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Agricultural Research Service
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# Agricultural Research

## Optimizing Water Use

Stories on
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Agricultural Research Service • *Solving Problems for the Growing World*

# FORUM

## Challenge for Sustaining Agriculture

## Making the Most of Our Water Supply

With the climate changing and the world's population expected to rise in the coming decades, supplying a hungry planet with food and fiber will become more of a challenge. By some estimates, food production will have to increase by up to 70 percent to feed the world over the next 40 years. Perhaps no resource is more critical to meeting that challenge than water.

How essential is water to agriculture? An estimated 17 percent of all harvested U.S. cropland is irrigated, accounting for about 56.6 million acres. Worldwide, more than 40 percent of the world's food is grown on irrigated land, including the majority of high-market-value crops, such as fruits, nuts, and vegetables.

There is no doubt that drought is a continuing threat. At the peak of last summer's record drought, more than 2,255 counties in 39 states were declared disaster areas, prompting U.S. Department of Agriculture Secretary Tom Vilsack to make $30 million in emergency funds available to struggling crop and livestock producers. By some estimates, the drought affected more than three-quarters of the nation's corn and soybean crops. Economic losses are shared by livestock producers, who are forced to pay more for feed made with corn and other grains.

At the Agricultural Research Service, our drought-related research is based on the belief that if we understand the effects of drought on crops and habitats, we will be better equipped to address them. This issue highlights some of that research. On page 4, there is an article about research at the ARS Water Reuse and Remediation Research Unit in Riverside, California, to help growers tap into irrigation water that drains off crop fields in California's San Joaquin Valley and reuse it to grow forage for livestock.

On page 6, you can read about work at the ARS Conservation and Production Research Laboratory in Bushland, Texas, to develop irrigation-scheduling tools for the Texas High Plains, where water levels in the Ogallala Aquifer are rapidly declining.

Researchers at the ARS Hydrology and Remote Sensing Laboratory in Beltsville, Maryland, have developed new tools that use satellites to assess soil water levels over large geographic areas. They've found that microwave and thermal radiative signatures of agricultural landscapes, detected by satellites, allow them to identify droughts earlier and collect more detailed information about their extent and severity. They shared the technology with the National Oceanic and Atmospheric Administration and other federal agencies, and it is being used to assist in quantifying drought conditions in the Horn of Africa.

ARS scientists are also using molecular tools at more than a dozen locations to identify genes in cotton, soybeans, wheat, and other crops that play key roles in determining drought tolerance. Their approaches range from using high-throughput phenotyping under drought conditions to DNA marker-assisted breeding techniques. Using molecular tools, researchers at the ARS Coastal Plains Soil, Water, and Plant Research Center in Florence, South Carolina, have discovered a family of genes in cotton that code for aquaporin proteins that function in plants as water-transport systems. Along with others discovered by the group, these genes are good candidates for improving cotton water-use efficiency.

ARS researchers in Ithaca, New York; College Station, Texas; and other locations are scanning the genomes of corn, wheat, and other crops, searching for genes that will prove useful in developing varieties with deeper roots and other traits that will help them grow with less water and under higher temperatures. At the ARS Soybean and Nitrogen Fixation Research Laboratory in Raleigh, North Carolina, scientists have developed and released the first-ever drought-tolerant soybean, which carries a gene that allows it to wilt more slowly than a normal soybean. Under drought conditions, slow-wilting types yield about 4 to 8 bushels per acre more than normal varieties and also show good yield potential when rain falls.

After many years of exploiting existing diversity within the genus *Phaseolus* (beans), ARS researchers in Mayagüez, Puerto Rico, have been able to combine genes for resistance to heat, drought, and disease and have released two high-yielding, stress-tolerant black bean varieties; two heat-tolerant kidney bean lines; and rust-resistant, heat-tolerant common bean lines.

Many experts say the 2012 drought was a harbinger of future problems and should be considered a call to action. Drought is expected to become more common, forcing farmers and ranchers to spend more time, energy, and resources trying to raise crops and livestock on drier soils with less water. Our goal is to help growers maximize the potential of every drop of water available so that they can continue to produce the kind of bounty we have come to expect in our supermarkets and neighborhood stores.

**Mark Walbridge**
ARS National Program Leader
Water Quality and Management
Beltsville, Maryland

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# Agricultural Research

Solving Problems for the Growing World

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| | |
|---|---|
| Editor: **Robert Sowers** | (301) 504-1651 |
| Associate Editor: **Sue Kendall** | (301) 504-1623 |
| Art Director: **BA Allen** | (301) 504-1669 |
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PEGGY GREB (D2827-2)

ARS scientists have found a way to map the structure of an elusive protein that gives certain plant viruses the ability to travel through plants, from plants to insects, through the insects, and back into plants. See story, page 20.

Cover: In California's San Joaquin Valley, high-clay saline-sodic soils can dry out and crack. Water is in short supply there, so ARS researchers are studying how to use spent, or already used, irrigation water to raise a salt-tolerant forage to improve the soil and feed cattle. Story begins on page 4. Photo by Peggy Greb. (D186-2)

PEGGY GREB (D188-39)

# Irrigation Wastewater: Waste Not, Want Not

Soil scientist Dennis Corwin on the salt-encrusted edge of an evaporation pond that received drainage water from nearby tile-drained, irrigated land.

**Agricultural producers** on the west side of California's San Joaquin Valley (WSJV) used to drain irrigation wastewater into Kesterson Reservoir, a series of holding ponds that were part of the San Luis National Wildlife Refuge. But selenium levels in the water became hazardous to waterfowl, so the storage facility was closed in 1987. Since then, farmers have been keeping the wastewater—which also contains salt and traces of arsenic, boron, and molybdenum—in evaporation ponds on their own land, which takes around 10 percent of the crop land out of production.

Agricultural Research Service soil scientist Dennis Corwin and his colleagues had another idea. They thought it might be possible to use the spent wastewater for irrigating salt-tolerant forage grown on marginally productive saline and sodic soils. Sodic soils contain large quantities of sodium, which is one of the minerals found in salt compounds. Saline soils are contaminated with salt compounds at levels that significantly limit plant growth.

If this approach worked, farmers would have a viable alternative to simply storing the spent wastewater until it evaporated, and they would be able to reclaim degraded soils and produce livestock forage in the bargain. Irrigating with wastewater could also help stretch water supplies in a region already struggling with limitations imposed by intermittent droughts.

Corwin and his partners conducted their investigation on a private 80-acre field in the WSJV. To the scientists, it was a worst-case example of the type of damage they hoped to reverse—the crusted saline-sodic soils at the site drained so poorly that irrigation was a waste of time and money.

"One end of the field had so much salt on the surface that it looked like it was covered in snow," says Corwin, who works at the ARS Water Reuse and Remediation Research Unit, part of the U.S. Salinity Laboratory in Riverside, California.

The first step was to install tile drains for channeling subsurface leachate off the field, which would be an essential part of the reclamation process. Then, geophysical mapping techniques developed at the U.S. Salinity Laboratory were used to generate a three-dimensional map of salinity and trace elements. After using this map to identify soil-sampling sites, the team collected samples at 1-foot depth increments five times—1999, 2002, 2004, 2009, and 2012—over the 12-year study period.

During the first 2 years of the study, a stand of salt-tolerant Bermuda grass was established at the site and irrigated with the spent wastewater. "We had cattle grazing at the site within 2 years of starting up," Corwin says. "The farmer was so pleased with the results that he used the same approach on other marginally productive fields that had damaging saline-sodic levels."

### In-Depth Findings

The striking results weren't just on the surface. The researchers determined that the irrigation wastewater was leaching salts and trace elements below the root zone, which resulted in a significant and rapid improvement in soil quality. They observed an overall decrease in levels of salts, boron, and molybdenum through

DENNIS CORWIN (D2829-1)

DENNIS CORWIN (D2830-1)

DENNIS CORWIN (D2831-1)

DENNIS CORWIN (D2832-1)

Sequence of photos showing the improvement and decline in forage yield in a field with poorly drained saline-sodic soils. From top to bottom: In 2000, before irrigation with saline drainage water began; in 2004, tile drains had been installed to facilitate drainage, and wastewater irrigation had begun to support the growth of livestock forage; in 2010, wastewater irrigation still supported flourishing stands of forage; in 2012—just 2 years after irrigation had stopped—the soils had returned to their former degraded condition.

the root zone. They also noted an overall decrease in the soil's sodium absorption ratio (SAR), which is determined by comparing levels of sodium, magnesium, and calcium.

From 1999 to 2004, salinity decreased 21 percent, SAR decreased 19 percent, boron decreased 32 percent, and molybdenum decreased 67 percent in the top 4 feet of the soil profile. After 2004, each continued to decrease and gradually leveled off by 2009.

## Backsliding

In the final 2 years of the study, drought had reduced water supplies so completely that all sources of drainage water had been used. Even backup degraded water, such as municipal wastewater, was no longer available for irrigation. As a result, irrigation stopped at the site, and rain became the only source of water that the field received.

During this time, salts, SAR, soil acidity, and levels of boron, molybdenum, and selenium increased throughout the soil profile. Salinity and SAR almost reached their 1999 levels, while soil acidity and selenium levels exceeded them. In just under 2 years after irrigation stopped, the soil had nearly returned to its original poor quality.

