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Agricultural Research

Selecting for Faster Growing Yellow Perch

Story on page 4.

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

FORUM

Valuing U.S. Aquaculture

In 2012, the average American ate 14.4 pounds of seafood—reflecting a slight drop from the 15 pounds eaten in 2011. However, this does not necessarily mean that we want to eat less fish. These figures are a result of an increasing U.S. population and a decrease in commercial fishery catches used for food, according to the National Oceanic and Atmospheric Administration.

Our appetite for seafood is expected to surpass the slowing rate of global harvesting from the wild, making the success of the U.S. aquaculture industry even more crucial in meeting the demand for fish and shellfish. Aquaculture, also known as "fish farming," is the propagation and rearing of animals and plants in aquatic environments, under controlled or selected conditions.

More than 91 percent of the seafood Americans consume is imported. While about half of all seafood consumed in the United States is produced from aquaculture, only about 5 percent of this seafood comes from the United States, which produces oysters, clams, mussels, catfish, salmon, trout, and yellow perch. As a nation, we are the world's third largest consumers of seafood, yet we rank 15th in total aquaculture production, according to a 2012 United Nations Food and Agriculture Organization report, "The State of World Fisheries and Aquaculture."

Although the United States is a small producer in the global aquaculture industry, it is a leader in advanced technology and has world-class research capacity. It has the natural resources, markets, and feed grains essential for commercial success, too.

Like crop, livestock, and poultry production, aquaculture is an agricultural industry, with a promising future for growth driven by innovations and entrepreneurs. It is the fastest growing animal-protein sector worldwide and is the most efficient animal-protein production system.

World economies will continue to compete for available seafood, both wild harvested and farmed, as demand increases. Simplifying regulatory barriers and streamlining the permit process can stimulate more investment and growth as demand for high-quality aquaculture products increases. Aquaculture investors are looking for locations near large markets, while small businesses are supplying local markets. The lack of access to sites in marine waters is creating strong markets worldwide for many farm-raised shellfish products. A concerted effort among governmental agencies can help make the United States an attractive country for investment to supply these markets.

The U.S. Department of Agriculture supports research through its Agricultural Research Service aquaculture national program to enhance production of freshwater and marine aquatic animals. Scientists investigate fish health, nutrition, genetic improvement, reproduction and early development, and production systems. They have recently started examining the development of ready-to-eat products and uses for processing trimmings.

ARS research includes breeding programs, in partnership with universities, to develop fast-growing finfish and shellfish that also have enhanced disease resistance, improved yield, and better reproduction. For example, the article on page 4 of this issue highlights some of ARS's work with yellow perch. Research also involves collaborations with ARS national programs dedicated to natural resources and sustainable agriculture, nutrition and food safety, and quality and utilization of agricultural products.

To help U.S. fish farmers remain internationally competitive, ARS scientists examine methods to reduce production costs, which include investigating alternative protein sources for fish feed and developing more efficient production systems and practices. This research helps to improve the quality, safety, and variety of aquaculture products available to consumers.

New projects under way are focusing on the health aspects of fish oil in the human diet. Farmed salmon is a main source of the fish oil consumed by people in the United States. Fish physiologists at ARS salmon and trout laboratories are working with ARS nutrition experts to explore the nutritional and health value of fish.

In Hagerman, Idaho, scientists are looking at genetic differences in the ability of trout to accumulate healthy fish oils—omega-3 fatty acids—in their flesh. They are investigating the potential to selectively breed fish for enhanced capacity, on the same feed, to yield a product higher in nutritional value.

Americans eat an average of one seafood meal a week. However, USDA's dietary guidelines recommend eating twice as much as that, because research suggests it improves cardiovascular health. It's also a good idea to choose seafood grown in the United States because of our strict environmental and food safety regulations. Buying U.S.-grown fish and shellfish ensures that consumers are getting food that meets rigorous state and federal standards while supporting America's aquaculture farmers.

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Agricultural Research

Solving Problems for the Growing World

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GLORIA SOLANO-AGUILAR (D3161-1)

The effect of diet is being evaluated in Ossabaw pigs at an ARS animal facility in Beltsville, Maryland. The researchers are studying this type of pig because it has a higher tendency to develop obesity-related diseases. Story begins on page 14.

Cover: ARS scientists are studying yellow perch to determine ways to better select fish for faster growth and better disease resistance. Story begins on page 4. Photo by Robert Colletta. (D3149-1).

ROBERT COLLETTA (D3149-1)

A genetically improved yellow perch being reared at the University of Wisconsin-Milwaukee School of Freshwater Sciences, where ARS scientists are studying fish growth and disease resistance.

Identifying Yellow Perch Gender To Improve Production

A plentiful supply of yellow perch was once available in the U.S. Great Lakes region, but that changed in the 1990s. Populations of this Midwest fish-fry favorite dropped dramatically due to the invasion of the zebra mussel, overfishing, and other factors.

Agricultural Research Service scientists are working closely with the University of Wisconsin-Milwaukee School of Freshwater Sciences to ensure that the aquaculture industry produces enough yellow perch to meet consumer demand. This high-value fish is very low in fat, high in protein and calcium, and has a mild, sweet flavor.

