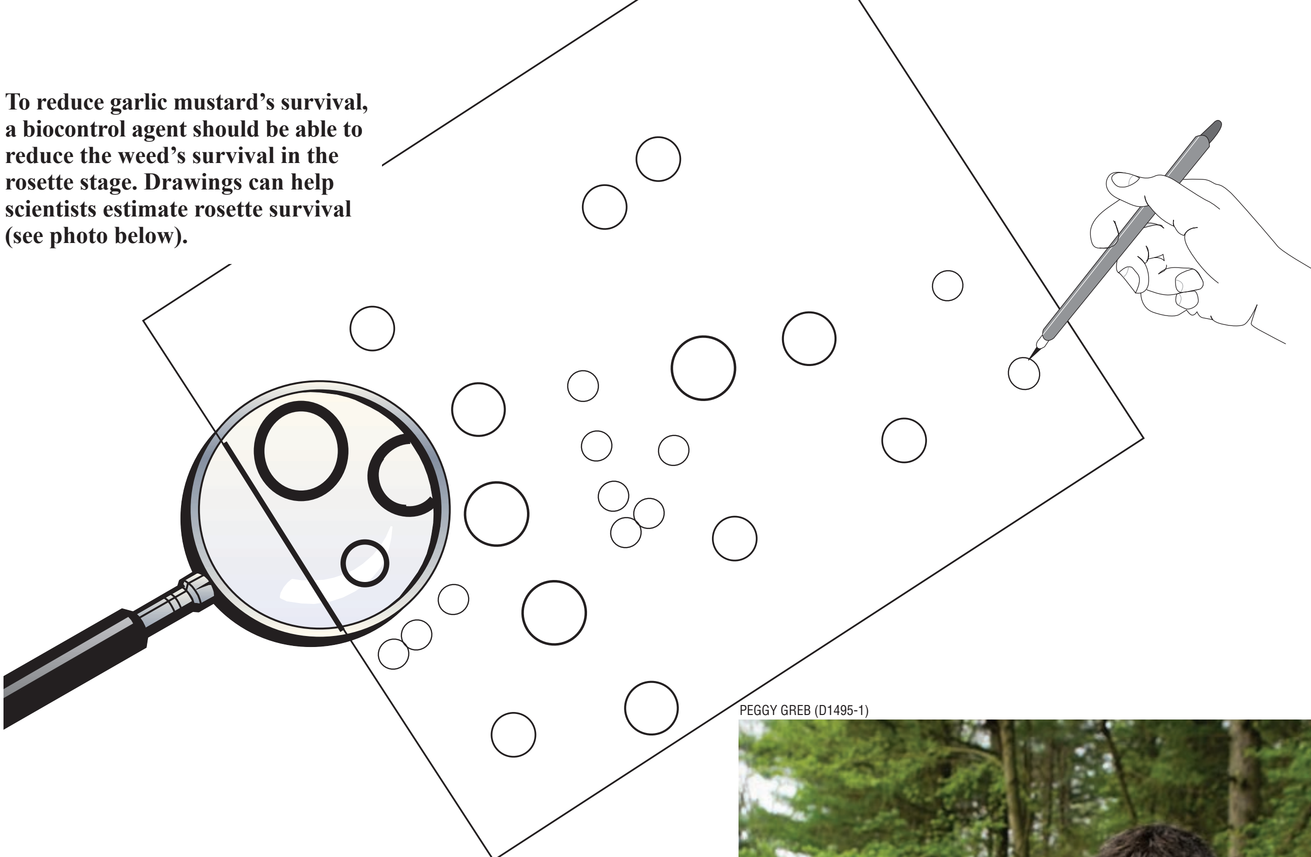
Well before Davis created the life-cycle model, CABI scientists began looking for and testing potential biocontrol agents to tackle garlic mustard. They collected data on the amount of damage each insect could inflict on the garlic mustard population. From a list of more than 70 natural enemies found to be feeding on garlic mustard in Europe, four *Ceutorhynchus* weevils were selected as the most promising control agents.

Combining the feeding information collected by CABI scientists and the demographic information of garlic mustard in North America, Davis used the computerized life-cycle model to assess each weevil's ability to inflict damage on the weed and inhibit its growth. One weevil, *C. scrobicollis,* came out on top.

High Hopes for Little Insect

The tiny *C. scrobicollis* has a life cycle of 1 year and produces one batch of offspring per lifetime. It lays its eggs on garlic mustard's leaf stems in the fall. When the eggs hatch in the spring, the larvae feed on the weed's root crown, the area from which the rosette's leaves grow and where nutrients are stored.

To reduce garlic mustard's survival, a biocontrol agent should be able to reduce the weed's survival in the rosette stage. Drawings can help scientists estimate rosette survival (see photo below).



PEGGY GREB (D1495-1)

In Urbana, Illinois, ecologist Adam Davis records the position of garlic mustard rosettes (clusters of green leaves low to the ground) on a sheet of transparent mylar. The locations are recorded in June and October of the first year and in June the following year to estimate rosette survival rates. Once garlic mustard rosette locations are recorded, they are then converted into digital coordinates in a GIS (geographical information system) program, permitting spatial analysis of rosette survival.

By feeding on the root crown, *C. scrobicollis* stops the flow of nutrients and water from the roots to the rest of the plant. It also damages the meristem, the area of the plant where new growth takes place. As a result, garlic mustard produces fewer seeds or, in areas with high weevil populations, dies prematurely in early spring without producing any seeds.

C. scrobicollis also appears to be monophagous, meaning it eats just one thing: garlic mustard. That means scientists won't have to worry about any unintended consequences when using this insect as a biocontrol agent.

During preliminary testing, CABI scientists believed *C. scrobicollis* was the best candidate to control garlic mustard. Putting the weevil's feeding data through Davis's life-cycle model confirmed their beliefs and created a stronger case for the permit process.

"The model gave teeth to the permit application to release this weevil in the United States," says Davis. "It provided a peek into the future as to the impact the weevil could have on the garlic mustard population here."

C. scrobicollis is currently in quarantine at the University of Minnesota. If all goes well, this beneficial weevil may soon be roaming North America to find a nice garlic mustard meal.—By **Stephanie Yao,** ARS.

This research is part of Crop Protection and Quarantine, an ARS national program (#304) described on the World Wide Web at www.nps.ars.usda.gov.

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Flu Fighters

Identifying, Treating, and Preventing the Flu With Help From ARS Research

The first reports of a novel swine-origin influenza outbreak surfaced in the U.S. media in April 2009. The U.S. Centers for Disease Control and Prevention (CDC) first detected human infection in mid-April. Over the next 6 weeks, human infections were detected in more than 40 countries and nearly every U.S. state.

Early media reports referred to the virus—now known as “2009 influenza A (H1N1)”—as “swine flu,” a potentially confusing term for the public. Scientists initially concluded that the virus came from pigs because its genetic material was most similar to that of swine influenza virus. We now know that the 2009 H1N1 virus is a “triple reassortant virus,” meaning that it contains genetic material from swine, avian, and human influenza viruses—a mix that may help the virus spread quickly and pass between humans and pigs.

For some researchers, the story of a new influenza virus infecting pigs and people was all too familiar. As early as 2007, researchers from USDA’s Agricultural Research Service (ARS) and Animal and Plant Health Inspection Service (APHIS) had been monitoring a strain of influenza that could spread between pig and human populations.

In August 2007, several people developed flulike symptoms after exhibiting their swine at a county fair in Ohio. Many of the pigs became sick as well. Tests revealed the source of the illness: a triple reassortant influenza A (H1N1) very similar to viruses that are endemic in U.S. pig herds.

Scientists from ARS and APHIS characterized the virus and found that in pigs it was slightly more virulent than average. In light of the virus’s characteristics and its documented ability to spread from pigs to humans, the scientists advocated close monitoring of influenza in swine, birds, and other species.

In September 2008, ARS, APHIS, and CDC launched a collaborative effort funded by CDC to develop a national swine influenza virus (SIV) surveillance pilot program. The goal is to better understand the epidemiology of SIV infections and to improve diagnostic tests, preventive management, and vaccines for swine and humans. This program has been instrumental in implementing surveillance for the 2009 outbreak, but it’s not the first time ARS influenza work has had major benefits. ARS scientists have been at the forefront of avian and swine influenza research for decades.

Agency scientists have developed tests to rapidly identify poultry infected with avian influenza and vaccines to protect against the disease. They’ve also developed and tested vaccines for swine. And ARS scientists have contributed to public health by identifying flu strains, assessing vaccines, and developing international standards for inactivating flu virus in cooked products.

JAMES FOSSE (D1611-1)

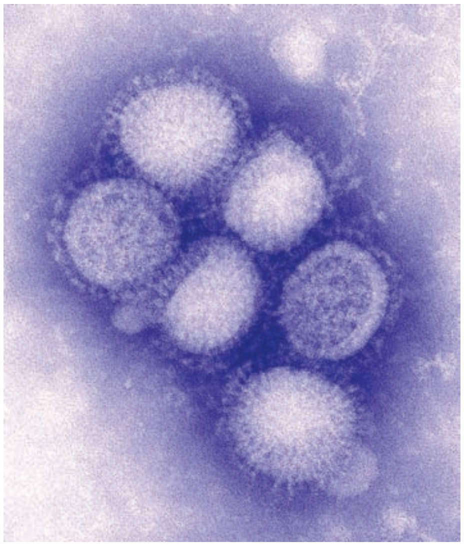
ARS veterinary medical officers Kelly Lager (left) and Amy Vincent collect a nasal swab from a piglet to test for novel H1N1 influenza virus.

Swine Surveillance

A 2008 paper co-written by ARS veterinary medical officer Amy Vincent states that swine influenza has undergone dramatic changes in the past decade, after nearly 80 years of relative stability. The increase in new subtypes and variants has complicated efforts to control the disease and increased the need for innovative strategies to fight it.