"I thought reclaimed soils in the WSJV might revert in 5 years, so I was amazed at how quickly it happened," Corwin says. "The shallow water table was unquestionably the reason for the quick reversion. Maybe some disking for weed control would have slowed the reversion, because when the weeds drew water up from the lower portion of the soil profile, they helped to bring back boron, salt, and molybdenum that had been leached just below the alfalfa's root zone."

At the end of the study, Corwin and his colleagues concluded that recycling drainage water for salt-tolerant forage crops could potentially free up as much as 84,000 acres of land currently used for evaporation ponds. The approach not only reduced drainage volumes, but also reclaimed marginally productive soil and used an alternative water source, which reduced demand on limited quantities of good quality water. However, Corwin noted that soils irrigated with drainage water would need periodic monitoring, particularly if they are left fallow for any length of time, to make sure that salts and trace elements did not begin to reaccumulate to potentially problematic levels.

"The first part of the study showed how quickly soils above a water table 5 to 6 feet deep can be brought back to productivity," he says. "The long-term study showed how the soil response levels off as irrigation continues—and how quickly soil can revert to its former condition once irrigation stops."

Corwin published his results in 2012 in the *Journal of Environmental Monitoring*.—By **Ann Perry,** ARS.

*This research is part of Water Availability and Watershed Management (#211) and Climate Change, Soils, and Emissions (#212), two ARS national programs described at www.nps.ars.usda.gov.*

*Dennis Corwin is with the USDA-ARS Water Reuse and Remediation Research Unit, 450 W. Big Springs Rd., Riverside, CA 92507; (951) 369-4819, dennis.corwin@ars.usda.gov.*✱

STEPHEN AUSMUS (D2810-1)

# Two Approaches for Optimizing Water Productivity

STEPHEN AUSMUS (D2811-10)

Above: Agricultural engineer Susan O'Shaughnessy views an irrigation prescription map constructed from data collected by an ARS wireless sensor system. The map shows variable crop water needs. Next to her, agricultural engineer Joaquin Casanova tests his prototype TDR (time domain reflectometry) probe.

Right: A variable-rate center-pivot irrigation system at Bushland, Texas. Technician Luke Britten (left) and Susan O'Shaughnessy (right) adjust wireless infrared thermometers in the field while technician Brice Ruthardt (center) uses a neutron gauge for soil water measurements.

**Agricultural Research Service** researchers in Bushland, Texas, are helping farmers make the most of their water supplies in a region where they depend on the Ogallala Aquifer, a massive underground reservoir under constant threat of overuse.

Steve Evett, Susan O'Shaughnessy, and their colleagues at the Conservation and Production Research Laboratory are developing and testing soil-water and plant-stress sensors and automated irrigation systems that will irrigate fields only as necessary. Automated systems are considered key to sustainable use of the aquifer and to helping growers reduce water and labor costs.

"As water becomes more precious and the costs to pump it continue to rise, we need to tap the potential of every drop used in agriculture. To do that, we need to develop the best systems possible for accurately scheduling and controlling irrigation," Evett says. The researchers are developing automated irrigation and sensor systems based on two approaches that complement each other, O'Shaughnessy says. One system applies water based on levels of crop water stress detected by wireless sensors mounted on aboveground moving pipelines of commercial irrigation systems.

In the other system, the researchers are adapting sensor technology designed for urban sites so that it will work in agriculture. It triggers irrigation based on soil water content detected by sensors in fixed locations in the soil.

"Each system has advantages and disadvantages. But the combination of these two networked systems in a single field would be ideal, providing the temporal frequency and spatial coverage needed for monitoring crop water stress and robust control of irrigation," O'Shaughnessy says.

## Sensing Water Needs From Above

Evett, O'Shaughnessy, and their colleagues have filed for a patent on the automated irrigation system. They verified its effectiveness in numerous field studies that compared it with manual irrigation control based on soil water monitoring with a neutron probe. The probe is a research standard for irrigation scheduling, but growers avoid it due to expense and regulatory burdens.

In one study, the researchers cultivated early- and late-maturing sorghum for 2 years. They used 16 prototype wireless sensors on a center-pivot irrigation system to monitor crop canopy temperatures. They chose sorghum because of its importance as a cash crop in the Southern High Plains and because it withstands water stress. Even so, irrigation plays a significant role in sorghum production in the region, tripling its yields.

Crop canopy temperature was monitored during the growing season as the pivot system moved across the field. Other instruments recorded weather data. The information was processed daily by a computer at the pivot point, which automatically scheduled and delivered irrigations when and where necessary.

"The sensor network was mounted on a six-span center pivot, but the technology could be adapted to other types of moving or static irrigation systems," O'Shaughnessy says.

An earlier system used to trigger irrigation manually was the Crop Water Stress Index. It calculated water stress based on canopy temperatures and weather factors measured at midday. Because cloud cover and other weather changes could make once-a-day measurements irrelevant to daily water use, the researchers developed a system using continuous measurements over the course of a day and calculated an Integrated Crop Water Stress Index (iCWSI). Irrigations were delivered automatically when and where iCWSI values exceeded a threshold established from previous data.

In a sorghum field, ARS technician Jourdan Bell (left) collects soil water content data from TDR (time-domain reflectometry) probes that measure crop water use. In the background, soil scientist Robert Schwartz observes grain fill in plants grown under deficit irrigation.

STEPHEN AUSMUS (D2813-7)

Besides comparing crop yields and water-use efficiency between automatic and manual control methods, the study also evaluated yields at "deficit irrigation" levels. This was important since growers in the region sometimes increase profits by irrigating less, which saves on water and pumping costs.

The study results, published in 2012 in *Agricultural Water Management*, showed that the automated method of irrigation scheduling was just as effective as the manual method at both the full and deficit irrigation levels, producing similar grain yields and water-use efficiency levels.

Through a cooperative research and development agreement (CRADA), Evett and his colleagues are modifying Nebraska-based Valmont Industries' commercial irrigation systems in ways that will make them more useful to growers. The research team is integrating the ARS-developed sensor networks and irrigation-control system with the company's variable-rate and center-pivot irrigation systems.

ARS researchers in Florence, South Carolina; Maricopa, Arizona; Portageville, Missouri; and Stoneville, Mississippi are working with Evett and Valmont on the CRADA as part of an ARS multilocation research plan. The scientists are also developing sensor technology that will allow irrigation levels to be set based on site-specific data, which can be updated based on changing weather conditions.

## Sensing Water Status Below the Soil Surface

For the automated irrigation system using underground sensors, Evett and his colleagues established a CRADA with Acclima, Inc., of Meridian, Idaho, to create a soil-water sensor designed to measure deeply and accurately. Evett and Bushland researchers Robert Schwartz and Joaquin Casanova are coinventors of the system.

Acclima makes sensor-based irrigation control systems with probes that use time-domain transmission technology, which measures the time required for an electromagnetic pulse to travel along an electrode embedded in the soil. Water slows the signal's travel, and the recorded speed is an accurate representation of the amount of water in the soil. A computer automatically activates water pumps and/or valves at predetermined soil water content levels.

Because Acclima's sensors are designed to control irrigation on tracts of grass, shrubs, and ornamentals, they only need to monitor water content to depths of about 4 to 6 inches. For use in agriculture, the probes need to be installed at depths of 50 inches or more and take readings at multiple depths.

Evett and his Acclima partners are developing new technology that uses time-domain reflectometry. They are using hollow, nonconductive, plastic tubes that can be drilled deeper into the soil. The tubes are divided into segments that attach to each other so they can be drilled down to any desired depth.

Prototype designs tested in water and test fluids, in clay and loam soils, and in the field have shown the feasibility of the approach, Evett says. ARS and Acclima have filed for a patent on the technology, which also includes the ability to assess soil salinity.

A new generation of relatively inexpensive wireless sensors is likely to make sensor network systems affordable in the near future, O'Shaughnessy says. Combining these sensor systems and improving and testing control algorithms based on years of data will increase the robustness and effectiveness of the irrigation automation solution.

Says Evett, "This is the future of irrigation, getting water where it is needed when it is needed, and limiting water use to the exact amount that is needed."—By **Dennis O'Brien,** ARS.

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

*To reach scientists mentioned in this article, contact Dennis O'Brien, USDA-ARS Information Staff, 5601 Sunnyside Ave., Beltsville, MD 20705-5129; (301) 504-1624, dennis.obrien@ars.usda.gov.* ✻

# Compounds in Whole-Grain Rice Varieties

**Whole-grain brown rice contains 15 vitamins and minerals,** including B vitamins, potassium, magnesium, and iron—all nutrients the body needs to grow and develop normally. In addition to these essential nutrients, there are bioactive phytochemicals in rice, as well as in other whole grains, vegetables, fruits, beans, nuts, and seeds. Although the role of these plant chemicals in terms of human health has not been proven, a body of evidence suggests that some phytochemicals could be nutritionally beneficial.

Now, studies headed by chemist Ming-Hsuan Chen, who is with the Agricultural Research Service's Dale Bumpers National Rice Research Center in Stuttgart, Arkansas, have provided knowledge about the chemical composition and potential bioavailability of compounds in a representative group of rice varieties.