Using traditional breeding methods, scientists in the ARS Dairy Forage and Aquaculture Research Unit are developing genetically improved yellow perch by selecting for faster growth. One challenge has been the difference in growth rate between male and female yellow perch; females tend to grow faster and larger than males. But differences in growth rate alone are not sufficient to identify gender, says ARS fish physiologist Brian Shepherd. If only large fish are selected, there will be a strong bias toward females. The biggest males are also needed, but scientists had been unable to distinguish between smaller females and larger males.

To improve fish performance, scientists must also understand physiological differences between young female and male fish. But since gender identification is difficult until fish mature, it can be challenging to separate them for these kinds of studies.

"For example, if you're trying to genetically improve cattle, pigs, or sheep, imagine how difficult it would be to separate animals and manage them if you could not tell the sexes apart until they mature," Shepherd says. "It's problematic in yellow perch and other fish species."

Attention to Appearances

Shepherd and his colleagues developed criteria, based on the shape and color of the anal and reproductive openings, to identify the gender of yellow perch during early growth. The step-by-step procedure provides a systematic way to segregate males and females that are 3 inches or more in length. The process is fast, easy, and reliable. It involves an algorithm—a checklist that includes the size of the fish, the shape of specific parts, and other criteria.

The method is more than 97 percent accurate and provides a practical tool to cost-effectively sort females from males, Shepherd says. Gender separation can be used to develop yellow perch brood stocks, conduct gender-specific experiments, and identify the fastest growing females and males for growth performance.

"Because fish are unharmed, the algorithm can also be used to distinguish males from females during field surveys," Shepherd adds.

In the study, scientists examined different geographical strains of yellow perch and considered factors such as maturity and environmental conditions that may affect external characteristics related to gender. They examined yellow perch that were about 3 inches to 11 inches long and identified female and male characteristics that could be confirmed in four distinct geographical strains.

Scientists segregated the fish according to length within each strain and then determined gender based on external appearances. Fish have two openings—the anal and the urogenital—on their abdomen in front of their tails. The shape of the urogenital opening in males is round and generally larger in circumference than the anal opening, whereas females tend to have a V- or U-shaped opening that is smaller in circumference than the anal opening.

"Males usually have a more brownish-red coloration of that area than females do, especially when they're sexually immature," Shepherd says.

Scientists confirmed gender by looking at the internal gonads of fish. "We had

ROBERT COLLETTA (D3158-1)

At the University of Wisconsin-Milwaukee School of Freshwater Sciences, ARS fish physiologist Brian Shepherd (left), animal caretaker Timothy Paul (middle), and student Eric Vang collect data from male and female yellow perch as part of research to genetically improve the fish.

greater than 90 percent accuracy in correlating external gender with the true gender in females," Shepherd says. "Geographical locations of strains had significant effect on accuracy, so a stepwise checklist [algorithm] was developed to help producers determine whether the criteria would apply to their populations. With our algorithm, we were able to reliably identify the gender of yellow perch that were just 3.1 inches in length. That's a pretty small fish."

Without effective gender identification methods, scientists were retaining smaller females thinking they were males. The new system allows them to identify the biggest females and males for producing the next generation of yellow perch.

Determining Disease Resistance

In other research, Shepherd and his colleagues studied the yellow perch's immune response. Male and female fish were given a compound called "lipopolysaccharide," which mimics a bacterial infection. The female's immune response was lower than the male's, suggesting the females recovered faster or cleared the compound more effectively.

"Though more work is needed, this suggests that females may have a more robust immune system than males," Shepherd says. "Therefore, we aim to produce all-female yellow perch populations that will grow faster and have greater disease resistance. That should really be a tremendous advantage to producers."—By **Sandra Avant,** ARS.

This research is part of Aquaculture, an ARS national program (#106) described at www.nps.ars.usda.gov.

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Brian Shepherd and Timothy Paul select yellow perch from a tank for traits desired in breeding.

ROBERT COLLETTA (D3148-1)

Supershedders' Role in Spreading *E. coli* Scrutinized


TERRY ARTHUR (D3139-1)

About 2 percent of cattle are supershedders of *E. coli* O157:H7 bacteria. Just one supershedder in a pen can spread the bacteria to the hides of most of the other animals in the pen.

Though you can't tell just by looking at them, some of the cattle grazing in a pasture, or noshing high-energy rations in a feedlot pen, may be "supershedders"—meaning they shed high levels of pathogenic *Escherichia coli*—such as *E. coli* O157:H7—in their manure.

Supershedding could increase the amount of *E. coli* O157 that makes its way from pasture or feedlot pen into packinghouses where steaks, roasts, ground round, or other in-demand beef products are prepared.

Often referred to simply as "O157," this bacterium is apparently harmless to cattle. But in people, it can cause vomiting, severe stomach cramps, diarrhea, or other illness, such as hemolytic uremic syndrome—a sometimes deadly form of kidney failure.

In the United States, O157 is associated with about 95,000 infections every year, according to estimates from the U.S. Centers for Disease Control and Prevention. Some of these infections are attributed to eating O157-contaminated ground beef that was not properly cooked. Findings from studies led by Agricultural Research Service microbiologist Terry Arthur may help keep beef safe to eat by adding to our knowledge of supershedding. Arthur is with the agency's Roman L. Hruska U.S. Meat Animal Research Center in Clay Center, Nebraska.