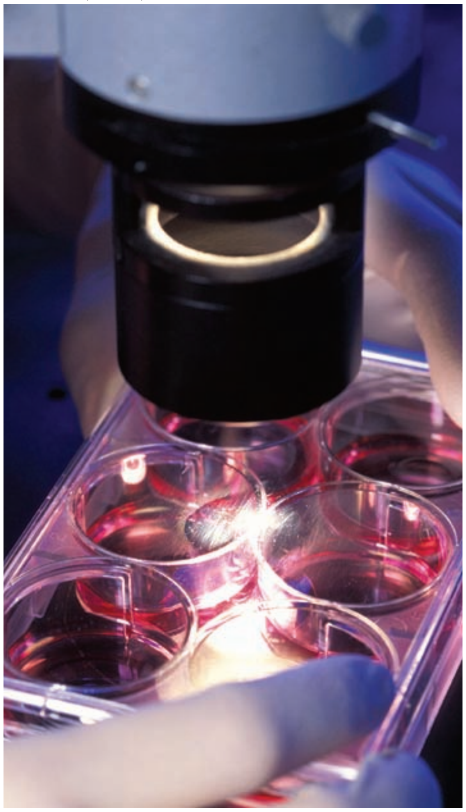
One such strategy is the pilot SIV surveillance program,

CDC INFLUENZA LABORATORY (D1610-1)



Transmission electron micrograph of the H1N1 virus.

SCOTT BAUER (K11003-1)



In studies to evaluate swine flu vaccines, cells are observed for signs indicating they are infected by live swine influenza A virus.

in which APHIS coordinates with the National Animal Health Laboratory Network to screen potential SIV cases submitted from veterinary diagnostic laboratories. ARS then characterizes virus samples of special interest. The agencies also share virus isolates.

"We hope the pilot program will eventually become a permanent fixture," Vincent says. "Having an established program would enable us to track not only where SIV occurs, but whether the virus undergoes significant mutations. This information could be essential in the event of an outbreak."

The virus behind the current outbreak—like the virus from the Ohio county fair—is a type of influenza A known as "H1N1." Though these two viruses have some substantial genetic differences, they both have surface proteins from swine influenza viruses. The surface proteins of these viruses are very different from those of human seasonal influenza A (H1N1) viruses, which means that most humans do not have any cross-reactive antibody against them. Other viruses that have caused human illness, but originated in animals, include H5N1 and H7 viruses from poultry.

At the beginning of the 2009 H1N1 outbreak in humans, pork producers had two important questions: Is the virus capable of infecting pigs? And if so, how will they respond? To find out, Vincent and her colleagues challenged young pigs with the virus in a biosafety level 3 facility at the ARS National Animal Disease Center (NADC) in Ames, Iowa. They observed that the pigs developed clinical signs of influenza consistent with those seen in endemic SIV infections. The scientists are also examining antisera and existing vaccines to determine whether the immunity that develops in response to infection or vaccination will protect pigs against the current outbreak.

Pigs, Poultry, and Pathogenicity

Because the new 2009 H1N1 virus contains genetic material from avian influenza viruses, two more questions must still be addressed: Is the virus capable of infecting avian species? And are poultry and other birds potential vectors for influenza in humans? Though the answers to these questions are still unknown, ARS scientists have a strong foundation of avian influenza research on which to build.

Scientists at the ARS Southeast Poultry Research Laboratory (SEPRL) in Athens, Georgia, have developed and evaluated avian influenza vaccines, helped assess public health threats, evaluated virus virulence, and helped develop protocols for inactivating flu viruses in food products. More recently, they've been evaluating how specific viruses pass from one animal to another.

Domestic pigs generally catch influenza from other pigs, but they're also susceptible to infection from humans and birds. Fortunately, several highly pathogenic strains of avian influenza virus don't appear to cause significant disease in pigs. That's the conclusion of a study led by Aleksandr Lipatov (now with the CDC). The research team also included Yong Kuk Kwon, Luciana Sarmento, Erica Spackman, David Suarez, and David Swayne at SEPRL, and Kelly Lager at NADC.

"The finding is significant because pigs are thought to be vessels for mixing avian influenza and human influenza to allow genetic reassortment that could set the stage for pandemic avian influenza," says Swayne.

In the studies, piglets were exposed to four different strains of highly pathogenic H5N1 avian influenza viruses. Two swine influenza viruses were used as controls. The studies show that none of the H5N1 virus strains caused significant or fatal disease in pigs, but they did cause mild to moderate inflammation in the lungs.

Infection with either swine influenza virus resulted in much more severe symptoms and pneumonia.

In related research, scientists at NADC and SEPRL have collaborated to develop two sets of diagnostic tests to quickly and accurately differentiate the new 2009 H1N1 virus from endemic forms of swine and avian influenza viruses already circulating in the United States.

Research efforts like these are essential to ensuring the health and safety of people and livestock in the United States and around the world.—By **Laura McGinnis** and **Sharon Durham,** ARS.

This research is part of Animal Health, an ARS national program (#103) described on the World Wide Web at www.nps.ars.usda.gov.

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Bacterial Backtalk
Swine, Stress, and *Salmonella* Infection

PEGGY GREB (D1509-1)

Microbiologists Brad Bearson (left) and Shawn Bearson prepare to inoculate a pig with *Salmonella enterica* serovar Typhimurium to investigate *Salmonella* colonization in swine.

The complex cellular signaling that takes place between bacteria and host is called "crosstalk." In animal production environments, this system is effective—and disruptive to animal health and food safety. So ARS microbiologists Brad and Shawn Bearson are looking for ways to cut off the communication.

The husband-and-wife team is learning how to interpret the crosstalk between swine and the foodborne pathogen *Salmonella enterica* serovar Typhimurium (*S.* Typhimurium). Brad works at the ARS National Soil Tilth Laboratory in Ames, Iowa, while Shawn is located across town at the ARS National Animal Disease Center.

The researchers studied how *S.* Typhimurium responds when it is exposed to norepinephrine, a hormonal neurotransmitter that helps animals regulate their physiological response to stress. As part of this regulation, norepinephrine secretion increases when stress levels increase—a situation swine commonly face during transport.

Microbe on the Move

The researchers found that *S.* Typhimurium is able to respond to norepinephrine via a two-component system involved in monitoring the bacterial environment. This phenomenon is called "microbial endocrinology." An adaptation that mirrors similar systems in *E. coli* and other pathogens, it enhances the pathogen's potential for motility (movement) and colonization.

One component—a protein called "QseC"—is embedded in *S.* Typhimurium's membrane. When norepinephrine increases, QseC alerts a response regulator called "QseB" inside the bacterial cell. QseB responds to this alert by regulating a cascade of early, middle, and late genes that increase the pathogen's motility.

Although multiple two-component sensing-and-signaling systems are present in bacterial cells, this is the first time that the response of QseC to norepinephrine has been characterized in *S.* Typhimurium. In addition, this is the first time it has been described in a large-animal model.

"Performing experiments in a large-animal model is important," Shawn notes, "because in the small-animal model—the mouse—*S.* Typhimurium infection is systemic. But when pigs are infected with *S.* Typhimurium, they don't usually develop systemic infections; instead, they develop gastrointestinal (GI) tract infections. So the large-animal model is much more applicable to food safety and livestock health—and maybe to human health as well."

To learn more about how QseC supports *S*. Typhimurium in the swine GI tract, the researchers developed a strain of *S*. Typhimurium with a genetic mutation that inactivated the *qseC* gene. This mutation, in turn, inactivated the QseC protein. They inoculated 13-week-old pigs with both the mutant strain and a wild-type *S*. Typhimurium strain. After 1 week, they took samples of GI tract tissue from the inoculated swine and examined the samples for bacterial colonization.

The researchers found that the wild-type strain was able to establish significantly more colonies in the swine GI tract than the mutant strain.

Bearson and Bearson also found that swine inoculated with the mutant bacterium shed significantly fewer pathogens. This has potential food safety consequences, because even asymptomatic pigs can carry and shed *S*. Typhimurium, which can then infect other swine around them.

"All of our results indicate that QseC regulates a variety of genes that affect pathogen motility, fitness, and invasive effectiveness," Brad says. "Just as important, our results show that this two-component sensing system is a key factor in the ability of *S*. Typhimurium to successfully colonize the swine GI tract."

Secrets to Success

Once the researchers had a clearer understanding of how *S*. Typhimurium responds to fluctuating norephinephrine levels, they began to look for a medication that could disrupt the response. They decided that phentolamine—a compound already used medicinally in humans—was a likely candidate for in vitro testing.

In these studies, the scientists exposed wild-type *S*. Typhimurium to either norepinephrine or a combination of norepinephrine and phentolamine. They observed that phentolamine eliminated the pathogen's norepinephrine-enhanced motility.

PEGGY GREB (D1510-1)

Brad Bearson analyzes cultures for the presence of *Salmonella enterica* serovar Typhimurium in swine feces.

But *S*. Typhimurium's success in swine isn't just due to its increased motility when norepinephrine levels increase. It also has a mechanism for acquiring iron from its host to support its own growth and replication.

In blood serum, a protein called "transferrin" binds to iron. This binding action blocks efforts by opportunistic bacteria to use the metal for their own survival and reproduction.

But norepinephrine can also bind to iron. When this occurs, *S*. Typhimurium has receptors that bind to the norepinephrine, which allows the pathogen to access the sequestered iron. On the other hand, too much iron can be toxic, and *S*. Typhimurium has developed genetic mechanisms to regulate iron acquisition and uptake.

The researchers identified key *S*. Typhimurium genes involved in the pathogen's ability to access iron from norepinephrine. They also found 36 genes involved in the bacterium's iron response that were down-regulated when exposed to norepinephrine, protecting the pathogen from iron toxicity.