Rice is a cereal grain, along with corn, oats, red and white wheat, and barley. The USDA-ARS National Small Grains Collection (NSGC) in Aberdeen, Idaho, for example, contains more than 18,000 rice samples of various colors, called "accessions," from around the world. These accessions provide a valuable resource to breeders for developing new rice varieties with desirable agronomic and nutritional features.

Rice is categorized into seven color classes based on bran color: white, light brown, speckled brown, brown, red, variable purple, and purple.

Rice bran, an outer layer of whole grain rice, is a rich source of the phytochemical known as gamma-oryzanol and of two forms of vitamin E—the tocopherols and the tocotrienols. These nutritional compounds have been linked to preventing oxidative damage in foods and to having a wide spectrum of biological activities.

The team used several assays and analytical methods to determine the profiles of tocopherols, tocotrienols, and gamma-oryzanol in five color classes of bran: white, light brown, brown, red, and purple. They found a wide variation in the concentrations of the two forms of vitamin E and of gamma-oryzanol in the brans of all five color classes studied. "This suggests that breeders can screen the NSGC accessions in all color classes for a high vitamin E and oryzanol content," says Chen.

PEGGY GREB (D2846-6)

Center: ARS researchers studied five color classes of rice bran: white, light brown, brown, red, and purple/black. The team analyzed the phytochemicals and vitamin E concentrations in each class of rice. They discovered that the red and purple rice brans have higher phenolic and flavonoid concentrations than the lighter brans. Vitamin E concentration differed among rice brans but was not associated with bran color.

Studies have shown that pigmented or darker colored cereal grains, such as red and purple, have higher amounts of some phytochemical compounds than nonpigmented varieties. The team also analyzed other phytochemicals—specifically phenolics and flavonoids—in the same five color classes of bran. The researchers wanted to measure the concentrations of both the extractable and the cell-wall bound amounts in the three lighter rice varieties—white, light brown, and brown—and in the two darker rice varieties—red and purple.

Cell-wall bound phenolics and flavonoids in rice grains are of interest because previous studies by other researchers have shown that cell-wall bound phenolic compounds can be broken down by digestive enyzmes and by microflora in the gut. This liberation from the cell wall may mean that these compounds become available for absorption in the body. In addition, the amount of absorbable phytonutrients in rice may be underestimated because typically the cell-wall bound forms have not been measured, says Chen.

To measure phenolic and flavonoid compounds in each rice variety studied, solvents were used to release their extractable amounts, and an alkali solution was used to liberate their cell-wall bound amounts.

The study showed that the red and purple rice brans had higher extracted and cell-wall bound phenolic and flavonoid concentrations than the lighter-colored rice brans measured. Also positive, in the white and light-brown whole-grain rice brans, which are commonly sold in the market, the bound portion of phenolics was found to be about 30 to 50 percent of the total phenolics.

"We found one purple rice bran variety that was both high in phenolic compounds as well as vitamin E and oryzanols" says Chen. Measuring the specific kinds and amounts of phytochemicals in various foods is needed before clinical trials can be conducted to investigate their potential health benefits in humans.

The study findings were published in 2011 in the *Journal of Food Science* and in 2012 in *Food Chemistry*.

### Consumer Reaction Is Key

Another ARS study has shown flavor differences arising from various colored brans in whole-grain rice. Food technologist Karen Bett-Garber analyzed and characterized rice flavors at the ARS Food Processing and Sensory Quality Research Unit in New Orleans, Louisiana. This sensory research enhances the understanding of the different flavors associated with colored rice brans as perceived by consumers.

"Milled, white rice has been characterized in terms of flavor, but colored whole-grain rice has not," says Bett-Garber. She created a glossary of colored-rice flavors, and flavor differences, of each whole-grain rice type she studied, which will help researchers, breeders, and marketers in describing the flavor of colored whole-grain rice varieties.

The bran layers, along with their unique phytochemical profiles, of all of the rice varieties that Bett-Garber studied were retained (meaning they had not been removed via milling and polishing). For the study, 10 panelists trained in descriptive analysis developed 25 descriptors to define cooked whole-grain rice flavor. They then evaluated the flavor of 22 rice samples with bran that was white, light-brown, dark-brown, red, or purple.

The resulting glossary describes the flavor attributes, as well as other differences, between bran color and types of whole-grain rice. For example, brown rice was described as having more intense grainy-starchy, cooked cereal, and popcorn-buttery flavors. Purple (black) rice was higher in oily, dark-berry, medicinal and smoky-burnt flavors. And red rice had greater intensities for beany, animal-wet dog, and earthy flavors. All in all, the darker rice cultivars tended to have more bitter taste and astringent mouthfeel. The study was published in 2012 in *Journal of Sensory Studies*.

Challenges for future research and breeding programs include enhancing the nutritional value of rice while preserving and improving its flavor. Together, these studies shed light on the chemical composition and consumer-acceptance potential of colored whole-grain rice varieties.—By **Rosalie Marion Bliss,** ARS.

*This research is part of Plant Genetic Resources, Genomics, and Genetic Improvement (#301) and Quality and Utilization of Agricultural Products (#306), two ARS national programs described at www.nps.ars.usda.gov.*

*Ming-Hsuan Chen is with the USDA-ARS Dale Bumpers National Rice Research Center, 2890 Highway 130 E., Stuttgart, AR 72160; (870) 672-9300, ext. 230, ming.chen@ars.usda.gov.*

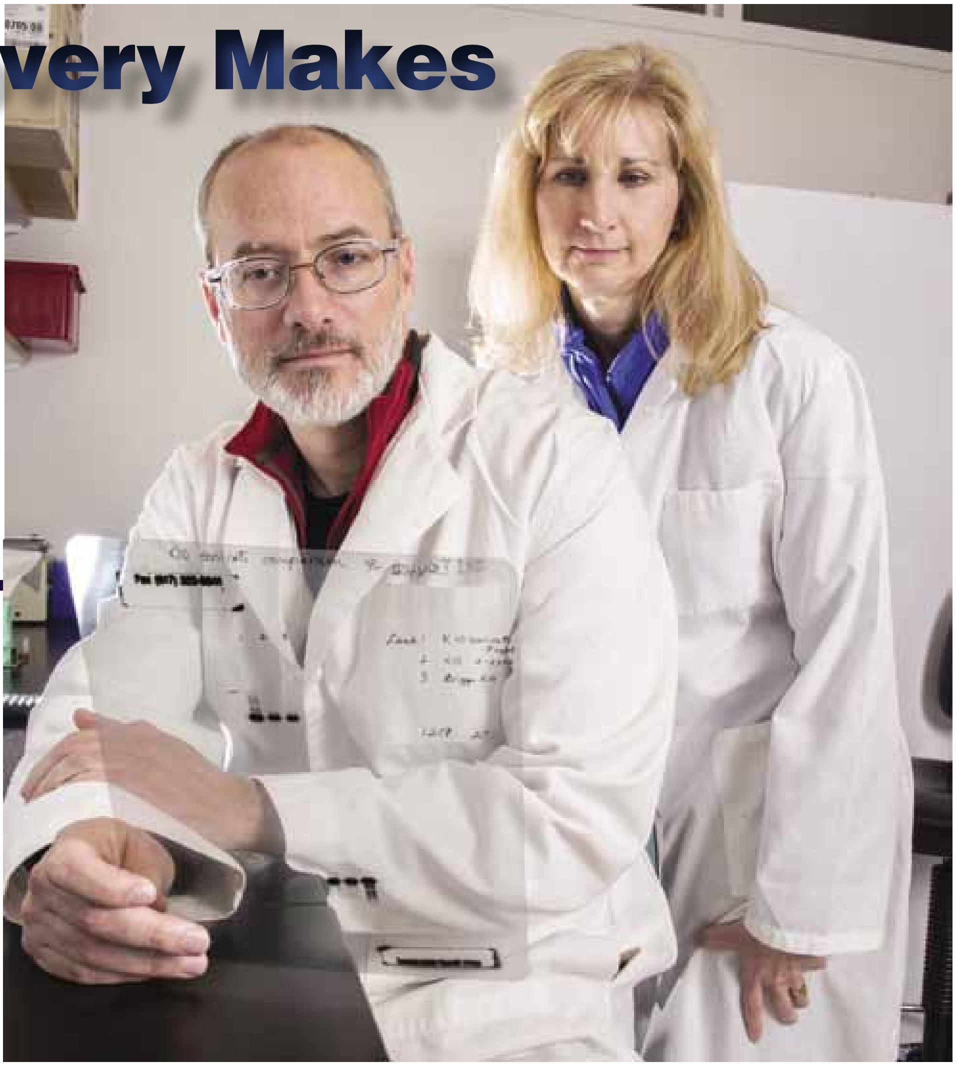
*Karen Bett-Garber is with the USDA-ARS Food Processing and Sensory Quality Research Unit, Southern Regional Research Center, 1100 Robert E. Lee Blvd., New Orleans, LA 70124; (504) 286-4459, karen.bett@ars.usda.gov.* ✱

U.S. long-grain rice growing in a field. In the Food Processing and Sensory Quality Research Unit in New Orleans, Louisiana, food technologist Karen Bett-Garber has created a glossary of flavor differences in various-colored whole-grain rice brans.