The investigations may provide a scientifically sound basis for new and effective strategies to curb supershedding. What's more, the studies are a step toward a longer-term goal—shared by public health officials, food safety researchers, and beef producers, processors, and purveyors alike—of ensuring that no harmful *E. coli* occurs in any link of the beef-production chain—from ranch to fork.

What Defines a Supershedder?

Scientists generally agree that a supershedder is any animal that sheds 10,000 pathogenic organisms per gram of manure. "It isn't the amount of manure that's shed," Arthur emphasizes. "It's the amount of the pathogen in the manure."

Supershedding is a transitory condition that researchers currently think lasts less than a month. Regardless of duration, the basic problem with supershedding is the same: the copious amounts of O157 in the manure don't necessarily stay where the manure was deposited.

Instead, shedding may lead to spreading.

An animal that takes a soothing dust bath, for instance, may inadvertently roll over some *E. coli*-contaminated manure on the feedlot floor and end up with O157 cells stuck to its hide. Later, some of that manure-borne *E. coli* may spread to pen mates during the usual milling about. Or the microbes could be ingested during mutual grooming, another normal, everyday behavior of pastured or penned cattle.

E. coli O157 that's swallowed might then colonize a previously uninfected animal's gastrointestinal tract, and that animal's manure could later become a new source of infection in other cattle.

From Feedlot to Packinghouse

Understandably, high levels of O157 on cattle hides could stress packinghouse sanitation systems designed to prevent the spread of the pathogen.

To discover more about supershedding, Arthur and colleagues designed and conducted studies of 6,000 head of feedlot cattle and more than 13,000 manure, hide, and carcass samples. Some of their investigations are among the most detailed of their kind, to date.

The team was the first to gather data—representative of the entire U.S. cattle population—to estimate incidence of supershedding. Their analysis showed that supershedders may make up approximately 2 percent of a herd, on average.

In other work, the researchers monitored O157 contamination on hides of cattle in 10 feedlot pens and determined that supershedders were responsible for the majority of contamination.

There's more. Arthur and team showed that, in supershedders, *E. coli* colonization may occur throughout the digestive system, from mouth to tail-end. "If you are operating a packinghouse sanitation system with the expectation that O157 occurs primarily in the lower digestive tract, it's important to know that a supershedder is apparently an exception to that generalization," Arthur says.


A sponge rubbed on a cow's hide to sample contaminants, including soil, manure, and microorganisms.

PEGGY GREB (D625-1)

FRANK RENO (D3137-1)

Microbiologists Terry Arthur (left) and Norasak Kalchayanand analyze molecular fingerprint patterns for *E. coli* O157:H7 in studies of supershedding cattle.

One Strain To Blame?

In another "first," the researchers showed that supershedding was not restricted to any particular O157 strain. "Our work rules out the idea that a strategy should target a specific strain or strains to reduce supershedding," Arthur says. "The O157 in the manure samples collected for our research was mostly a mixture of strains in which no single *E. coli* predominated."

The research has also yielded criteria to gauge the success of candidate strategies for reducing or eliminating supershedding. Such interventions might include treating cattle with an O157 vaccine or adding an ingredient to their feed that helps suppress the pathogen. For an intervention to be deemed successful, the scientists say, two criteria must be met. First, none of the cattle in the pasture or pen would be supershedders. Second, the rate of fecal contamination (the number of cattle in a pen that are shedding O157 in their manure) would be kept below 20 percent. Though preliminary, these criteria are apparently the first statistically sound targets for the development and testing of a feedlot intervention.

"When you have a supershedder or more than 20 percent of the animals shedding O157 in their manure," Arthur says, "you have a dramatic increase in the number of hides contaminated with manure-borne *E. coli* O157. Hide contamination is typically 80 percent or higher in those pens.

"It may seem surprising that having 20 percent of the herd shedding O157 at low levels, or one animal supershedding, could lead to having 80 percent of the hides contaminated with O157. But cattle tend to congregate, and that promotes contamination.

"Right now, it's difficult to say what's 'normal' in terms of the number of cattle in a pen that shed O157. We've seen anywhere from 0 to 100 percent, and we're trying to define factors that are responsible for the differences."

Arthur's group of current and former ARS scientists, including Clay Center researchers Mick Bosilevac, Jim Bono, Dayna Brichta-Harhay, Norasak Kalchayanand, Mohammad Koohmaraie, John Schmidt, Steven Shackelford, and Tommy Wheeler, has documented these findings in peer-reviewed scientific articles published in 2009 and 2013 in *Applied and Environmental Microbiology*. ARS and the Beef Checkoff helped fund the research.

Other Aspects Also Probed

Related work at Clay Center, conducted by researchers Jim Bono and Jim Wells, along with colleague Andy Benson of the University of Nebraska-Lincoln and others, may fill in other pieces of the supershedder puzzle. In particular, their research may help answer the question of why some cattle are supershedders while others are not.

In one set of studies, these scientists are inventorying and comparing the microbes that live in the gastrointestinal tract of supershedders with those dwelling in non-supershedders. This work may provide clues about whether some microbial species and strains help O157 flourish or, conversely, whether some "beneficial" species outcompete and suppress it. Such data may be useful in developing approaches to help the beneficial strains proliferate in cattle.