The team is pleased with their results to date. But they still have key questions about how these findings can enhance food safety and livestock health.

"We're still dealing with significant unknowns," Shawn cautions. "We don't know how much norepinephrine increases in the GI tract during stress, so we can't pinpoint the levels that might require intervention and treatment."

"We also want to find out differences between *S*. Typhimurium and *E. coli* to exploit for targeted interventions against *Salmonella,*" Brad says. "We're going to start in vivo studies of phentolamine, and we're going to keep looking for other substances that interfere with *S*. Typhimurium colonization of the GI tract. We want to find a proven method for inhibiting the pathogen before the pigs even begin to experience stress."—By **Ann Perry,** ARS.

This research is part of Food Safety, an ARS national program (#108) described on the World Wide Web at www.nps.ars.usda.gov.

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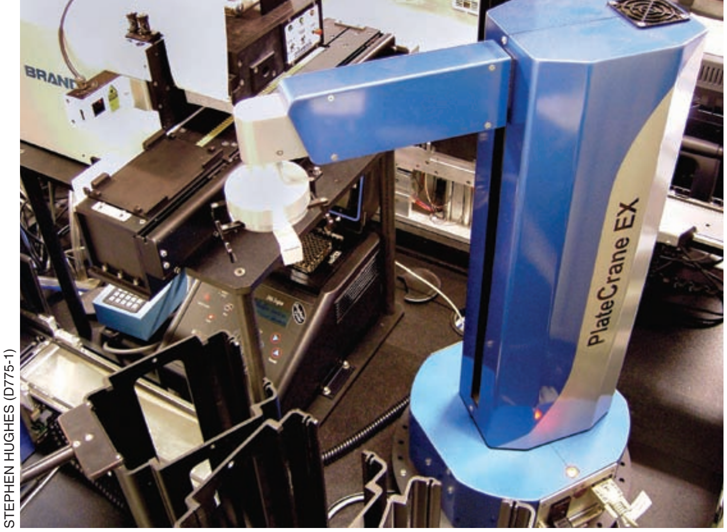
Robot-Controlled System Speeds "Designer" Yeasts for Making Ethanol



STEPHEN HUGHES (D775-1)

The blue robotic arm shown here moves plates of samples during automated tests.

A robot recently reported to work at ARS's National Center for Agricultural Utilization Research (NCAUR) in Peoria, Illinois, and its arrival was met with enthusiasm by many scientists there.

The robot in question doesn't walk or talk, however. Rather, it's the centerpiece of an automated system called the "plasmid-based functional proteomics work cell." The system's inventors expect it will greatly streamline studies aimed at harnessing the power of proteins for industrial applications, like making fuel ethanol from sugars in corn fiber.

ARS molecular biologist Stephen R. Hughes and Peoria colleagues developed the system, starting in 2004, with an engineering team at Hudson Control Group, Inc., of Springfield, New Jersey. Hudson president Philip J. Farrelly led the team.

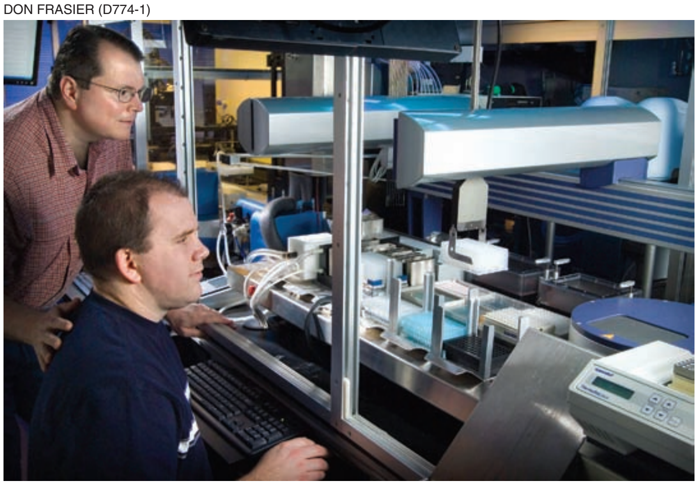
Ask Hughes about their patent-pending system, which was published in the May 2006 issue of *Proteome Science,* and you'll likely get a rapid-fire narration of its history and capabilities.

One thing is clear, though: It's the first system of its kind to fully automate several laboratory procedures that have traditionally been done one step at a time, by hand.

A short list of them includes: extracting genetic material from the cells of animals, plants, fungi, and bacteria; making DNA copies of these genes; inserting the copies into plasmids of *Escherichia coli;* culturing the bacteria so they'll multiply and can be stored; making "cDNA libraries" from these plasmids; plucking *E. coli* libraries of interest from plates on which they're grown; sequencing the nucleotide bases comprising the plasmid-bound cDNA copies; identifying these genes as well as the proteins they code for; and inserting desirable genes into *Saccharomyces* and other yeasts for improved fuel ethanol production.

Yes, the ARS center's robot does all that. Thanks to the fast, precise movements of its mechanized arm and computerized plate tracks, the robot will carry out these tasks hundreds or thousands of times faster than a human. That will prove critical in searching for genes to lift the yeasts over a major hurdle that's kept them from reaching peak performance as ethanol producers: their inability to metabolize sugars locked up in corn fiber.

DON FRASIER (D774-1)



Molecular biologist Stephen Hughes (left) and technician John Jackson use the automated "plasmid-based functional proteomic work cell" that may help them develop yeast strains to improve ethanol production.

Hughes, who's in NCAUR's Bioproducts and Biocatalysis Research Unit, says the system will also "allow us to build genes from scratch and mutate them—for example, to obtain enzymes with higher temperature stability."

Of particular interest is developing genetically engineered yeasts that can survive conditions inside the large industrial vats where corn-fiber sugars like xylose are fermented into ethanol. At the same time the yeast is fermenting the sugars, it could also be making other valuable products, such as pesticides, building blocks for biodegradable plastics, and anticancer compounds.

"What makes this effort unique in the ethanol-for-fuel research field is that we're using genetics to make a new organism that fits into existing processing plants, rather than developing a whole new process built around the organism, as everyone else is doing," comments Farrelly. "In other words, we're making a 'bug' to fit the process, rather than making a process to fit the bug."

Currently, only corn's starch component is converted. With the *Saccharomyces* yeasts now used, this equates to 2.8 gallons of ethanol from a bushel of corn. Developing the fiber-hydrolyzing strains, however, could realistically help squeeze 10 percent more ethanol from the grain crop by 2013, the researchers say.

Besides engineering the yeasts to do such work at high temperatures, the researchers are also looking to develop strains that tolerate lower pH levels or other ethanol-plant conditions.

"Using the robot to isolate improved yeast strains will benefit not only commercial ethanol partners," says Hughes, "but also farmers, by increasing the markets for agricultural commodities."—By **Jan Suszkiw,** ARS.

This research is part of Bioenergy and Energy Alternatives (#307) and Quality and Utilization of Agricultural Products (#306), two ARS National Programs described on the World Wide Web at www.nps.ars.usda.gov.

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In the Midwestern United States

Growing Biofuel Crops Sustainably

As crop residues are being used to produce ethanol and other biofuels, a delicate balance has to be struck between how much is removed for energy and how much is left on the ground to protect soil from erosion, maintain soil organisms, and store carbon in the soil.

The Agricultural Research Service has scientists in 18 states who are searching for that balance as a main goal of the Renewable Energy Assessment Project (REAP).

Collaborators include universities participating in the Sun Grant Initiative, which is funded through the U.S. Departments of Transportation, Energy, and Agriculture.

Because corn is currently the most widely used biofuel crop, the REAP team is especially interested in determining where, when, and how much corn stover can be harvested without harming soil productivity. "Corn stover" refers to the parts of the plant that remain after grain harvest—the leaves, stalks, and corncobs.

Dave Archer, an ARS agricultural scientist at Mandan, North Dakota, has drawn a 10-mile-radius circle around the University of Minnesota's Morris campus for an intense study that could serve as a national model for answering questions about the use of plant residue for fuel.

STEPHEN AUSMUS (D1622-3)

STEPHEN AUSMUS (D1622-11)

At the University of Minnesota-Morris Biomass Gasification Facility (top), gasification researcher Jim Barbour and ARS soil scientist Jane Johnson evaluate potential biomass feedstocks. Close-up (bottom) shows some of the feedstocks, including pressed corn stover (being handled by Johnson) for use in an institutional-scale unit.

Corncob Heat

The Morris campus plans to heat its buildings with gas released by a controlled burning of corn stover in a process known as "gasification." Archer found that farmers inside the 10-mile circle can produce enough corncobs or other stover to supply the university's gasification plant once it begins operating. And they can do so sustainably—paying attention to long-term profitability and environmental quality.

Archer says, "We're using this gasification plant's corn stover needs as a stand-in for the much larger needs of commercial ethanol plants, such as one that is near Morris, but outside the 10-mile circle. That plant is beginning to gasify corncobs and other biomass to replace some of the natural gas used in producing ethanol."

To prepare for these new bioenergy uses for corn and to establish procedures for making similar cost-benefit calculations for corn bioenergy operations around the country, Archer used the ARS Environmental Policy Integrated Climate (EPIC) model to study the economics and environmental soundness of farmers' supplying the university with corncobs or other corn stover.

"The university's plant needs about 10,000 tons of biomass a year," Archer says. "The circle area produces an average of 22,595 tons of corncobs a year."

Archer used the EPIC results to compute the costs of harvesting the corncobs or corn stover, transporting the materials on the farm, and replacing soil nutrients lost from the plant biomass removal, while considering short-term effects on crop yields and soil erosion.