KEITH WELLER (K7577-1)

# New Discovery Makes Detecting Johne’s Disease Easier

JAMES FOSSE (D2815-1)



At the National Animal Disease Center, in Ames, Iowa, microbiologists John Bannantine and Judy Stabel review results of a western blot experiment using the MAP specific monoclonal antibody.

**Before a disease can be treated, it must first be identified.** But that’s not always easy, especially in the case of Johne’s disease, which affects cattle, sheep, goats, deer, and other ruminants.

Johne’s disease—also known as “paratuberculosis”—is a costly, contagious disease that causes diarrhea, reduced feed intake, weight loss, and sometimes death. Annual estimated losses to cattle producers range from $40 to $227 per infected animal. For the U.S. dairy industry alone, losses exceed $220 million each year.

For years, scientists have been hampered by the fact that any antibody—a protein produced by the immune system to fight infections and foreign substances—used to detect the Johne’s disease bacterium also reacted to other environmental bacteria or maybe the pathogen responsible for bovine tuberculosis. This caused false-positive test results.

“You may think cattle are infected, based on a positive antibody test result, but they may simply have been exposed to nonpathogenic mycobacteria that’s ubiquitously present in the environment,” says microbiologist John Bannantine at the Agricultural Research Service’s National Animal Disease Center (NADC) in Ames, Iowa.

That problem, however, is now history. Bannantine and his colleagues at NADC have found an antibody that’s 100 percent specific in detecting *Mycobacterium avium* subspecies *paratuberculosis* (MAP)—the cause of Johne’s disease.

“No one else in the world has been able to find such a specific antibody that binds only to MAP strains, until now,” says Bannantine, who’s in NADC’s Infectious Bacterial Diseases Research Unit.

### A Wild Goose Chase

After identifying the new antibody—called “17A12”—from mice immunized with MAP strain K-10, scientists decided to dig a little deeper to determine the protein this novel antibody binds to.

Initially, researchers believed they had found a new gene encoding the antibody-binding protein that was not originally identified in the MAP K-10 genome. They called it “UP1” (Unique Protein 1).

“In 2005, we sequenced the genome of a MAP cattle strain, and then annotated it—finding all the genes and identifying where they start and where they stop,” Bannantine says. “But the 17A12 antibody bound to a protein encoded by a gene that was not annotated in the MAP genome. This was unusual, but we were excited about finding a potentially new gene.”

STEPHEN AUSMUS (K11050-8)

ARS scientists at Ames, Iowa, have discovered a specific antibody that will help develop a test for the Johne’s disease bacterium in cattle.

To confirm this unusual finding, Bannantine developed additional monoclonal antibodies to the UP1 protein. Only one of these new antibodies reacted with the native protein produced by MAP. It also happened to bind to the same area of the protein as the initial antibody—17A12.

"When we expressed the UP1 protein in *E. coli*, it reacted with the monoclonal antibody, and we saw this as confirmation that UP1 was real, even though it wasn't annotated in the genome," Bannantine says.

**Finding the Right Protein**

But some of the data just didn't add up. After additional experiments, it turned out that UP1 was not a real gene after all.

"It completely blew my mind. I had never had a day in the lab like that where there was such a revelation," Bannantine says. "When we first got the results contradicting UP1 as a real gene, it was scary because we thought we had made some big mistakes. Then slowly all the pieces started to fit together, and we knew what we actually had. It was incredible."

Although UP1 was not a gene, it had an epitope—a portion of a molecule to which an antibody binds. This epitope mimicked a similar epitope in the real gene.

In fact, Bannantine says, out of the seven amino acids that make up the epitope in UP1, one amino acid was different from the real epitope encoded by *MAP1025*, a gene that *was* annotated in the MAP genome.

With this new discovery, scientists not only found the real antibody-binding protein, but could now focus on the reason why the new antibody was so specific to MAP.

**Solving the Mystery of Specificity**

A sequence analysis showed that the *MAP1025* gene was present in the non-MAP strains, which did not explain why the antibody was specific to *M. paratuberculosis* and not to environmental contaminants. To find the answer, Bannantine sequenced *MAP1025* from several paratuberculosis and non-paratuberculosis strains.

He found a single nucleotide change in the sequence encoding the epitope. This change affected the first amino acid out of the seven that make up the epitope, which is what created the specificity of *M. paratuberculosis*, Bannantine says.

Researchers can now accurately detect the Johne's disease pathogen and know for certain that it's not a contaminant.

"We finally have a specific antibody to detect live mycobacteria in the tissues or feces of cattle. This could never have been done before," Bannantine says.

Scientists have received a patent for the new antibody and are moving forward to develop diagnostic tests that will confirm the presence of the Johne's disease bacterium.

"We can now use this antibody to enrich the bacterium when it is present in low concentrations, such as in unpasteurized milk samples," Bannantine says. "Improved testing and diagnostics is really the big benefit of this antibody."—By **Sandra Avant,** ARS.

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

*John Bannantine is in the USDA-ARS Infectious Bacterial Diseases Research Unit, National Animal Disease Center, 1920 Dayton Ave., Ames, IA 50010; (301) 337-7340, john.bannantine@ars.usda.gov.*✱

## Examining Johne's Vaccines and Bovine TB Tests for Cross Reaction

Vaccines play a critical role in reducing the severity of Johne's disease, but using them can become a balancing act if the vaccine cross-reacts with tests for other cattle diseases like bovine tuberculosis (TB)—an issue in states where wildlife are tracking TB back into dairy herds.

"Producers are concerned that if they vaccinate against Johne's, they will not be able to discriminate whether their herd has bovine tuberculosis or Johne's," says microbiologist Judy Stabel, in the National Animal Disease Center's Infectious Bacterial Disease Research Unit.

Stabel looked at the cross reactivity of an effective U.S. commercial vaccine with TB diagnostic tests. Several calves were vaccinated against Johne's disease, and blood samples were taken over a period of 1 year. Immune and serological responses of calves were then measured using novel TB diagnostics that will be available in the future.

"These new serology tests for TB diagnostics are very promising," Stabel says. "They're similar to the stick pregnancy tests for humans, except you use serum."

The tests consist of small sticks coated with bovine TB antigen. The antibody in the serum reacts to the antigen if it is positive.

With vaccinated calves, no reactivity was found using the TB tests, which is good because it means that those tests will not wrongly identify cattle with Johne's disease as being positive for bovine TB, Stabel says.

"We also did skin testing, which is one of the main tests in the field for bovine tuberculosis, and found similar results," she adds.

The results of these studies showed that calves could be vaccinated against Johne's disease without interfering with bovine TB diagnostics.

A major advantage of the new serologic tests is the cost and ease of use, Stabel says. "Animals can be handled one time to obtain a blood sample rather than being handled twice to administer and read a skin test. That reduces veterinary costs."—By **Sandra Avant,** ARS.

*Judy Stabel is in the USDA-ARS Infectious Bacterial Diseases Research Unit, National Animal Disease Center, 1920 Dayton Ave., Ames, IA 50010; (301) 337-7304, judy.stabel@ars.usda.gov.*✱

# House Fly Virus Stops

STEPHEN AUSMUS (D1058-1)

Common house fly, *Musca domestica*.

**The house fly is often considered merely a nuisance.** But these flies are capable of transmitting animal and human pathogens that can lead to foodborne diseases, including *Escherichia coli*, *Salmonella*, and *Shigella* bacteria.

Insecticides are important for control, but house flies are particularly good at developing resistance, and their larvae tend to stay deep enough within their gooey food to avoid exposure to sprays.

Scientists at the Agricultural Research Service's Center for Medical, Agricultural, and Veterinary Entomology (CMAVE) in Gainesville, Florida, are looking at new methods that target adult flies. A promising biological control agent—salivary gland hypertrophy virus (SGHV)—was recently discovered. Once infected with the virus, female flies do not produce eggs, and male flies do not mate.

Entomologist Chris Geden in the Mosquito and Fly Research Unit at CMAVE partnered with scientists at the University of Florida (UF) and Aarhus University in Denmark to study the distribution and host range of the virus as well as the effectiveness of different application methods.

### A Virus That Works

SGHV is one of three viruses of a newly discovered family called "Hytrosaviridae." The other two viruses are one that infects tsetse flies in Africa and one that infects a pest of flower bulbs in Europe. These viruses are all very host specific, that is, they only occur in the insects they infect, Geden says.

As SGHV replicates in the salivary gland in female flies, something also happens within the reproductive system.

"The salivary glands become huge, ovaries remain small, and the fly can never lay any eggs," Geden says. "The virus hijacks the fly's protein-manufacturing control system. All the protein that would normally go into the ovaries to develop a fly's eggs is diverted to produce virus particles."

The virus may serve as a potential insect-sterilization agent by reducing the fertility of flies, says Drion Boucias, an entomology professor with UF's Institute of Food and Agricultural Sciences.

"More importantly, studies on this unique virus may provide clues as to how to reduce the yolk protein, providing a template for the development of novel insect birth-control chemistries," Boucias says.

Flies are believed to acquire SGHV when they feed. Infected flies carry disease-causing pathogens on their feet and in their intestines. Each time they feed, infected flies regurgitate massive numbers of virus particles on food. Healthy flies then feed on the contaminated food and pick up virus particles.

The team of researchers wanted to find out if they could infect female flies and stop egg development.