In another line of inquiry, geneticists Larry Kuehn and Warren Snelling are scrutinizing the genetic makeup of supershedders to determine whether supershedding is a gene-controlled trait. If it is, it may be possible to breed the trait out of tomorrow's beef cattle herds and help end this troublesome phenomenon.—By **Marcia Wood,** ARS.

In Clay Center, Nebraska, technician Frank Reno collects swab samples from cattle hides to test for *E. coli* O157:H7.

TERRY ARTHUR (D3138-1)

This research is part of Food Safety, an ARS national program (#108) described at www.nps.ars.usda.gov.

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SANGHOON KIM (D3151-1)

Transparent Coating for Glass, Plastics—and More—May Have Bright Future

A nanoparticle-based coating from ARS, applied to the right half of this plastic container, prevents water from beading and reducing visibility.

When rain falls on a glass window or a plastic skylight cover, it often forms beads. That's because these surfaces typically are hydrophobic, meaning they repel water.

But a transparent, nanoparticle-based coating developed by Agricultural Research Service scientists can quickly change surfaces from hydrophobic to hydrophilic, so that rain doesn't bead up, and your visibility isn't impaired.

When raindrops come in contact with the spray-on coating, they spread, instead of beading, explains chemist Sanghoon Kim, who headed the nanoparticle studies.

Besides its potential use on windows, the coating might also be applied to solar panels, to help keep dirt from interfering with their performance.

What's more, Kim and colleagues have observed that the coating works well on other materials, including Plexiglas and metals such as stainless steel.

Kim, chemist Atanu Biswas, and physical scientist Kervin Evans—all at the ARS National Center for Agricultural Utilization Research in Peoria, Illinois—created the coating's nanoparticles using only a few off-the-shelf laboratory chemicals, including an ag-sourced protein.

From start to finish, production of the nanoparticles takes less than an hour, involves simple procedures with inexpensive chemicals, and doesn't require specialized equipment or costly heating.

In a proof-of-concept experiment, the researchers used bovine serum albumin as their protein and ethyl cyanoacrylate as the starting material that's key to creating the nanoparticles. Bovine serum albumin is a cattle industry coproduct. Ethyl cyanoacrylate is a major component of what's commonly known as "super glue."

Simply stated, the nanoparticles were formed when ethyl cyanoacrylate reacted with bovine serum albumin in a solution of acidified ethanol. Spinning this mixture in a centrifuge removed any byproducts of the reaction, resulting in nanoparticles suspended in the solution.

Applying the coating is quick and easy. All that's needed is to spray it onto clean glass or other recommended surface, then rinse with water.

Kim, Biswas, and Evans documented their results in a peer-reviewed scientific article published in 2013 in *Colloids and Surfaces B: Biointerfaces.*

In a followup study, Kim and colleague Yeon Seok Kim, of the National Institute of Standards and Technology, used gliadin—a protein from wheat—to demonstrate that their process is applicable to both plant- and animal-derived proteins. An article published this year in the *Journal of Nanoparticle Research* has details.

Typically, plant proteins are less expensive than those from animal sources. Regardless of which agricultural source is chosen, informal cost estimates suggest that the new coating could be made at the same or less cost than hydrophilic coatings already on the market.

The fact that the Peoria coating is rain-ready in about a minute apparently makes it unique. ARS is seeking a patent for the research, and the scientists are looking for industry partners, such as manufacturers of window-cleaning products, to commercialize the invention.—By **Marcia Wood,** ARS.

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

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Foraging Worms Guarantee Slug Bait Failure

WILLIAM E. GAVIN (D3144-1)

Pacific Northwest producers use slug bait to control grey field slugs like these, but the bait is often consumed by earthworms instead.

Grey field slugs in the Pacific Northwest used to be kept in check by practices such as conventional tilling and burning postharvest straw residues. But many producers are now using no-till management and leaving leftover straw on the fields to enhance soil quality and prevent erosion. The downside to these sustainable practices is that they create a much more hospitable environment for slugs, which have become an agronomic nightmare.

"They'll eat anything," says Agricultural Research Service agronomist George Mueller-Warrant. "In the middle of the night, the ground is covered with them."

These nocturnal mollusks, *Deroceras reticulatum,* are such pests that in western Oregon, producers of grass seed, wheat, and other field crops spend around $3.7 million every year for around 2.9 million pounds of slug bait. The bait is often in the form of metaldehyde—either as pellets, granules, or liquid—or iron phosphate pellets.

However, earthworms have also flourished under the no-till, no-burn agricultural regime. Foraging at night, they regularly make a meal of the slug bait with no apparent ill effects.

So Mueller-Warrant worked with ARS plant physiologist Steve Griffith and research leader Gary Banowetz to determine how much—and how quickly—slug bait was being lost to worms. All three scientists work in the ARS Forage Seed and Cereal Research Unit in Corvallis, Oregon.

The researchers treated 15 grass seed production fields and greenhouses with different types of slug bait and then spent 4 hours every night observing the foraging behavior of the worms. Averaged over all observations, half of the bait pellets disappeared in less than 2½ days.