Economics and Corn Stover Fuel

"Ethanol plant operators use a rule of thumb that says that it's only worth producing ethanol from corn stover if they can buy it for no more than $50 a ton," Archer says. "If corncob harvest added only $5 per acre to corn-harvest costs, the EPIC model simulations showed that area farms could sell the corncobs profitably for anywhere from $17.95 to $33.71 a ton."

The EPIC model showed that harvesting 40 percent of the corn stover would raise erosion rates on most soils by 0.25 tons an acre per year. Crop residues left on the soil after grain harvest tend to slow down precipitation runoff and protect soil from water erosion.

The more soil erodes, the more phosphorus washes away with it. The EPIC model showed that

STEPHEN AUSMUS (D1620-1)

ARS soil scientists Jane Johnson and Don Reicosky (retired) look for signs of erosion on plots used to test rates of biomass removal. In the background, technician Rochelle Jansen collects trace gas samples to test for carbon dioxide, nitrous oxide, and methane.

soil erosion and phosphorus losses could be reduced by harvesting stover from areas less susceptible to erosion, removing stover at lower rates, and choosing tillage and cropping practices with soil conservation in mind.

Soil Conservation and Corn Stover Removal

No-till or other forms of conservation tillage leave more crop residue on the soil surface by eliminating plowing. Cropping practices include long-term diverse rotations and cover crops. Archer's colleague, Don Reicosky, then an ARS soil scientist at Morris but now retired, says that "without these changes nationally, crop residue removal could cause soil carbon levels to begin another nosedive similar to when prairie sod was busted open and intensive agriculture began, around 1870."

Reicosky has done studies that document this, showing that rotations with diverse kinds of crops slow down the rate of carbon loss over the years. He has found that intensive tillage releases carbon dioxide from soils, so using conservation tillage can significantly reduce losses of soil carbon to the atmosphere.

In the EPIC simulations, Archer used data from studies conducted by Reicosky and Jane Johnson, also a soil scientist at Morris. Johnson is measuring corn stover and corncob yields under various levels of tillage and the amount of corn stover needed to protect soil and maintain soil carbon levels. Johnson says, "In general, conservation tillage reduces the amount of residue needed to maintain soil carbon levels. But more plant residue is needed to maintain soil carbon than to control soil erosion."

The Morris scientists are researching whether the coproducts of gasification and pyrolysis can be used as a soil amendment to replace lost carbon and nutrients and help maintain soil quality. If so, this may further increase the amount of corn stover that could be harvested for biofuel.

Archer says that when Johnson finishes her study of minimal residue requirements to maintain soil carbon levels, he will use that information to update his EPIC analysis. He also says there is need for other research. For example, more information is needed on the cost of the cob harvest, and his study did not include costs of transporting cobs to the university's gasification plant.

Still, removal of some residue for bioenergy may also remove a barrier to the adoption of no-till in the northern Midwest states: the concern that leaving too much residue slows the soil's spring warm-up and drying, thus delaying planting time.

Cover Crops May Offset Carbon Losses

Using perennial groundcover roots and shoots as alternative sources of organic material is another potential way to add enough carbon to the soil to offset the carbon lost when stover is removed.

At the ARS National Soil Tilth Laboratory in Ames, Iowa, agronomist Jeremy Singer wants to identify perennial cover crops for this purpose.

Singer's research is part of a study of optimizing corn management for biofuel production. He's evaluating perennial grasses and a legume to assess their potential as groundcovers to mitigate soil erosion and enhance soil organic matter, even in fields where all the corn grain and stover are harvested for biofuel production. This research is partially funded by the U.S. Department of Energy through its Sun Grant Regional Partnership.

The project, which Singer is conducting with colleagues from Iowa State University at Ames, has three goals: to determine the relative competitiveness of groundcover species growing with corn; to identify whether a genetic component exists that enhances corn's ability to compete with perennial groundcovers; and to develop management systems that minimize competition between corn and the groundcover while maximizing soil carbon contributions, soil erosion protection, and nutrient cycling.

Singer will be overseeing the component on developing management systems. "A perennial cover crop extends the function of a winter cover crop through the whole season," Singer notes. "Besides adding soil carbon to maintain critical soil functions, it also enhances water infiltration, holds nitrogen in the soil, helps to suppress weeds, and reduces water runoff."

STEPHEN AUSMUS (D1624-10)

Baseline soil sampling is done to characterize a farmer collaborator's field for sustainability of harvesting corn cobs for bioenergy. Here, soil scientist Jane Johnson (right) and technician Brooke Knick separate the samples by depth before lab analysis for carbon, nitrogen, and other nutrients.

Two Likely Candidates

The first season of the study was disrupted by spring floods and summer hail. But even after these challenges, the harvest results indicated that white clover and Kentucky bluegrass were likely cover crop candidates that warranted additional study. One of the grasses studied, creeping red fescue, added notable amounts of carbon to the system, but its vigorous growth hindered growth of the corn crop.

Singer and his colleagues now need to see whether their initial findings will hold up with further research. Then they need to combine their findings with other research on corn hybrids that can share their space with perennial cover crops and still provide good yields.

When the best groundcover has been identified, using no-till and other reduced-tillage practices in the corn-groundcover system will reduce the amount of fossil fuel needed to prepare and plant the crops. This, in turn, will likely decrease greenhouse gas emissions—another prospective plus for farmers and fields alike.—By **Don Comis** and **Ann Perry,** ARS.

This research is part of Bioenergy and Energy Alternatives (#307) and Agricultural System Competitiveness and Sustainability (#216), two ARS national programs described on the World Wide Web at www.nps.ars.usda.gov.

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JEREMY SINGER (D1634-2)

Agronomist Jeremy Singer (left) and technician Dustin Wiggins observe that a creeping red fescue cover crop appears to be hindering growth of the corn crop.

JANE JOHNSON (D1627-1)

The amount of corn stover that can be harvested from corn grown in rotation with soybean is being determined in both no-till and chisel-plowed fields. Returning stover and reducing or eliminating tillage protects soil (above), while harvesting the leaves and chisel plowing exposes soil (below).

JANE JOHNSON (D1626-1)

From Lubricants to Packaging Materials

ARS Scientists Just Say "No" to Petroleum

Screech!

Few sounds are more nerve-wracking than the scraping of metal on metal. It's the job of lubricants to prevent that annoying noise by making sure that—when metal touches metal—everything moves smoothly and quietly.

Machines that have moving parts—your car's engine or the hydraulic pump of a huge earthmover, for instance—almost always require lubricants. Most lubricants are made of so-called "base oil" that's blended with additives to boost performance.

In the United States, the demand for additives, already at nearly 2 billion pounds a year, is expected to increase at a respectable 2 percent a year for the next 5 years. That's why Sevim Z. Erhan and colleagues have developed a new process for making biobased additives, ones suitable for use in formulating greases; crankcase, two-cycle, and marine engine oils; and hydraulic, transmission, and drilling fluids.

The additives would be made from the predominant fat molecules—triglycerides—in natural oils of familiar crops like soybean, corn, or canola or from lesser-knowns like pennycress, camelina, and crambe. Fully biodegradable, these vegetable-oil-based additives are just slightly darker, and somewhat thicker, than the clear, light-yellow cooking oils used in home kitchens across America.

The additives would provide an alternative to today's petroleum-based products—and a potentially profitable market for growers in the Midwest and elsewhere.

Erhan did the work while a chemist at the ARS National Center for Agricultural Utilization Research in Peoria, Illinois. She's now director of the agency's Eastern Regional Research Center in Wyndmoor, Pennsylvania.

The new-age additives, says Erhan, offer many benefits and, to date, no downside. "You can use them with either biobased or conventional lubricants," she points out. And the novel additives meet all the standard criteria for a top-notch, antifriction, antiwear additive, namely, impressive viscosity and liquidity, high flashpoint, and stability despite temperature extremes.

In small-scale laboratory tests to evaluate wear and friction, for example, the plant-oil additives performed as well as or better than commercial petroleum-based additives. And since they're fully biodegradable, proper disposal is fast, easy, and inexpensive.

What's more, the additives can be produced with the same equipment that chemical companies already have in place for making traditional additives.

It's no wonder that several leading makers of petroleum-lubricant additives have already expressed an interest in the scientists' process for making the biobased additives.

Erhan and coinvestigators Brajendra K. Sharma of Pennsylvania State University-University Park, and Atanu Adhvaryu, formerly with the university, describe the technology in their U.S. patent, issued in 2007. Importantly, the procedure and the resulting additives are eco-friendly. "No harsh solvents are used, and very

NCAUR (D1643-1)

At the NCAUR lab in Peoria, chemist Sevim Erhan (now center director at the Eastern Regional Research Center in Wyndmoor, Pennsylvania) formulates a biodegradable lubricant using an ARS patented biobased antiwear additive.

Wrapping Up With Dairy-Derived Ingredients

Speaking of saying "no" to petroleum-based products, many food manufacturers want to incorporate renewable materials into their operations to help reduce food-packaging waste.

Most food packages are made of multilayer films that are thin, continuous sheets of synthetic polymers. But consumers and food retailers are concerned about the waste generated during the manufacture of such packaging. Many, it seems, are interested in replacing petroleum-based packaging with biobased packaging.

At the ARS Eastern Regional Research Center in Wyndmoor, Pennsylvania, scientists are developing strong, biodegradable dairy-based films that are better oxygen barriers than petrochemical-based films. "We are involved with finding the technology to improve dairy films so that they can eventually perform as plastics," says research leader Peggy Tomasula, with the center's Dairy Processing and Products Research Unit.

PEGGY GREB (D1642-1)

Close-up of the additive being placed onto ball bearings before friction test at right.

PEGGY GREB (D1641-1)

Visiting chemist Brajendra Sharma uses a standard four-ball test method to measure friction and wear properties of vegetable-oil-based antiwear additives. The additives can be used in either biodegradable or conventional lubricants and greases.

little energy and water are required," says Erhan.