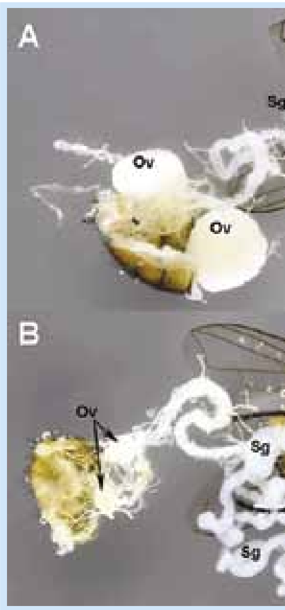
"It's a way of managing the fly population at the adult level by limiting its ability to reproduce," Geden says.

### The Right Approach

Generally, the virus's infection rate is very low—around 0.5 to 1 percent of the fly population, Geden says. But sometimes a "hot spot"—an area of significant SGHV activity—can be found. For example, one of the biggest hot spots was found at a large dairy farm in Gilchrist County, Florida, where the virus infection rate was about 37 percent.

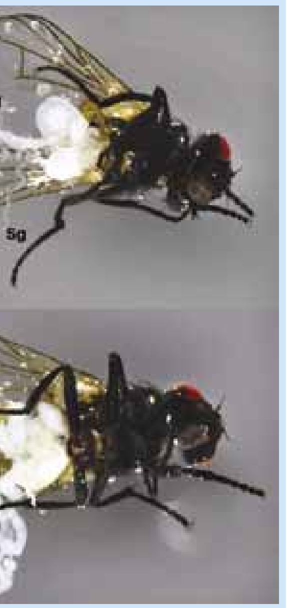
"We thought the best approach to increase the infection rate was to develop baits that contain the virus, but we were never able to get high infection levels using baits," Geden says.

The most effective method of infecting flies was direct application of a crude homogenate—a mixture of infected flies and water.



Entomologist Chris Geden and student assistant Rachel Di
virus to determine its effects on fly mortality and reproducti

# Flies from Reproducing



©LYLE BUSS, UNIV. OF FLORIDA (D2679-1)

Comparison of a healthy fly (A) and a fly infected with SGHV (B). The fly with SGHV shows underdeveloped ovaries (ov) and overdeveloped salivary glands (sg).

"If we dip the flies in the homogenate or allow them to walk on a surface treated with it, then bingo, we achieve high infection rates; 56 percent of the Danish flies and 50 percent of the Florida flies became infected," Geden says.

In laboratory tests, scientists used SGHV-infected house flies collected from livestock farms in Denmark and a strain of Florida house fly—"Orlando normal"—reared at CMAVE. Virus obtained from one of the infected Danish house flies was injected into the Florida flies, which were found to be highly susceptible to it.

STEPHEN AUSMUS (D2618-1)

illard sort stable flies before injecting them with SGHV
on.

None of the other four fly species injected with the virus showed any symptoms.

The study also showed that the Danish and Florida SGHV strains had a similar ability to produce infection. Experiments using Danish and Florida strains of virus as food baits produced infection rates in house flies of 22 percent and 26 percent, respectively. Spraying flies directly with the virus resulted in 18 percent and 22 percent of the flies becoming infected.

## Stopping Stable Flies

Two other species—the black dump fly and the stable fly—were also severely affected by SGHV. Stable flies are an important economic pest that affects cattle, pigs, horses, and other large animals. They can also be a problem in recreational areas.

"When we injected stable flies, not only did they become infected, but they also died very quickly, and of those that didn't die, hardly any had developed ovaries," Geden says.

"We found that infected stable flies produced 50 percent to 75 percent less feces, suggesting that they aren't feeding on blood as often or as well as healthy flies. Flies that had the virus not only didn't lay eggs, but also didn't bite as much."

Researchers also found that the virus was developing and replicating in the salivary gland, ovaries, body fat, and other tissues in stable flies. Virus-infected flies had a much shorter lifespan than uninfected flies.

## Enhancing Management Techniques

Developing an effective method to infect flies with SGHV in the field could have a significant impact on reducing fly abundance and biting rate, scientists agree.

"I think these novel management strategies of filth fly populations will play an increasingly important role as the human population continues to increase and boundaries between the suburbs and animal-production facilities are reduced," Boucias says.

While SGHV shows great promise in controlling fly populations, it's not a quick fix, Geden says.

"This is not an insecticide. It's not something you would put out when people are complaining about flies at picnics and expect to get a fast reduction," he says. "This would be part of an integrated management program in which you would go out early in the year when natural fly populations are just beginning to increase, hit them with the virus to knock down their reproductive ability, and come back 2 to 3 weeks later and do it again."—By **Sandra Avant,** ARS.

*This research is part of Veterinary, Medical, and Urban Entomology (#104) and Animal Health (#103), two ARS national programs described at www.nps.ars.usda.gov.*

*Chris Geden is in the USDA-ARS Mosquito and Fly Research Unit, Center for Medical, Agricultural, and Veterinary Entomology, 1600-1700 S.W. 23rd Dr., Gainesville, FL 32608; (352) 374-5919, chris.geden@ars.usda.gov.*✱

Stable fly, *Stomoxys calcitrans*.

STEPHEN AUSMUS (D2620-17)

# Improved Vitamin B12 Test May

STEPHEN AUSMUS (D2837-9)

A new vitamin B12 assay developed by ARS scientists at the Western Human Nutrition Research Center, Davis, California, requires only a tiny drop of blood rather than a standard 1-milliliter sample.

**Vitamin B12 helps your body perform many vital chores,** including forming healthy red blood cells; keeping your brain functioning smoothly; and processing (metabolizing) the fats, proteins, and carbohydrates in foods that you eat.

Like all vitamins, B12 is a micronutrient, meaning that we need it in only very small amounts.

We get B12 from animal products—meats, fish, poultry, eggs, cheese, and yogurt, for instance—or from B12-fortified foods, notably breakfast cereals. We can also obtain it from nutritional supplements, such as B12 tablets or multivitamin pills. People who need to boost their B12 levels quickly may do so via shots or other means prescribed by their physicians.

In the United States, the very young and the elderly are among the groups at risk of becoming B12 deficient. Newborns whose mothers are deficient in the vitamin may begin their lives with low stores of it. The problem may be compounded if these babies are breastfed, because the B12 levels in their mother's milk may be inadequate.

Seniors may have a different set of problems. If they lack sufficient gastric acid in the stomach, for instance, they may be unable to absorb enough of the vitamin from their food.

Now, a team of Agricultural Research Service scientists based at the Western Human Nutrition Research Center in Davis, California, has developed and tested an improved method for measuring a marker, or indicator, of the body's stores of B12 in blood. Importantly, the blood sample can be very small in volume.

That's an advantage in both medical and research situations. For example, samples that are taken from newborns and infants for health examinations at a hospital, or perhaps for use by medical or nutrition researchers, are typically very small.

ALISON H. KEENAN (D2838-2)

In the Obesity and Metabolism Research Unit at Davis, California, ARS chemists John Newman and Theresa Pedersen review data from a UPLC-ESI-MS/MS system used to measure methylmalonic acid, an indicator of vitamin B12, in a blood sample.

# Help Young and Old Alike

What's more, a small amount of sample is often the norm in complex, large-scale studies in which samples from volunteers have to be allocated among many researchers, each of whom may need it for a different research purpose. These studies can help answer vital questions about the vitamin, such as: How does B12 contribute to growth and development in the first years of life? How does it affect brain function in our later years? What is the extent of B12 deficiency in the United States and in other countries?

## Reliable Results in About 4 Minutes

The ARS team has shown that a specimen of only 25 microliters of blood plasma or serum—the equivalent of about one-half of a drop of water—can be analyzed with speed, accuracy, reliability, and precision using a leading-edge analytical technology, UPLC-ESI-MS/MS, short for ultra-performance liquid chromatography-electrospray ionization-tandem mass spectrometry. If the assay is performed using robotics, the sample could perhaps be as small as 5 microliters.

ARS researcher John W. Newman at the nutrition center led a collaboration that produced the assay. His team built on earlier work in which scientists elsewhere used an older technology, liquid chromatography-tandem mass spectrometry, to measure the same indicator compound, or biomarker, that Newman's group selected, namely, methylmalonic acid, or MMA.

## What is MMA?

MMA is a compound that can build up in the bloodstream if there isn't enough vitamin B12 in the body for an enzyme, methylmalonyl-CoA mutase, to function properly. That's why MMA is broadly accepted as a reliable marker of the B12 that's functioning in your body, or what nutrition researchers refer to as your "functional B12 status."

The 25-microliter sample size needed for the new MMA assay represents a 4- to 10-fold reduction in the volume of specimen required for other MMA-based B12 tests, says Newman. His team was able to reduce the required sample volume and, at the same time, increase precision and accuracy of MMA analyses, by using modern instruments and by carefully adjusting the procedures used to handle and analyze the tiny samples.

During the 4 minutes that it takes to process a sample, MMA molecules are subjected to a complex sequence of steps. Each step helps ensure that only MMA will be detected, identified, and measured.