The worms approached bait pellets the same way they approached all other potential food sources, and 20 percent of the time they ate it on the spot. But they usually took the pellet back into their burrows, sometimes at the rate of 3 every hour. The scientists noted that the worms strongly preferred the metaldehyde or iron pellets to other forms of the bait (liquid or granular).

The research team also surveyed earthworm populations but did not find any significant correlation between population density and rate of bait disappearance: At all observation sites, all of the bait disappeared by 7 or 8 days after it had been applied. The team's calculations indicated that earthworm population densities everywhere were more than sufficient to remove all slug bait.

Oregon producers currently apply around 10 pounds of slug bait per acre 2 to 5 times a year to more than 185,000 acres of grass seed fields. But since much of the bait in the less-expensive pellet form is quickly consumed by worms, the scientists suggest that in fields of high-value crops, it might be more cost effective to apply fewer rounds of the more expensive granular and liquid bait, which the worms typically ignore.—By **Ann Perry,** ARS.

This research is part of Agricultural System Competitiveness and Sustainability, an ARS national program (#216) described at www.nps.ars.usda.gov.

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Dissecting a Potato Pathogen's Hiding Place in Its Insect Vector

Researchers are leaving no stone unturned when it comes to finding new ways of managing zebra chip disease and the insect that can spread it to potato crops.

One scientist, Agricultural Research Service entomologist Rodney Cooper, is using fine-tipped forceps and a fluorescent microscope to study the organs and tissues of the potato psyllid, a tiny, cicada-like insect that can transmit the bacterium *Candidatus* Liberibacter solanacearum, the cause of zebra chip.

Cooper combines the procedure with fluorescent genetic markers to understand where and how Liberibacter survives in the psyllid—from the moment it is ingested by the insect pest to the time it is injected into new plants.

"Our understanding of the basic biology and ecology of this pathogen, as well as its interactions with the insect vector and host plant, is incomplete," says Cooper, who is in ARS's Fruit and Vegetable Insect Research Unit in Wapato, Washington. Filling this information gap will have wide-ranging benefits, he adds—from improved experimental designs and data interpretation to better decisionmaking on how best to detect, control, and prevent zebra chip.

The disease is named for the dark bands it causes inside tubers. The bands become more pronounced once the potatoes are cut and fried to make products such as chips. Other symptoms include curled-in leaves and tissue discoloration. Zebra chip poses no consumer danger, but it affects tuber appearance and taste and can diminish marketability at a cost to producers and processors.

JOSEPH MUNYANEZA (D3165-1)

Zebra chip symptoms in potato slices from infected tubers in uncooked (left) and fried chips (right). The disease poses no consumer danger, but it affects potato taste, appearance, and marketability.

The primary disease-control method is to spray potato crops with psyllid-killing insecticides, but researchers hope to provide growers with more sustainable approaches, particularly resistant varieties. In addition to expediting such efforts, information from Cooper's psyllid dissections may eventually set the stage for targeting Liberibacter directly.

Once a psyllid specimen is dissected, its excised organs and tissues are subjected to "fluorescent in situ hybridization."

JOSEPH MUNYANEZA (D3164-1)

A potato psyllid nymph is yellow-green, flat, sedentary, and usually found on the underside of leaves. Potato psyllids transmit the bacterium that causes zebra chip disease in potatoes.

This procedure uses special probes to bind to complementary segments of bacterial DNA, which, if present, glows (fluoresces) green. Using this microscope-aided method, Cooper and colleagues have observed Liberibacter in four main areas of the psyllid: the gut, hemolymph (blood), bacteriomes (organs where symbiotic bacteria reside), and salivary glands (where it gets injected into plants during psyllid feeding).

Of the organs and tissues examined, Liberibacter appeared most often in the psyllid's gut. Indeed, bacteria fluoresced green in the guts of 66 percent of psyllid specimens examined. It was also found in 40 percent of salivary glands and bacteriomes.

The pest's nymph stage proved less hospitable to Liberibacter, which rarely appeared in organs other than the gut, the researchers observed.

"This may represent a bottleneck or barrier in the infection route, which could be manipulated and extended into the adult stage. If the adults don't transmit Liberibacter, then they're less of a threat," says Cooper, who, along with coauthors Joseph Munyaneza and Venkatesan Sengoda, published the work in the *Annals of the Entomological Society of America,* in January 2014.—By **Jan Suszkiw,** ARS.

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

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Advance May Speed Development of Seed Rot-Resistant Soybeans

CROP GENETIC RESEARCH UNIT (D3155-1)

GENETIC RESEARCH UNIT (D3156-1)

Left: Using a fluorescence microscope, plant pathologist Shuxian Li observes the pathogen *Phomopsis longicolla* that causes *Phomopsis* seed decay of soybean. Above: Healthy soybean seeds (top) and seeds infected by *P. longicolla*.

Soybeans have been called the "wonder crop" for all the products that can be made from the versatile legume—including cooking oil, tofu, livestock feed, and biodiesel, to name just a few. But America's second-largest field crop next to corn is no match against *Phomopsis longicolla*, a seed rot fungus, which, along with other species, cost soybean producers in 16 southern states over 2 million bushels in losses in 2012.