In brief, the vegetable oil is allowed to react with several chemicals, for various periods of time, including a 4-hour stint at a boil. Savvy chemists would summarize the process as "reacting triglyceride oils with thiols to yield poly (hydroxy thioether) derivatives."

Plant Starch—Not Polystyrene

Just as the environmentally sound additives might help lessen America's dependence on petroleum, so too might starch-based foams replace polystyrene products. An example: the rigid, custom-fit foam pieces used inside cardboard cartons to keep computer monitors securely in place during shipping and storage. Those foam pieces could just as easily be made from wheat, potato, or corn starches instead of petroleum.

That's according to Gregory M. Glenn, a plant physiologist with the ARS Western Regional Research Center in Albany, California.

Glenn and co-inventor Simon K. Hodson, formerly with EarthShell, LLC, of Lebanon, Missouri, have patented two "green" processes for making top-performing, starch-based foams for a multitude of everyday products—shipping liners, disposable dinnerware, and more.

These biofoams, which look like familiar polystyrene foam goods, are strong, durable, and versatile, Glenn says. They can be manufactured to the same range of densities as conventional foams and can be die-cut or molded into a seemingly limitless array of shapes, sizes, and thicknesses.

The starch-derived foams aren't waterproof, however, so a moisture barrier needs to be added. But such barriers are nothing new and, in fact, can be derived from plant sources, such as corn, so that the finished biofoam product would still be made exclusively from renewable, biodegradable natural resources.

The Shape of Things To Come

Glenn has worked with both of the new processes in small-scale experiments at

PEGGY GREB (D1639-1)

Food technologist Artur Klamczynski (left) and plant physiologist Greg Glenn prepare batches of starch-based dough which will be further processed by extrusion to form heat-expandable pellets.

She wrote "Using Dairy Ingredients To Produce Edible Films and Biodegradable Packaging Materials," a book chapter in the soon-to-be-published *Dairy-Derived Ingredients: Food and Nutraceutical Uses,* by London-based Woodhead Publishing.

The chapter focuses on films made from dairy proteins, with an emphasis on those based on casein and whey, the major proteins found in milk. It also covers research efforts to improve the proteins' mechanical and barrier properties so that these natural materials could eventually be used in a variety of applications.

As a dairy ingredient, casein shows good adhesion to different substrates. But while casein is an excellent barrier to oxygen, carbon dioxide, and aromas, it is a weak barrier to moisture. "The water-soluble nature of these proteins is a big problem," says Tomasula. "Much of the research on edible casein films to date is directed to improving their water-vapor barrier properties. The use of dairy-based films as part of multilayered packaging systems will be the focus of future research."—By **Rosalie Marion Bliss,** ARS.

his laboratory. Both technologies rely on an extruder—a standard piece of equipment—to heat and mix a dough-like blend of starch and other all-natural compounds.

For one process, the extruder squeezes out long strings of the dough, referred to as a "thermoplastic melt," that are later cut into small beads, about half the size of a marble. At various points in the process, the beads puff and expand. That happens, for instance, when the beads are put into the cavity of a heated mold, to press them into the desired shape.

Expanded beads will eventually touch one another, creating a strong matrix that's much like the bead matrix of polystyrene foams.

The second process that Glenn and Hodson developed results in a mix that emerges from the extruder as a continuous hollow tube. As it moves, it is slit along one length so that instead of being rounded, it opens flat, just like a book that's opened to lie flat on a table.

The now-flat foam is then rolled up—like a bolt of cloth—and stored. Later, it can be heated, unrolled, and die-cut into the desired product.

To enhance the strength of the foams, natural fibers—from a softwood such as southern pine—can be added to the mix before it goes into the extruder, reinforcing the foam for heavy-duty jobs.

The mounting environmental costs of landfills increasingly stuffed with non-biodegradable polystyrene may eventually make Earth-friendly, biobased foams the preferred choice for tomorrow.—By **Marcia Wood,** ARS.

This research is part of Quality and Utilization of Agricultural Products, an ARS national program (#306) described on the World Wide Web at www.nps.ars.usda.gov.

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SCOTT BAUER (K5052-5)

For Sustainability Smarts: The National Agricultural Library's "Sustainable Agriculture Information Tools" Website

You're interested in learning more about sustainable farming, but you don't want to spend a lot of time surfing the web.

No worries.

Expert librarians at the National Agricultural Library in Beltsville, Maryland, and Washington, D.C., have done the homework for you, compiling handy resources at www.nal.usda.gov/afsic/sustain.shtml.

Scroll down to the "Learn More" headings to find an interesting potpourri of clickables, all leading you to reliable, authoritative resources. All are carefully vetted by the pros at the library's Alternative Farming Systems Information Center.

Get backgrounded quickly—and become fluent in the lingo—by skimming "Sustainable Agriculture: Definitions and Terms."

Check out the comprehensive "Sustainable Agriculture Automated Database Searches." Anywhere from thousands to millions of documents were sifted and winnowed to find those most relevant and of the highest quality.

Curious about scientific journals? You'll want to view the library's top picks, posted at "Sustainable Agriculture: Top 10 Research Journals."

Have college-age kids or grandkids who might be interested in studying sustainable ag? They'll definitely want to read "Educational and Training Opportunities in Sustainable Agriculture," which covers everything from the nation's longest-running undergraduate degree program in organic farming (Washington State University-Pullman) to the University of New Hampshire's new "eco-gastronomy" program, an eclectic blend of nutrition, food, and farming.

Updated frequently, the library's sustainable agriculture website is widely regarded as among the best of its kind.—By **Marcia Wood,** ARS.

ELIZABETH KRIETMEYER (D766-1)

Chemist Randal Shogren performs a test to determine how much sorbitol citrate is needed to prevent scale, the crusty buildup of calcium carbonate from so-called hard water. Sorbitol citrate could be used as an inexpensive, environmentally friendly detergent additive.

Corn: A New Ingredient for Detergents?

A greener future could be in store for laundry and dishwashing detergents, thanks to new, environmentally friendly ingredients developed by Agricultural Research Service (ARS) scientists and Folia Inc., of Birmingham, Alabama.

Under a 4-year cooperative agreement, the ARS-Folia team has developed detergent additives called "cobuilders" that prevent buildup of crusty deposits known as "scale." In hard-water regions, scale can cause harm ranging from discolored clothing and cloudy dishes to diminished cooling and washer damage.

Phosphate-based cobuilders were once used to soften water and improve detergent cleaning power by preventing crystallization of calcium carbonate as scale. Now, the petroleum derivative polyacrylic acid is used. But it isn't biodegradable, notes chemist Randal L. Shogren, with ARS's National Center for Agricultural Utilization Research in Peoria, Illinois. "It goes down the drain and into the water supply, where its accumulation could be a problem."

In 2001, Folia asked Shogren and ARS chemical engineer J.L. Willett about developing a biodegradable alternative to polyacrylic acid. A cooperative research project ensued. In studies with Graham Swift (then Folia's chief technology officer), two former ARS postdoctoral researchers—Sergio Gonzalez and Ken Doll—and Daniel Graiver of Michigan State University-East Lansing, the ARS scientists explored the scale-stopping potential of citric acid and sorbitol.

Derived mainly from cornstarch, both compounds "are plentiful, renewable, and inexpensive resources," says Shogren. "We combined them and heated them to form biodegradable polyesters."

In a solution, calcium carbonate generally starts forming crystals within 1 minute. But adding the new corn-based polyester cobuilders prevented crystal formation for 10-30 minutes. Although less polyacrylic acid is needed to do the same, the biobased polyesters have the advantage of degrading naturally after use.

Adding biodegradable cobuilders to detergent isn't a new idea, and it has been demonstrated before. But the high costs of using solvents to produce them has restricted their sales to niche markets.

To get around the costly solvent problem, the team fused the sorbitol with the citric acid under heat and passed the mixture through a twin-screw extruder, producing the polyesters.

"This work is part of a larger effort to develop reactive extrusion as a continuous means of making biobased products. It's faster and more efficient than batch processes," adds Willett, who oversees the ARS center's Plant Polymer Research Unit.

According to Howard Bowman, Folia's current chief technology officer, the company has sent samples of the patent-pending, corn-based cobuilders to detergent makers for independent testing. With ARS's help, Folia seeks commercial-scale production capabilities of at least 1,000 pounds of cobuilder per hour.—By **Jan Suszkiw,** ARS.

This research is part of Quality and Utilization of Agricultural Products, an ARS National Program (#306) described on the World Wide Web at www.nps.ars.usda.gov.

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New Leads on **Nitrous Oxide**

Agricultural practices, particularly the use of nitrogen-based fertilizers, generate significant levels of the greenhouse gas nitrous oxide (N_2O). But the mechanisms behind these emissions are still nebulous, and some studies suggest that N_2O emissions may be underestimated by as much as 40 percent at some sites.

"We need to know much more about how agricultural practices affect the production and consumption of greenhouse gases, including N_2O," says microbiologist Tim Parkin, who works at the ARS Air Quality of Agricultural Systems Research Unit in Ames, Iowa.

Scientists already know that N_2O emissions rise as the applications of nitrogen-based fertilizers increase. Parkin and his Ames colleagues collaborated with scientists from AgCert International Limited under a cooperative research and development agreement to learn more about how different soils and fertilizers affect N_2O emissions.

The team used chamber studies to assess variation in the emissions of N_2O, carbon dioxide, and methane from a sandy loam mix and a clay soil. The fertilizers used were urea-ammonium nitrate (UAN) and a swine manure slurry.

The researchers tracked greenhouse gas emissions for 8 weeks and found that overall N_2O emissions were highest from soils amended with swine manure slurry. The emissions peaked shortly after the first fertilizer application and reached a slightly lower peak soon after the first simulated rain.