## Real-World Samples Examined

The team has tested the assay not only with samples containing known quantities of purified MMA, but also with serum samples collected from a real-world B12 study. In that study, blood samples were collected at three different intervals from 139 healthy female volunteers, age 20 to 59. Each volunteer received a B12 shot at the outset of the investigation and then took either a 500-microgram B12 tablet or a look-alike placebo every day for 3 months.

By the end of the study, the MMA concentrations in 86 percent of the unsupplemented volunteers were regarded as within normal range, as compared to 98 percent of those who were supplemented.

Newman, a chemist at the center and an associate adjunct professor in the Department of Nutrition at the University of California-Davis, collaborated in developing the MMA assay with Lindsay H. Allen, center director and an adjunct research professor of nutrition; and with three other ARS colleagues: chemist Theresa L. Pedersen, physical scientist William R. Keyes, and nutritionist Setareh Shahab-Ferdows. They documented their research in a peer-reviewed article published in 2011 in the *Journal of Chromatography B*.—By **Marcia Wood,** ARS.

*This research supports the USDA priority of improving children's nutrition and health and is part of Human Nutrition, an ARS national program (#107) described at www.nps.ars.usda.gov.*

*John W. Newman and Lindsay H. Allen are with the USDA-ARS Western Human Nutrition Research Center, 430 W. Health Sciences Dr., University of California, Davis, CA 95616; (530) 752-1009 [Newman], (530) 752-5276 [Allen], john.newman@ars.usda.gov, lindsay.allen@ars.usda.gov.* ✻

In the United States, the very young and the elderly are groups at risk of becoming deficient in vitamin B12.

PEGGY GREB (D2824-1)

# Rooting Out a Novel Stress Syndrome in Pigs

Undergoing surgery, even a minor procedure, can be stressful for anyone. But for people who have malignant hyperthermia, a hereditary disease that's triggered by certain drugs used for general anesthesia, it can also be dangerous.

Research into this rare, life-threatening condition, which causes a fast rise in body temperature, severe muscle contractions, and sometimes death, was limited until the discovery of a similar disorder in pigs, referred to as "porcine stress syndrome." The classical syndrome is associated with poor response to stressors like transport and with poor-quality pork. It has been eliminated from commercial herds in the United States, but stress-related issues, most often associated with transportation, continue to cause substantial losses—an estimated $50 million per year—to the U.S. swine industry.

These issues indicate that there may be another stress-related syndrome affecting the health and well-being of pigs in the United States. In response to these concerns, scientists at the Agricultural Research Service's Roman L. Hruska U.S. Meat Animal Research Center (USMARC) in Clay Center, Nebraska, have identified a previously undetected genetic stress syndrome that is most likely affecting the swine industry. They are mapping the defect in pigs to get to the root of the problem.

### A Big Undertaking of a Huge Gene

Molecular biologist Dan Nonneman and his colleagues—geneticist Gary Rohrer, agricultural engineer Tami Brown-Brandl, and veterinary medical officer Shuna Jones—have mapped the stress disorder to a genetic mutation located in the dystrophin gene—the largest known gene in the mammalian genome.

"We believe this is the defect because we found that there's about half as much dystrophin protein in affected pigs as there is in their normal siblings," says Nonneman, who's in the USMARC Reproduction Research Unit.

The syndrome was first discovered in two male siblings in the USMARC swine herd after they were transported from one facility to another at the center. Compared to their pen mates, the 3-month-old pair showed very atypical signs of stress—squealing, open-mouth breathing, skin discoloration, loss of mobility, and then death.

"This novel porcine stress syndrome is not a malignant hyperthermia like the classical stress syndrome; it's a defect in dystrophin," Nonneman says. "We hope to identify predictive markers that will allow us to determine the prevalence of this disease in commercial swine populations."

Left: Molecular biologist Dan Nonneman and agricultural engineer Tami Brown-Brandl view an electrocardiograph (ECG) from an unaffected littermate. Abnormal heart rates and ECGs collected under anesthesia can identify piglets affected with porcine stress syndrome.

Scientists re-mated the original sire (male) and dam (female) of the affected pigs to produce additional litters. At 8 weeks of age, the piglets underwent general anesthesia, and their heart rates and electrocardiographs (ECG) were monitored. Pigs identified as having the stress syndrome had abnormal ECG readings and sometimes died, whereas the heart rate of unaffected pigs remained steady. In some cases, piglets that did not undergo the anesthesia challenge were identified as having the stress syndrome when they had a stress response or died during typical procedures, such as weighing.

“We also looked at the enzyme creatine phosphokinase,” Nonneman says, “which is used to determine tissue damage after a heart attack. When muscle cells get damaged, this enzyme leaks out. Therefore, it’s used to monitor heart and other muscle diseases.”

Researchers found that creatine phosphokinase was about three times higher in pigs suspected of having the disorder.

“What we saw scattered throughout the muscle were regions with defects or injuries,” Nonneman says. “The whole muscle was not affected. The defect seemed to be interspersed throughout the tissue.”

### Detecting the Defect

A critical issue was to identify a phenotype that could accurately determine whether pigs were affected or not. After several generations were produced from the original sire and dam—a pedigree of 250 offspring, including 49 affected pigs—scientists genotyped the animals using the Illumina Porcine 60K SNP Beadchip. A glass slide that contains thousands of DNA markers, the beadchip shows relationships between 60,000 markers simultaneously.

One chromosomal region containing the dystrophin gene was found to be associated with the syndrome.

“I won’t say that we have the mutation, but I think we have found the defective gene,” Nonneman says. “This syndrome maps to the dystrophin gene—DMD, which stands for Duchenne muscular dystrophy. DMD involves muscle weakness that worsens quickly and can lead to death under stressful situations.”

The gene is located on the X chromosome. The condition is found primarily in males because they inherit only one X chromosome from their mother, who has one normal and one affected X chromosome if she is a carrier. Males born to females that carry the disease each have a 50-percent chance of inheriting the syndrome.

“Diagnosing pigs with the disorder is difficult because female carriers do not seem to be affected,” Nonneman says. “Also, pigs that have the syndrome show no signs of the disorder unless they undergo stress.”

Piglets seem to be more susceptible when they are about 8 weeks old, an age when they are transported from nursery to grower facilities.

### Useful in Muscular Dystrophy Research

The discovery of the defect may provide a unique biomedical model for cardiomyopathy—a heart muscle condition—associated with muscular dystrophy in humans, Nonneman says. Scientists at USMARC and Iowa State University are investigating the possibilities.

“Muscular dystrophy has a very wide range of severities and mechanisms as to how they occur,” Nonneman says. “There’s a lot of attention on developing therapies—some of which look very promising—for these diseases in humans. The pig provides critical information because of its similarities to human physiology.”

In the meantime, scientists are using next-generation sequencing technology, which produces millions of DNA sequences simultaneously, to completely sequence the chromosomal region in affected and normal animals and to identify all the DNA variation in dystrophin. The main goals are to identify the mutation, test as many commercial pigs as possible, find out how prevalent the new stress syndrome is, and develop strategies in cooperation with the pork industry to eliminate it from the U.S. pig population.

“We can use DNA markers to identify affected animals and then cull them out of the herds, choosing not to breed them. That’s what was done with the classic porcine stress syndrome,” Nonneman says.

Nonneman and his team plan to investigate whether the stress disorder affects meat quality as well as the growth and overall health of animals. They are also looking at cardiovascular effects in older pigs.—By **Sandra Avant,** ARS.

*This research is part of Food Animal Production, an ARS national program (#101) described at www.nps.ars.usda.gov.*

*Dan Nonneman is in the USDA-ARS Roman L. Hruska U.S. Meat Animal Research Center, State Spur 18D, Clay Center, NE 68933; (402) 762-4367, dan.nonneman@ars.usda.gov.* ✱

Dan Nonneman and technician Sue Hauver use an antibody to evaluate levels of dystrophin on an immunoblot of heart tissue from a family of normal and affected pigs. Animals affected with porcine stress syndrome have reduced amounts of dystrophin in their heart and muscle.

PEGGY GREB (D2823-1)

STEPHEN AUSMUS (D646-1)

# A Modeling Milestone for Soil Phosphorus Management

ARS and University of Arkansas scientists conduct rainfall simulation experiments that provided phosphorus runoff loss data used to develop and test the APLE computer model.

**The U.S. Department of Agriculture's Natural Resources Conservation Service** (NRCS) is looking for ways to upgrade the Phosphorus Index, a simple management tool developed during the 1990s to gauge the risk of phosphorus losses from agricultural fields. In developing a national nutrient-management policy, NRCS allowed states to modify the original index—a matrix of source and transport factors that contribute to phosphorus loss—with inputs to account for local variations in soils, climate, management, and water quality goals.

But this resulted in widely different state-by-state phosphorus indices that often didn't agree with each other on how to manage phosphorus. "We'd put in the same numbers and get different results, depending on the state," says Agricultural Research Service soil scientist Peter Vadas, who works at the U.S. Dairy Forage Research Center in Madison, Wisconsin. Additionally, many of these indices were not tested against monitoring data to show that they gave reliable results, or they didn't quantify phosphorus loss—such as losses in pounds per acre—which made them difficult to test against field data.