Applying fungicides, rotating soybeans with nonhost crops, and tilling the soil are among strategies used by growers to prevent *P. longicolla* from causing *Phomopsis* seed decay (PSD), a disease which degrades the seed and reduces the quality of the protein and oil constituents. "Breeding for resistance to PSD is the most effective long-term strategy," notes Shuxian Li, a plant pathologist in the Agricultural Research Service's Crop Genetics Research Unit in Stoneville, Mississippi.

As part of a *Phomopsis* resistance program there, Li has sought to learn more about how the fungus inflicts harm at the cellular level. Towards that end, she and colleagues enlisted the aid of *Agrobacterium tumefaciens,* a soil bacterium commonly used in genetic engineering procedures to endow plants with new traits. In this instance, the team used the bacterium to "shuttle" genes for an antibiotic marker and green fluorescent protein (GFP) into the nuclei of the fungus's cells. This resulted in new *P. longicolla* strains that produce the protein and emit a green glow when exposed to light in the blue-to-ultraviolet range.

"Green fluorescent protein is amazingly useful in scientific research, because it allows researchers to look directly into the inner workings of cells," says Li, who collaborated with Burton Bluhm and others at the University of Arkansas in Fayetteville. "Using this transformation method, we can monitor how the fungus infects plants."

The researchers compared the characteristics of seven GFP-modified strains to an unmodified "parent" isolate, and they confirmed the presence of the protein by using a molecular test method called "Southern blot analysis" and by direct observation with a confocal laser scanning microscope. A paper published in the March 2013 issue of the *Journal of Microbiological Methods* describes their research in detail.

Li plans on inoculating soybean seedlings with the modified strains to study how the infection process unfolds within the tissues of both resistant and susceptible soybean germplasm lines. She expects use of the GFP-expressing strains will also help to identify sources of PSD resistance that may escape detection using conventional disease-screening methods, such as field observation or visual assessment of seed on culture medium in the laboratory.

With the recent sequencing of *P. longicolla's* entire genomic code, use of the transformed strains will allow researchers to probe the function of specific genes for virulence directly in soybean plants, potentially unlocking new clues to protecting this important crop. Use of the soybeans with GFP, however, will be limited to scientific research.—By **Jan Suszkiw,** ARS.

This research is part of Plant Genetic Resources, Genomics, and Genetic Improvement (#301) and Plant Diseases (#303), two ARS national programs described at www.nps.ars.usda.gov.

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Taming Extreme Environments by Exploring Algae in Space

EUROPEAN SPACE AGENCY (D3140-1)

In Kazakhstan, not far from the Russian border, the Foton-M2 capsule is back on Earth after more than 2 weeks in space.

It may sound like the opening scene in a low-budget science fiction movie: Scientists send algae into space—some of it mutant—to see if it will grow. But an Agricultural Research Service scientist and an international team of researchers have in fact sent algae into a low Earth orbit to study the effects of space on photosynthesis and plant growth. The research, funded in part by the European Space Agency, is part of an effort to find new ways to produce food and biofuels in extreme environments.

Autar Mattoo, a plant physiologist with ARS's Sustainable Agricultural Systems Laboratory in Beltsville, Maryland, and his colleagues placed samples of the alga *Chlamydomonas reinhardtii* in airtight "photo cells" and had them launched in a Russian-made Soyuz space capsule from the Baikonur Cosmodrome in Kazakhstan.

The *C. reinhardtii,* often studied as a model for photosynthesis, spent 15 days in orbit getting doses of cosmic radiation while under light and temperature conditions that would ensure growth on earth. Four mutants of *C. reinhardtii* with alterations in an important gene were also sent up. Mattoo and his colleagues from the National Research Council of Italy, based in Rome, and Martin-Luther University in Wittenberg, Germany, exposed the same control and mutant algae to similar conditions in an Earth-based laboratory to compare results.

During photosynthesis in normal conditions, a protein-pigment complex known as "Photosystem II" (PS II) must constantly be repaired to fix damage caused by sunlight and ultraviolet radiation. As part of that repair process, a protein known as "D1" is continuously being replaced. Research has shown that mutations of the D1 protein in the PS II complex can either increase or decrease photosynthetic activity.

With this study, the researchers wanted to assess the effects of microgravity, cosmic rays, high-energy particles, and the ionizing radiation of space on the PS II complex, photosynthesis, and plant growth. They also wanted to see if the effects would differ in a simple model for photosynthesis, an alga, with the D1 gene altered in specific ways.

The scientists' goal is to engineer critical components of photosynthetic machinery to increase their efficiency and stability

MARIA TERESA GIARDI (D3143-1)

At the Institute of Crystallography in Rome, Italy, Giuseppina Rea selects algae cultures for the space flight.

EUROPEAN SPACE AGENCY (D3141-1)

MARIA TERESA GIARDI (D3142-1)

Above: At the Baikonur Cosmodrome in Kazakhstan, the Soyuz rocket is being prepared for launch. The Foton-M2 capsule containing the mutant algae samples is held in the green nose of the rocket. Right: Project coordinator Maria Teresa Giardi and fellow engineers hold the experiment materials before they are loaded into the capsule.

so that one day crops will produce higher yields and grow under extreme environments. The work is considered vital to meeting future demands for crop productivity and biofuels.