High levels of N_2O emissions were measured from sandy loam soils amended with either UAN or slurry. But on clay soils, only those amended with slurry—and not with UAN—had elevated N_2O emissions.

STEPHEN AUSMUS (D1512-29)

ARS soil scientist Rodney Venterea (foreground) and technician Jason Leonard collect gas samples from chambers used to measure flux of nitrous oxide and other greenhouse gases from experiments in a corn field in Becker, Minnesota.

The team concluded that the difference in emissions probably stems in part from key differences between the two soils: cation-exchange capacity (CEC) and water-filled pore space. Higher CEC in clays could reduce the availability of nitrogen for conversion into N_2O, and the higher percentage of pore water in the sandy loam could prompt higher denitrification, which in turn boosts N_2O emissions.

STEPHEN AUSMUS (D1514-13)

University of Minnesota technician Sonya Ewert (left) and ARS soil scientist Rodney Venterea use a gas chromatograph to determine amounts of greenhouse gases in samples collected from the field.

Looking for Ways To Reduce N_2O Emissions

Two hundred miles north of Ames, at the ARS Soil and Water Management Research Unit in St. Paul, Minnesota, collaborator Rod Venterea is asking similar questions.

Venterea's group has been comparing N_2O emissions after application of either urea or anhydrous ammonia fertilizer.

These are two of the most commonly used nitrogen fertilizers in the United States and the world. But U.S. use of anhydrous ammonia has declined by 15 percent over the past three decades, while urea use has nearly tripled.

"Because of their widespread use and changing use patterns, it is important to measure N_2O emissions from each fertilizer," Venterea says. And as it turns out, there is a large difference in N_2O emissions between anhydrous ammonia and urea—at least in the corn cropping systems Venterea has been studying.

Over several consecutive growing seasons, Venterea found that the amount of N_2O emitted from fields fertilized with anhydrous ammonia was, on average, twice as high as emissions from fields fertilized with urea. These results strongly suggest that N_2O emissions may decline in the future as more urea and less anhydrous ammonia is used.

"But this may not be the case in all soils," Venterea notes. He believes that acidic soils emit the most N_2O when anhydrous ammonia is applied and that more alkaline soils may emit lower levels. Plans to confirm this hypothesis with field testing are under way.

Venterea is also working with the agricultural equipment company John Deere to test different techniques for injecting anhydrous ammonia into the soil, which may reduce N_2O emis-

DEALING WITH CLIMATE CHANGE

sions and increase crop uptake of nitrogen from the soil. He's also working with other ARS collaborators and specialty fertilizer companies Agrium and AGROTAIN International to determine whether their controlled-release and inhibitor products can lower N_2O emissions, reduce nitrate leaching to groundwater, and mitigate other impacts. (See sidebar.)

Tillage and N_2O

Another area that Venterea's group has been studying is how farmers can reduce N_2O emissions when using reduced tillage. Reduced tillage (including no-till) is being increasingly looked at for its environmental benefits. "Using less tillage can be a win-win situation," Venterea says. "It can save on fuel costs, protect the soil from erosion, and conserve water and nutrients." In some cases, it may also help store carbon in the soil.

Venterea's work has shown that N_2O emissions with reduced tillage can be minimized by injecting the fertilizer below the upper 2 to 3 inches of soil. "This upper layer is where all the microorganisms that support N_2O emissions are concentrated in a reduced tillage soil, so you need to avoid placing the fertilizer there," he says.

Maximizing Measurement Estimates

In a crop field, it's a challenge to take comprehensive measurements of anything. So most measurements of N_2O emissions are conducted using chambers that are placed on the soil surface for short periods. The rate of N_2O emissions is then estimated based on how quickly N_2O accumulates inside the chamber.

But placement of the chamber itself can disturb the emission rate. "This so-called chamber effect is something we've known about for decades, and it continues to hinder us in making very accurate measurements in the lab," Venterea says.

So Venterea developed a spreadsheet-based error-calculation tool, based on gas-transport theory, that allows researchers to estimate the error caused by any given chamber type in a given soil. Venterea has posted the spreadsheet on the World Wide Web at www.ars.usda.gov/SP2UserFiles/person/31831/CEAT2.0.xls.

"The goal is to help people design measurement systems that minimize errors and also to estimate the amount of error they are getting," Venterea says. —By **Ann Perry,** ARS.

This research is part of Global Change (#204) and Soil Resource Management (#202), two ARS national programs described on the World Wide Web at www.nps.ars.usda.gov.

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STEPHEN AUSMUS (D1533-6)

In a nitrogen fertilizer source study, soil scientist Ardell Halvorson (right) and technician Mary Smith prepare gas samples from field sampling for gas chromatograph analysis of nitrous oxide, carbon dioxide, and methane.

Specially Formulated Fertilizers Reduce Greenhouse Gas

Climate and soil conditions in the Central Great Plains make nitrogen fertilizers a necessity for ensuring sufficient yields of corn, barley, dry beans, and soybeans. But using nitrogen fertilizers leads to release of nitrous oxide (N_2O), a major greenhouse gas, into the atmosphere. They are one of the reasons an estimated 78 percent of the nation's N_2O emissions come from agriculture.

At the Soil Plant Nutrient Research Unit in Fort Collins, Colorado, soil scientist Ardell Halvorson is examining the effects of altering the types and amounts of fertilizers growers apply to minimize N_2O emissions and maximize crop yields.

Halvorson is comparing N_2O emissions from no-till corn fields treated with conventional nitrogen fertilizer (urea) or either of two specially formulated urea fertilizers: one with controlled-release polymer-coated pellets or one with stabilizers added to keep more of the urea in the soil as ammonium for a longer period. He chose a no-till system because it is known to reduce carbon dioxide emissions.

After 2 years, he found that the specially formulated fertilizers drastically reduced N_2O emissions. The controlled-release fertilizer cut N_2O emissions by a third, and the stabilized fertilizer cut them almost in half.

Halvorson's results so far are limited to the irrigated fields and cool, semi-arid conditions at Fort Collins. But N_2O releases are the result of a complex interplay of varying conditions. Soil organic matter, rainfall distribution, climatic conditions, and soil variability affect microbial activity and potential for N_2O release from nitrogen application. So Halvorson is expanding the study, with financial support from the fertilizer industry and cooperation of other ARS locations, to see how the fertilizers respond at seven sites around the United States.—By **Dennis O'Brien,** ARS.

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Soybean & Wheat Response to Climate Change

PEGGY GREB (D1548-1)

Global greenhouse gas emissions are projected to rise dramatically in the next 40 years, with increased outputs of carbon dioxide (CO_2) being the main culprit. In light of our changing environment, Agricultural Research Service scientists in Urbana, Illinois, and Raleigh, North Carolina, are examining how the increase in greenhouse gases, particularly CO_2 and ozone, will affect two of the world's most widely planted crops: soybeans and wheat.

Open-Top Chambers Offer Insight

At the Plant Science Research Unit in Raleigh, ARS plant physiologists Fitzgerald Booker, Kent Burkey, and Ed Fiscus are assessing how climate change will affect soybean and wheat growth rates, crop yield, and soil chemistry by exposing the crops to the elevated levels of CO_2 and ozone projected for the year 2050.

Soybeans, wheat, and other crops grow more when CO_2 levels are elevated, because there is more carbon captured during photosynthesis for the plants to use. But those same plants are damaged and stunted by elevated levels of ground-level ozone, a gas created when sunlight "cooks" automotive and industrial pollutants that originate from combustion of carbon-based fuels.

Levels of both gases are rising. The Intergovernmental Panel on Climate Change, an international panel of highly regarded scientists, estimates that CO_2 levels could be about 1.5 times greater than the current 380 parts per million by 2050. The panel also estimates that daytime ozone in the summertime, now at about 50-55 parts per billion, may rise 20 percent over the same period.

Besides assessing the effects of future air concentrations on both crops, the researchers are conducting a 5-year project to determine whether a widely accepted no-till cropping system will improve soil quality and sequester carbon when levels of the gases rise.

"We know no-till increases carbon sequestration in the soil. The question is, What is going to happen with elevated levels of CO_2, and how are changes in other atmospheric gases, namely ozone, going to affect that?" Booker says.

The Raleigh researchers have 16 open-top chambers, divided into 4 treatments: 4 with elevated ozone, 4 with elevated CO_2, 4 with both gases elevated, and 4 controls. They are pumping the chamber air with up to 40 percent more ozone and CO_2 than what is found in today's ambient air. They are also charcoal-filtering the air so they can reduce ozone in the control chambers and tease out the impact that different ozone and CO_2 levels, by themselves, have on the plants.

In addition, the researchers are putting the postharvest residues, such as plant stems, empty pods, and dead leaves, back into the chambers, essentially

INSTITUTE FOR GENOMIC BIOLOGY/UNIVERSITY OF ILLINOIS (D1546-2)

ARS scientists Carl Bernacchi (left), Don Ort (center), and Lisa Ainsworth in a plot of soybeans treated with elevated carbon dioxide (CO_2) at the SoyFACE Global Change Research Facility at the University of Illinois Research Farm in Urbana. In the foreground is an automatic retractable awning used to capture rainfall in order to investigate the interaction of drought and elevated CO_2 on soybean. Behind the scientists is an array of infrared heaters used to raise the temperature of a portion of the plot to investigate interactions with anticipated global warming.

Plant physiologist Fitzgerald Booker prepares to place an optical scanner into a tube that will be positioned in the soil to photograph soybean roots. The photos will be analyzed to determine changes in root length and distribution caused by exposure to elevated carbon dioxide and ozone, which are pumped into the open-top field chambers.

making them small, self-contained plots that mimic conditions found in no-till systems. To measure changes in the soil's carbon and nitrogen content makeup, the researchers take samples twice a year. The CO_2 pumped into the chambers has a specific isotope marker so they can track it from the air through the plants and into the soil. They are analyzing the soil cores by depth to determine where composition changes may occur and also the amount of bacteria and fungi found in each layer to see whether microorganism populations or communities change.