So Vadas worked with colleagues to develop the Annual Phosphorus Loss Estimator (APLE), a user-friendly spreadsheet program that predicts field-scale phosphorus loss in runoff for a whole year. His ARS partners included hydrologist Carl Bolster, in Bowling Green, Kentucky; soil scientist Philip Moore, in Fayetteville, Arkansas; and agricultural engineer Michael White, in Temple, Texas. University of Wisconsin researcher Laura Good and Purdue University researcher Brad Joern also partnered on the project.

## Field Studies Meet Spreadsheet Simulations

The scientists developed APLE so it would be as easy to use as earlier phosphorus indices. It could be used in many different states to quantify field-scale phosphorus loss and soil phosphorus changes over 10 years for a given set of runoff, erosion, and management conditions. They included information and data that has been established for decades, as well as important innovations, especially for phosphorus loss from manures and fertilizers.

The scientists started out by programming APLE with data from 21 field studies that measured the impact of surface-applied manure and fertilizer on phosphorus loss (see box). The 21 studies represented a wide variety of field sizes, crop and manure management conditions, manure types, and geographic locations across the United States.

Then the scientists expanded APLE to include dissolved phosphorus loss from soil and sediment phosphorus loss from erosion. They tested APLE with field data from another 28 studies that monitored phosphorus loss in runoff from fields for at least a year.

MIKE BRYANT (D2819-1)

Hydrologist Carl Bolster prepares a soil sample to determine soil phosphorus concentration, an important input parameter to the APLE model.

The team showed that APLE could reliably quantify phosphorus losses in runoff for many different situations and could produce more reliable estimates than some existing phosphorus indexes. From there, Vadas and his colleagues again expanded APLE to simulate changes in soil phosphorus over 10 years. This application would be important for farmers concerned about accumulating too much soil phosphorus or wondering how long it might take to reduce the phosphorus that had already accumulated.

The team compared APLE soil phosphorus simulations with field results from 25 studies that monitored changes in soil phosphorus from 1 to 29 years. Many of the agronomic conditions in the studies varied considerably. Simulation scenarios included predictions for total soil phosphorus for a variety of soil layer depths; increase in soil phosphorus over time resulting from consistent phosphorus amendments; decrease in soil phosphorus over time in the absence of soil phosphorus amendments; and phosphorus stratification levels when phosphorus was applied but not tilled into the soil. APLE estimates were consistently in line with field measurements.

Results from these studies have been published in *Transactions of the ASABE, Journal of Environmental Quality,* and elsewhere. Some of the equations used in APLE have also been incorporated into the phosphorus indexes used in Wisconsin and Arkansas.

### Fixes for Phosphorus Management

Most recently, the scientists have been using APLE to improve the assessment of phosphorus losses from agricultural fields and whole farms. For instance, Bolster led a project demonstrating that APLE can be used to rapidly and easily develop weighting factors for determining the relative contribution of phosphorus losses from different sources, such as soil, manure, or erosion. Weighting factors for most indices have already been developed with input from field experts, but Bolster's results showed that APLE-generated factors significantly improved the correlation between index outputs and a large and diverse set of published field data.

In collaboration with scientists from the University of Wisconsin in Madison and Platteville, Vadas has been adapting APLE to simulate phosphorus loss from pastures grazed by beef and dairy cattle and from barnyards and exercise lots on cattle farms. With these improvements, APLE can be used to develop whole-farm estimates of phosphorus losses and the most effective strategies for reducing phosphorus losses from cattle farms. These practices could include barnyard improvements for capturing discharge, soil conservation practices that reduce erosion, or manure application practices that reduce exposure to runoff water.

"APLE has all of the current science, and it shows that we do have good equations to predict phosphorus loss," Vadas says. "We have decades of research on soil phosphorus processes, but we needed to find a way to integrate this information so that people can make sense of it. Now we have a good model that can expand on different scenarios very quickly and efficiently and help producers and policymakers make informed decisions about managing phosphorus."

APLE is free to download at tinyurl.com/phosphorusloss and includes supporting technical documentation and a user's manual.—By **Ann Perry,** ARS.

*This research is part of Agricultural and Industrial Byproducts (#214), Climate Change, Soils, and Emissions (#212), and Water Availability and Watershed Management (#211), three ARS national programs described at www.nps.ars.usda.gov.*

*Peter Vadas is at the U.S. Dairy Forage Research Center, 1925 Linden Dr. West, Madison, WI 53706; (608) 890-0069, peter.vadas@ars.usda.gov.* ✱

## What's In a Model?

Many variables were used to revamp the Annual Phosphorus Loss Estimator

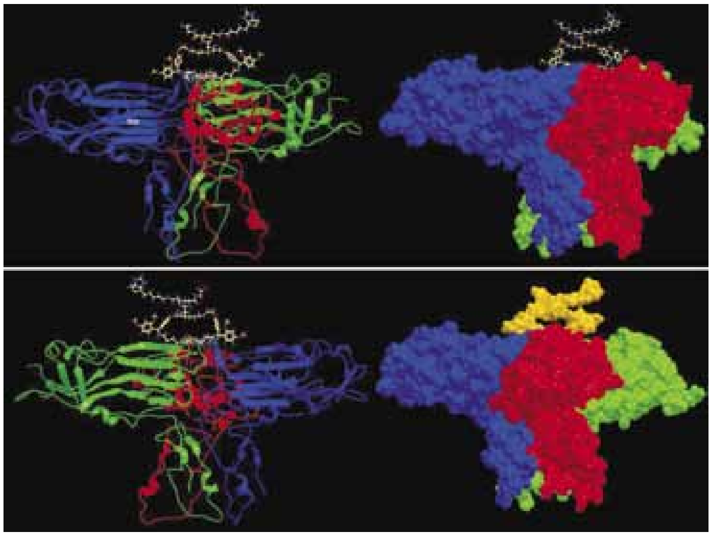
- soil test phosphorus levels
- percentage of clay and organic soil matter
- annual removal of soil phosphorus via crop uptake
- total number of cattle grazing days in the field
- field dimensions
- amount of manure (wet mass) applied
- percentage of solids in the manure
- total phosphorus content of manure
- percentage of manure incorporated into the soil
- depth of manure incorporation
- mass of phosphorus applied as fertilizer
- depth of phosphorus incorporation
- degree of soil mixing from tillage or biological activity
- annual precipitation, runoff, and erosion

# New Technologies for Studying Crops and Crop Diseases

PEGGY GREB (D2825-1)

Michelle Cilia, a molecular biologist in the Biological Integrated Pest Management Research Unit in Ithaca, New York, examines infected potato plants in the greenhouse for symptoms of virus infection.

MICHELLE CILIA, JAMES BRUCE (D2833-1)



Cross-linking measurements with protein interaction reporter technology enabled ARS scientists and their University of Washington colleagues to model the structure of the building blocks of infectious potato leafroll virus particles. The scientists are applying this technology so they can understand insect transmission of plant viruses and the mechanisms viruses use to infect plants.

Agricultural Research Service scientists in New York and California have developed very different technologies that share a common thread. They offer scientists new, innovative ways to probe what happens when a crop is threatened by drought or disease.

Michelle Cilia and Stewart Gray, ARS scientists at the Robert W. Holley Center for Agriculture and Health in Ithaca, New York, and their colleagues at the University of Washington have found a way to map the structure of an elusive protein that gives certain plant viruses the ability to travel from plants to insects, through the insects, and back into plants.

Andrew McElrone, a plant physiologist in the Crops Pathology and Genetics Research Unit in Davis, California, has adapted computed tomography (CT) scan technology to vastly improve on the tools available for studying how water and pathogens move through vascular plant tissue called “xylem.” The work has opened a window into how grapevines respond to drought stress and is shedding light on vascular networks in a number of other crops.

### Luteovirus Proteins: It’s the Shape That Counts

To move from plant to plant, luteoviruses, such as potato leafroll virus and soybean dwarf virus, need to stay in the infected plant’s phloem tissues, which carry sugars and other substances in the plant, so they can be ingested by a feeding aphid. Once inside the aphid, the virus must pass through the insect’s gut and salivary tissues before it can be passed into another plant by the aphid. To accomplish that remarkable journey, viruses need to assemble proteins into larger packages known as “virions.”

Each luteovirus species is very particular and can only be transmitted by a few species of aphids. Cilia, Gray, and their partners James Bruce and Juan Chavez of the University of Washington think that the outside shape, or topology, of the virion plays a major role in that specificity, determining whether a virus will be able to move through the aphid and infect a plant.

“As a virion, the virus moves from plants to aphids, through aphids, and back into plants, and the topology of the virion guides the transmission process,” Gray says.

A minor structural protein of these viruses is instrumental in guiding the virion on its journey through the insect. “These proteins extend from the shell of the virion and act like extension cords, giving the virion the flexibility it needs to make necessary connections in the plant and aphid," Bruce says. Flexibility is critical to how these proteins function, but it also makes them extremely difficult to study. Until now, there has been no structural information for these proteins. Such information is crucial for developing new ways to disrupt virus transmission.

In tests with potato leafroll virus, the researchers used protein interaction reporter (PIR) technology, a tool developed in Bruce's lab, to study protein interactions. The lab developed a unique set of chemical compounds, or PIR cross-linkers, that would interact with the structural proteins that guide the virion on its journey. These PIR cross-linkers enabled the team to capture a molecular snapshot of the structural proteins as they exist in the virion. Coupled to high-resolution mass spectrometry, the advanced molecular design of the PIR cross-linkers enabled the scientists to visualize for the first time the critical topological features of the virion. The results were published in the *Journal of Proteome Research*.