They found that some aspect of the space environment inhibited the ability of the control *C. reinhardtii* and two of the four mutant strains to photosynthesize and grow, both in space and later when they were brought back to Earth. However, two other mutant strains flourished, both in space and when they returned. The results, published in *PLOS One* in May 2013, highlight the importance of the D1 protein both in photosynthesis and as a target of environmental signals.

Future research will focus on developing D1 mutants of *C. reinhardtii* with enhanced stability and greater capacities for photosynthesis and growth in extreme environments.—By **Dennis O'Brien,** ARS.

The research is part of Plant Genetic Resources, Genomics, and Genetic Improvement, an ARS national program (#301) described at www.nps.ars.usda.gov.

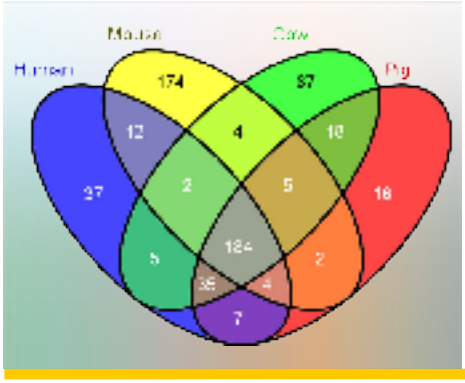
*Autar Mattoo is with the USDA-ARS Sustainable Agricultural Systems Laboratory, 10300 Baltimore Ave., Beltsville, MD 20705-2350; (301) 504-6622, autar.mattoo@ars.usda.gov.**

STEPHEN AUSMUS (D3153-7)

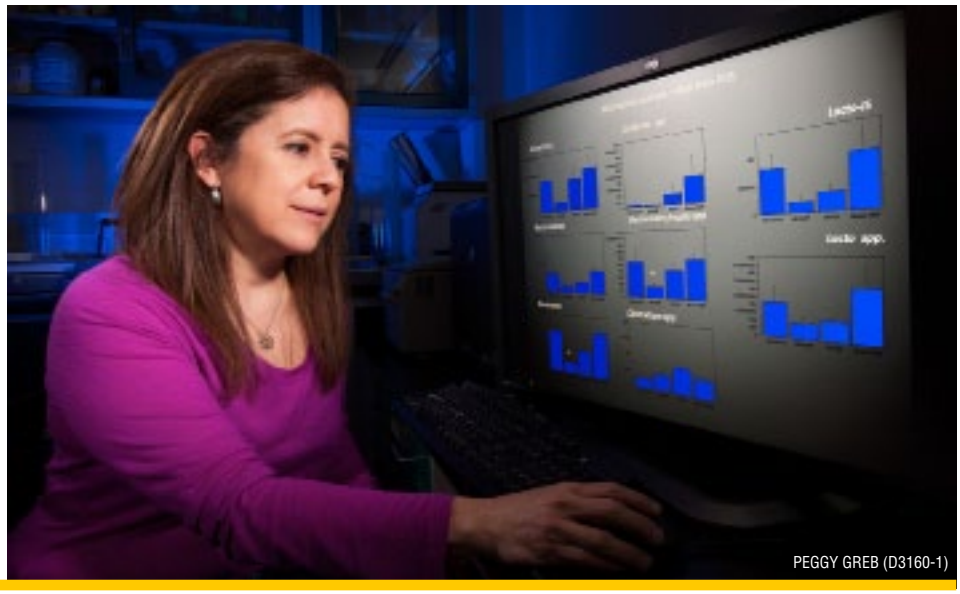
Plant physiologist Autar Mattoo analyzes data collected on algae during the space flight. The screen on the right shows the crystal structure of the D1 protein with mutation sites.

Pigs Useful in Immune and Obesity Research

HARRY DAWSON (D3162-1)



By comparing the number of shared immune response genes among humans, pigs, mice, and cows, ARS scientists discovered a high degree of similarity between the immune responses of pigs and humans.



PEGGY GREB (D3160-1)

Microbiologist Gloria Solano-Aguilar evaluates intestinal bacterial data from pigs consuming high-fat and low-fat diets. This data is helping researchers understand obesity in pigs, which can be a model for humans.

Nutritionist Harry Dawson and microbiologist Gloria Solano-Aguilar, both scientists at the Agricultural Research Service's Beltsville [Maryland] Human Nutrition Research Center (BHNRC), have teamed with scientists from ARS and other organizations to use the pig as an animal model to promote both human and animal health. This research focuses on assessing the effect of nutrition on immune and inflammatory responses.

Dawson helped develop and continues to curate the publicly available Porcine Translational Research Database of genes and proteins for comparison with those prominently studied in rodents and humans. "This database contains functional information on more than 5,800 genes commonly studied in humans, pigs, and mice, including about 2,240 that have been sequenced at BHNRC." The database can be found at tinyurl.com/porcinedata.

The database contains "manually annotated" genes, meaning that all genes and protein sequences included in the database, as well as information about their functions, were manually entered. Annotated genes can also be entered by computer software programs that predict the structure and identity of genes and proteins based on algorithms.

"These computer programs, while fast, are prone to error that can be corrected only by manual annotation," says Dawson.