After completing 3 years of the project, preliminary results show a trend for higher levels of soil carbon in chambers with elevated CO_2 but not in chambers with elevated ozone. Elevating CO_2 also reduced protein levels in wheat flour by 7 percent to 11 percent, but it had no effect on soybean seed protein.

SoyFACE: New Technology, New Understanding

Researchers in Urbana and cooperators with the University of Illinois at Urbana-Champaign have been working on a project called "SoyFACE"—short for Soybean Free Air Concentration Enrichment—that also measures how the projected increases in CO_2 and ozone will affect soybean production.

FACE technology, which was first used for crop research by ARS soil scientist Bruce Kimball, ARS plant physiologist Jack Mauney (now retired), and scientists from the U.S. Department of Energy's Brookhaven National Laboratory, allows testing of plants in open-air field conditions. ARS scientists Don Ort, Lisa Ainsworth, and Carl Bernacchi, in the Photosynthesis Research Unit, and Randall Nelson, in the Soybean/Maize Germplasm, Pathology, and Genetics Research Unit, use the technology to produce atmospheric conditions predicted for the year 2050.

Horizontal pipes arranged in an octagon about 70 feet in diameter surround each test plot. A computer measures wind direction and speed, then releases concentrated amounts of CO_2 and ozone. The wind carries the gases across the soybean crop.

"At the start of the project, we sought to understand how CO_2 and ozone affect soybean independently," says Ort. "We found soybean yield increases by about 12 percent at the elevated CO_2 concentrations predicted for 2050, half of what previous studies estimated. We also found that increased ozone is quite harmful to yield, reducing it by about 20 percent. In fact, our study showed current levels of ozone are already suppressing soybean yield by up to 15 percent."

The results of the individual studies led the scientists to examine the combined effects of CO_2 and ozone on soybean. They found that elevated CO_2 partially offsets the ozone damage. These findings with SoyFACE confirm general results obtained with open-top chamber studies of ozone and CO_2 effects on crop yield conducted at Raleigh and other locations. But the ability of SoyFACE technology to test these principles in the open air, without the environmental modifications caused by the chambers themselves, means greater confidence in our understanding of how plants respond in the real world, including the actual estimates of impact on crop yields. Furthermore, there is much to be learned about how other interacting factors that affect ozone uptake may come into play by mid-century.

The scientists recently began studying how combined factors will affect soybean production. They are looking at drought and increased temperature, drought and increased CO_2, and elevated temperature and CO_2. They'll also be analyzing how microbial communities and soil carbon storage are affected by these changes.

These projects provide valuable information that will help breeders develop soybean cultivars better adapted to an ever-changing climate.—By **Stephanie Yao** and **Dennis O'Brien,** ARS.

This research is part of Air Quality (#203), Global Change (#204), and Plant Biological and Molecular Processes (#302), three ARS national programs described on the World Wide Web at www.nps.ars.usda.gov.

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PEGGY GREB (D1550-1)

To assess oxidative stress in soybean grown at SoyFACE under elevated ozone concentrations, ARS molecular biologist Lisa Ainsworth (back) and graduate student Kelly Gillespie use a liquid-handling robot to perform a high-throughput assay.

GRACEnet A Unified Response to Climate Change

Most of the greenhouse gases believed to be warming the planet come from the burning of fossil fuels, but agriculture can play an important role in mitigating those emissions.

Green plants capture carbon dioxide through photosynthesis and store carbon in their tissues. Soil stores organic carbon, but can emit methane and nitrous oxide. The amount of carbon stored and gas released depends on how and whether the land is tilled, the amount of crop residue left in the field, soil moisture, application and form of fertilizer, and type of crops planted.

ARS scientists are looking for ways that agriculture can help reduce greenhouse gases through improved tillage and fertilizer use and cropping systems that sequester more carbon in the soil and keep it out of the atmosphere.

The concept behind GRACEnet (Greenhouse Gas Reduction through Agricultural Carbon Enhancement Network) is simple. Scientists are comparing traditional systems with alternatives, using regional approaches and the same protocols to test soils, measure plant growth, and keep track of weather conditions and greenhouse gas releases. GRACEnet researchers are spread out over 32 sites across the country, but they communicate often and meet periodically. They work towards four goals: a national database on greenhouse gas flux and soil carbon storage; regional and national guidelines; upgraded computer models to estimate the effects of cropping systems and other crop management practices on net greenhouse gas emissions; and scientific papers to guide federal and state policymakers.

The overall goal of the 5-year project is to develop strategies and tools that producers can use to reduce agriculture's climate-change footprint, says Ronald Follett, GRACEnet lead scientist and a senior supervisory scientist at the ARS Soil Plant Nutrient Research Unit in Fort Collins, Colorado.

Regional approaches are necessary because the factors that determine how much greenhouse gas is released—such as soil quality, types of crops raised, rainfall patterns, and temperature ranges—vary tremendously from one location or region to the next. Besides, those scientists best equipped to come up with alternatives for farmers are the ARS researchers who work closely with them, according to Follett.

"The scientists at each site are in touch with producers and local farmers, so they're familiar with local issues affecting them," he says.

Lessening the Effect of Biofuel Crops

As part of his contribution to GRACEnet, Follett and his collaborators are studying the effects of raising corn for biofuels on a Nebraska field where bromegrass had previously been growing for 13 years. Planting grass became popular in the 1990s as a way to save highly erodible soils and store carbon in the soil. Farmers nationwide were encouraged, sometimes with cash payments, to stop cultivating their land and to plant native and perennial

STEPHEN AUSMUS (D1540-2)

Plant physiologist Jack Morgan (left) and soil scientist Ron Follett (right) discuss research projects at ARS GRACEnet sites across the United States while physical science technician Ed Buenger conducts mass spectrophotometer analysis of soil samples for carbon and nitrogen.

STEPHEN AUSMUS (D1621-4)

Using closed vented chambers, biological science aide Rochelle Jansen (right) and soil scientist Jane Johnson collect gas emissions from soil. Samples will be analyzed for carbon dioxide, nitrous oxide, and methane with a gas chromatograph.

grasses. But the demand for biofuels in recent years is prompting some farmers, particularly in the Midwest, to switch to corn.

Follett and his team converted the bromegrass field into no-till corn and controlled weeds chemically. He periodically collects soil samples at three depths and analyzes them for organic carbon. Previous studies predicted massive releases of organic carbon from fields converted from grass to corn or other energy crops. But that work, says Follet, "was based on the assumption that you had to plow extensively to raise corn in these areas, and that just isn't the case."

After 6 years of work, Follett found that by using a no-till method, there was no loss of organic carbon and that its levels didn't change significantly at any soil depth. The pattern held even in drought years, when dried, parched soil conditions should have exacerbated release of soil carbon.

STEPHEN AUSMUS (D1541-1)

Biological science aide Lindsey Dowswell prepares to separate soil aggregates while physical science technician Ed Buenger washes a separated aggregate sample off of a soil sieve into a container to determine the amount and origin of the carbon sequestered in the sample.

Alternative Ways To Grow Wheat

Soils in eastern Oregon are relatively low in organic carbon, and research is under way to prevent further losses. Wheat farmers there traditionally plant winter wheat one year, leave the field fallow for a season, and use conventional tillage before the next wheat planting.

Hero Gollany, an ARS soil scientist and GRACEnet participant in Pendleton, Oregon, is using alternative tilling and crop rotation systems to increase the amount of carbon sequestered in the soil and reduce release of greenhouse gases.

Gollany is looking at three scenarios. In one set of plots, she is comparing the traditional 2-year, wheat-fallow rotation with a 3-year cycle of no-till winter wheat, followed by a second crop of no-till winter wheat, followed by sorghum. She is measuring residue yields, soil organic carbon, and greenhouse gas emissions (carbon dioxide, methane, and nitrous oxide). Throughout the year, she obtains gas samples from specialized chambers installed in the field and measures the samples in the laboratory. During the growing season, she takes measurements once or twice a week, depending on the weather. Because precipitation usually increases nitrous oxide emissions from the soil, she also collects data after any rain or snowfall.

In scenario two, winter wheat is grown continuously, and the untilled soil is directly seeded each year. The third system is also wheat-fallow, but it uses sweep tillage and a rod-weeder to control weeds. Sweep tillage is a low-impact method of plowing 4 to 5 inches into the soil. It is less destructive to soil than conventional tillage and is believed to help soil retain more organic matter and moisture. Previous experiments show a 14-percent increase in soil organic carbon in the sweep tillage system compared to the moldboard system. Gollany is still evaluating results of the greenhouse gas measurements.

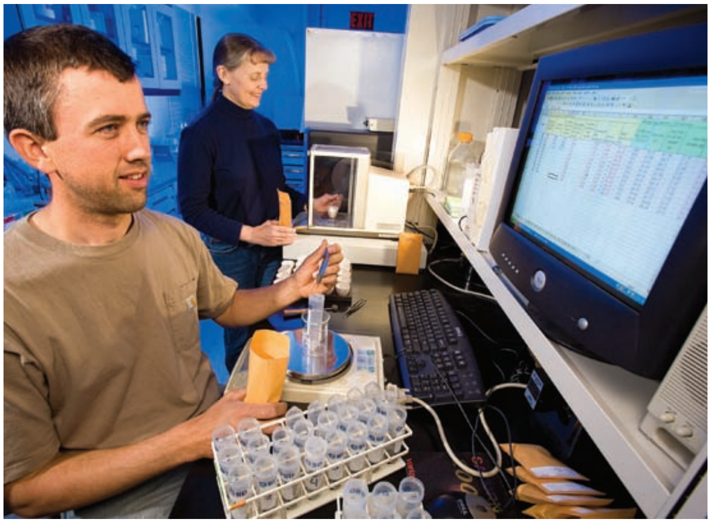
Gollany also is upgrading CQESTR, a computer model that simulates the effects of climate, soil type, cropping systems, and other management practices on soil organic carbon. Soil organic carbon is a key indicator of how well soil will hold moisture, provide nutrients, and remain productive. Gollany's goal is to make CQESTR more user friendly so land managers, researchers, and conservationists can use it in different ways. For example, it could be used to determine the effectiveness of carbon sequestration efforts among farmers participating in carbon trading programs.