"We are able to map topological features of the virus that no one has ever seen before," Cilia says. The next step will be to use PIR technology to discover how the virion and the structural proteins interact with plant and insect proteins.

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Injecting a liquid culture of a plant-infecting bacteria to deliver an infectious clone of the virus into plant cells.

### CT Scans To Study Plants

In Davis, McElrone has found a way to use high-resolution CT to study the vascular systems of grapevines and other plants in greater detail than ever before.

For his studies, McElrone used the microtomography beamline at the Advanced Light Source, a unique and powerful tool at the U.S. Department of Energy's Lawrence Berkeley Laboratory. He and his colleagues focused on the xylem tissue, which consists of tubelike elements that carry water and nutrients throughout the plant. Drought conditions increase tension on the water column in the xylem tubes, making them more susceptible to the formation of air bubbles, called "embolisms," that interrupt the water flow and lead to damage or even death of the vine.

With the instrument, McElrone created hundreds of images of the xylem tubes, showing the embolism-formation process in grapevines exposed to drought stress. The team developed software that extracts information from the images, allowing them to visualize how every vessel in a cross section of xylem tissue is connected to every other vessel, as well as the sizes and orientations of the connections.

Much of the work was funded with a National Science Foundation grant, and the software is available free to other researchers.

### Bridge Cells: A Key to Infection Susceptibility

Some pathogens target the xylem tissue. Using the CT technology, McElrone found that a key to infection susceptibility is the presence of specialized "bridge cells," which interconnect larger xylem tubes. His research has shown that how well the bridge cells link the xylem tubes could contribute to how well the bacterial pathogen

## Probing Common Threats

that causes Pierce's disease, which results in major damage in vineyards, will move through the grapevine. Grapevines susceptible to Pierce's disease have more bridge cells, which may allow pathogens to pass through them more easily.

The CT scanning technique is a vast improvement over the traditional method of studying individual cross sections of grapevine tissues under microscopes. Being able to study embolism formation and the connections between xylem tissues in such fine detail will help scientists determine how plants respond to water stress and other changing environmental conditions. It also should assist in efforts to breed crops better equipped to tolerate drought and resist disease, McElrone says.

McElrone has a manuscript accepted in the *Journal of Visualized Experiments* and an accompanying film that demonstrates the technique for other scientists. Much of his work has focused on grapevines, but he is working with other researchers to use the technology to study vascular tissues in other crops, including blueberries, citrus, sunflowers, and walnuts.

"This is a new technology and we are just beginning to appreciate the possibilities," McElrone says.—By **Dennis O'Brien,** ARS.

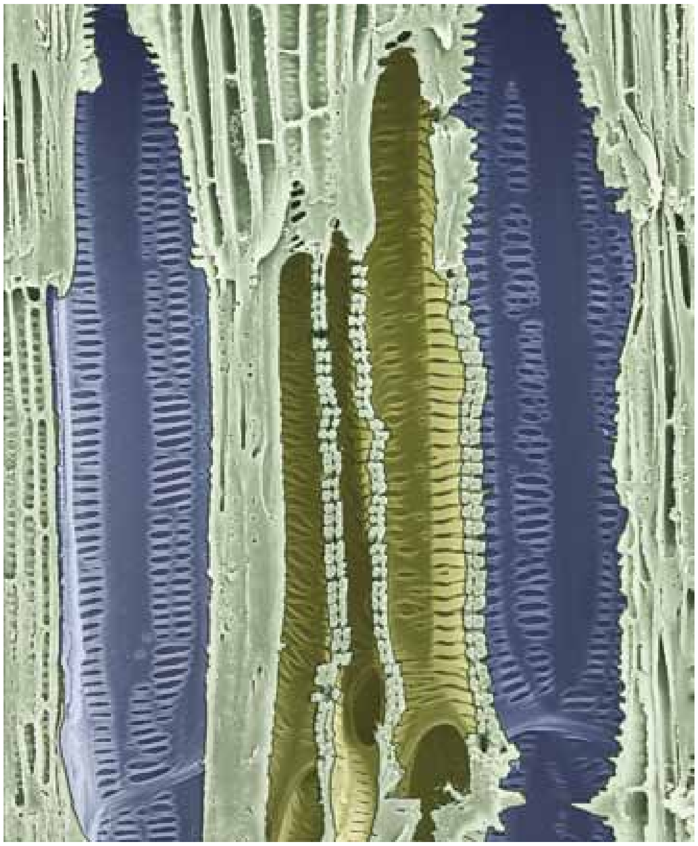
*The research is part of Plant Diseases (#303) and Crop Production (#305), two ARS national programs described at www.nps.ars.usda.gov.*

*To reach the scientists mentioned in this article, contact Dennis O'Brien, USDA-ARS Information Staff, 5601 Sunnyside Ave., Beltsville MD 20705-5129; (301) 504-1624, dennis.obrien@ars.usda.gov.**

Grapevines susceptible to Pierce's disease have more bridge cells, which may allow pathogens to pass through them more easily.

Right: Postdoctoral associate Stacy DeBlasio injects a liquid culture of a plant-infecting bacteria into plant cells.

Bottom: This scanning electron micrograph of grapevine xylem highlights the tubes that carry water and nutrients throughout the plant. The middle yellow section is a bridge that connects the two larger xylem vessels. These bridges increase the connectivity of the xylem transport system, differ in number and structure between grapevine species, and may play an important role in the spread of disease-causing pathogens.



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PEGGY GREB (D2828-1)

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

## Locations Featured in This Magazine Issue

*Locations listed west to east.*

Map courtesy of Tom Patterson, U.S. National Park Service

**Not shown:** Mayagüez, Puerto Rico

**Davis, California**
3 research units ■ 117 employees

**U.S. Salinity Laboratory, Riverside, California**
2 research units ■ 39 employees

**U.S. Arid-Land Agricultural Research Center, Maricopa, Arizona**
3 research units ■ 80 employees

**Small Grains and Potato Germplasm Research Unit, Aberdeen, Idaho**
1 research unit ■ 57 employees

**Conservation and Production Research Laboratory, Bushland, Texas**
2 research units ■ 55 employees

**Roman L. Hruska U.S. Meat Animal Research Center, Clay Center, Nebraska**
6 research units ■ 117 employees

**Grassland Soil and Water Research Laboratory, Temple, Texas**
1 research unit ■ 33 employees

**Southern Plains Agricultural Research Center, College Station, Texas**
4 research units ■ 132 employees

**Poultry Production and Product Safety Research Unit, Fayetteville, Arkansas**
1 research unit ■ 12 employees

**Ames, Iowa**
8 research units ■ 462 employees

**Stuttgart, Arkansas**
2 research units ■ 56 employees

**Jamie Whitten Delta States Research Center, Stoneville, Mississippi**
7 research units ■ 260 employees

**Madison, Wisconsin**
5 research units ■ 131 employees

**Southern Regional Research Center, New Orleans, Louisiana**
7 research units ■ 171 employees

**Animal Waste Management Research Unit, Bowling Green, Kentucky**
1 research unit ■ 16 employees

**Center for Medical, Agricultural, and Veterinary Entomology, Gainesville, Florida**
4 research units ■ 134 employees

**Coastal Plains Soil, Water, and Plant Research Center, Florence, South Carolina**
1 research unit ■ 38 employees

**Raleigh, North Carolina**
4 research units ■ 96 employees

**Robert W. Holley Center for Agriculture and Health, Ithaca, New York**
3 research units ■ 61 employees

**Henry A. Wallace Beltsville Agricultural Research Center, Beltsville, Maryland**
30 research units ■ 707 employees

**Tropical Agriculture Research Station, Mayagüez, Puerto Rico**
1 research unit ■ 40 employees

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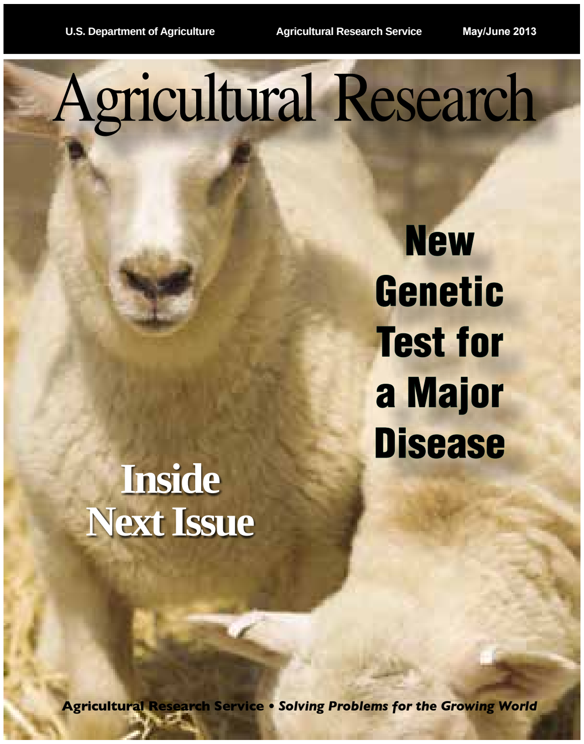

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