Seeking Ways To Reduce Greenhouse Gases

The cool soils of Minnesota have the potential to store a lot of the organic carbon that gives soils a dark or black color. So Jane Johnson, a soil scientist at the North Central Soil Conservation Research Laboratory in Morris, Minnesota, is exploring alternatives that will appeal to farmers, increase the carbon stored in the soil, and reduce the amount of greenhouse gases released.

Many farmers in Minnesota rotate corn and soybeans in a 2-year cycle, using aggressive tillage and fertilizer. Johnson rotated corn and soybeans on one set of plots that were fertilized and tilled every year with a chisel or moldboard plow. She is comparing that to a 4-year rotation with less-disruptive tillage, with and without adding fertilizer. The less-disruptive system is

STEPHEN AUSMUS (D1623-4)



Biological science technician Chad Rollofson (left) and soil scientist Jane Johnson weigh soil samples for analysis to determine how soil carbon and other chemical properties are changing due to management over time.

called "strip tillage" because only narrow bands of soil are tilled instead of the entire field. The four crops grown in rotation are corn, soybean, wheat, and alfalfa, with one crop grown each year during the 4-year cycle.

She also included unfertilized plots as a scientific control.

"We used realistic scenarios, ones that farmers were using or would use if there is a benefit to using them," Johnson says.

Like Gollany, Johnson uses the specialized chambers to measure the levels of carbon dioxide, methane, and nitrous oxide released throughout the entire year. Soil is collected with a probe to determine the amount of organic carbon sequestered in it.

Johnson is still analyzing data on carbon sequestration patterns. She found no consistent pattern to methane release. And no matter which tillage or crop rotation system was used, nitrous oxide emissions peaked during spring thaws when the sun's rays began to warm the soil.

Chisel and moldboard plowing increased carbon dioxide emissions for a short time. But measured over the course of a year, carbon dioxide emissions were no different from plots with intensive tillage than from plots without it.

Johnson is finding that when measured over the course of a year, greenhouse gas releases are largely the same under 2-year and 4-year rotation systems and that applying nitrogen fertilizer has less overall impact on nitrous oxide emissions than anticipated. The test plots were managed to match nitrogen application with nitrogen use, which may have reduced nitrous oxide emission.

"You have less nitrogen available for loss if it's being used where you want it, in the plant," Johnson says.

Her results show that reducing emissions is more complicated than just cutting back on nitrogen fertilizers and changing crop rotation cycles. "By shifting from one rotation to another, we're not affecting greenhouse gases all that much, and it looks as if it's more complex than reducing the amount of fertilizer," she says.—By **Dennis O'Brien,** ARS.

The research is part of Global Change, an ARS national program (#204) described on the World Wide Web at www.nps.ars.usda.gov.

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OREGON STATE UNIVERSITY (D1542-1)

Aerial view of GRACEnet test plots at the Columbia Plateau Conservation Research Center in Pendleton, Oregon.

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2007 Midwest Area Award Winners

Distinguished Senior Scientist of the Year

Norman R. Fausey
Soil Drainage Research Unit
Columbus, Ohio

For significant accomplishments in promoting efficiency and environmental stewardship in agricultural drainage water management, with extensive benefits to producers, industry, and the general public.

Area Early Career Scientist of the Year

Kristin D. Bilyeu
Plant Genetics Research Unit
Columbia, Missouri

For discovery of genetic mechanisms for lowered linolenic acid content in soybeans and significant impact on soybean breeding efforts for enhanced nutritional content.

Technology Transfer Awards

Superior Efforts

Low Glycemic Index Sweetener Team

Gregory L. Côté
Melinda S. Nunnally
Ting Carlson
Timothy D. Leathers
Sheila M. Maroney
Anton J. Woo

National Center for Agricultural Utilization Research Unit
Peoria, Illinois

For technology transfer activities related to research leading to the commercial low-glycemic-index sweetener known as Sucromalt, produced and marketed by Cargill Corporation.

2008 Midwest Area Award Winners

Area Senior Scientist of the Year

David M. Spooner
Vegetable Crops Research Unit
Madison, Wisconsin

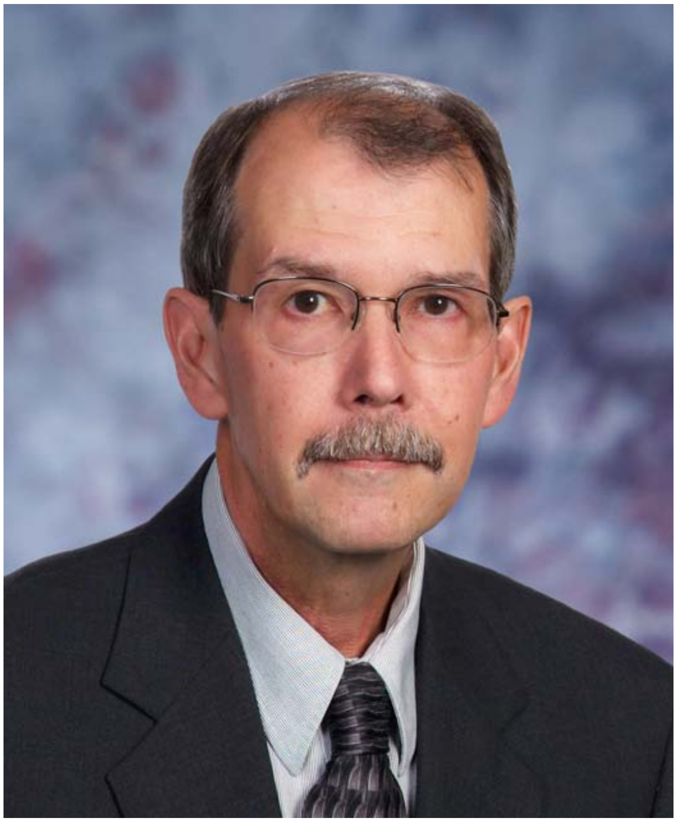
For outstanding research on the systematics, evolution, and domestication of potatoes, tomatoes, and their relatives, and characterization of plant germplasm for valuable genetic variation.

Area Early Career Scientist of the Year

Alejandro P. Rooney
Microbial Genomics and Bioprocessing Research Unit
Peoria, Illinois

For excellence in research on the genetics of agriculturally important organisms and the application of genetic theory to enhance American agriculture and biosecurity.

2009 Midwest Area Award Winners



Area Senior Scientist of the Year

Randy C. Shoemaker
Corn Insects and Crop Genetics Research Unit
Ames, Iowa

For an outstanding career in research on soybean genetics and genomics.

Area Early Career Scientist of the Year

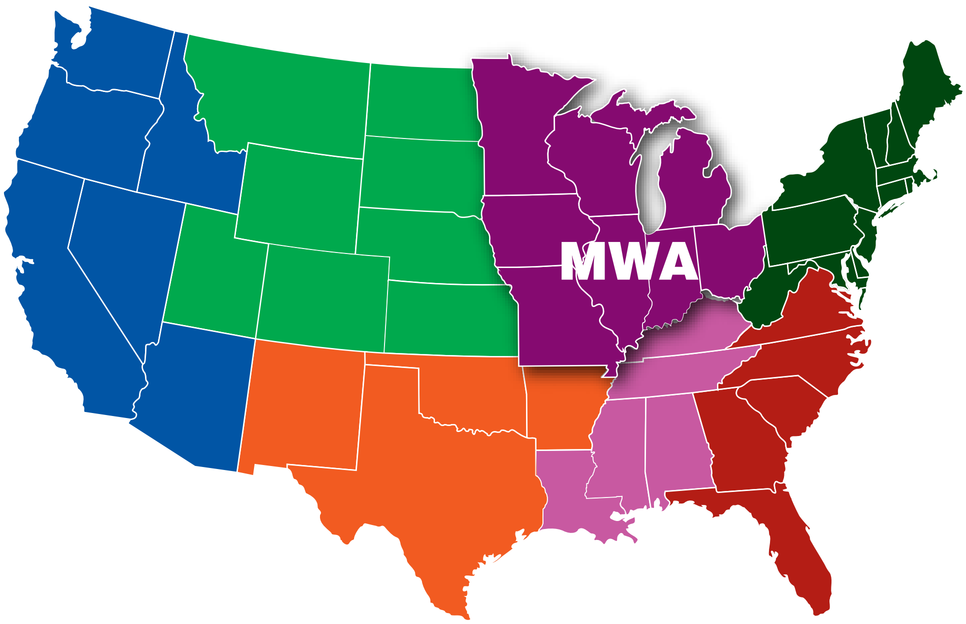
Elizabeth A. Ainsworth
Photosynthesis Research Unit
Urbana, Illinois

In recognition of far-reaching contributions in understanding the impact of global change on crop physiology and production.

Technology Transfer Awards
Superior Efforts

George E. Inglett
National Center for Agricultural Utilization
Research Unit
Peoria, Illinois

For outstanding accomplishments in the invention and technology transfer of the multifunctional food ingredient Z-Trim, which contributes to healthier foods for people around the world.



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