Immune System Similarities

In addition, Dawson conducted a comparative analysis and assessment of specific portions of the swine, mouse, and human genomes. He found that humans share far more immune-system-related genes and proteins with pigs than they do with mice. He reported that when a functional part of a protein is missing among one of the three species, the chance that it is preserved only in pigs and humans is nearly two times greater than the chance it is preserved only in mice and humans. Dawson's book chapter, "A Comparative Assessment of the Pig, Mouse, and Human Genomes," was published by CRC Press in 2011 in *The Minipig in Biomedical Research.*

The first complete pig genome sequence, version Sscrofa 10.2, was released by the Swine Genome Sequencing Consortium in 2012 (see box). As part of that effort, a subgroup called the "Immune Response Annotation Group" annotated more than 1,400 swine genes involved in the animal's immune response. This group included ARS's Dawson, molecular biologist Celine Chen, chemist Joan Lunney, and others.

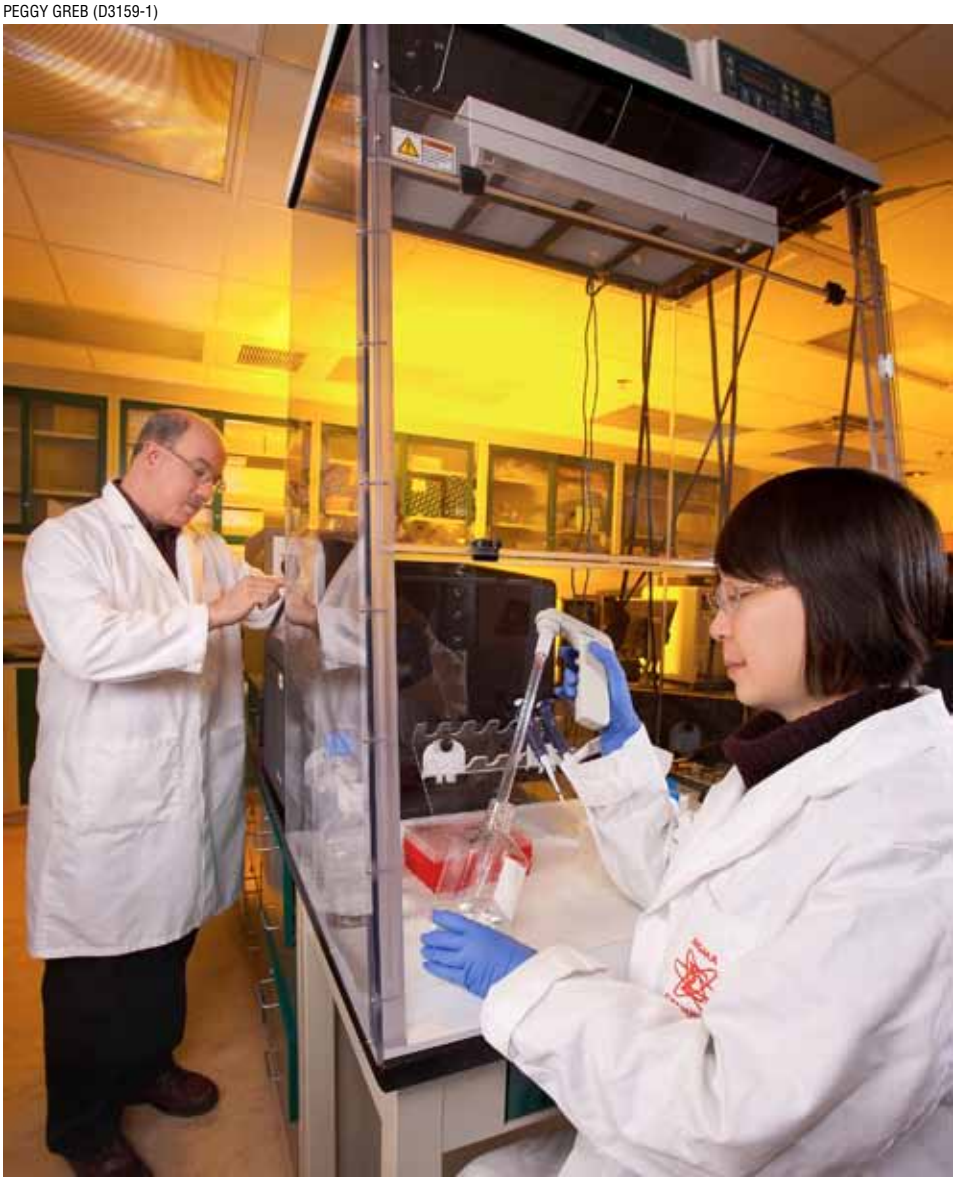
The group discovered that the immunity genes of pigs and humans are very similar and evolve at a similar rate in both species. These findings were reported in the journal *Nature* in 2012.

Later, the group published a study that further characterized the structure and function of the porcine immunome. An immunome is a collection, or reference set, of immune-system-related genes and proteins of a given species. The group provided new immune-response annotations for more than 500 porcine genes and 3,472 protein-coding transcripts.

"The porcine genome is not yet complete, and additional genes may be discovered," says Dawson. "But these comprehensive and integrated analyses provide important tools for measuring the porcine immune response." The findings were published in *BMC Genomics* in 2013.

These comparative studies provide compelling evidence for using swine in research on both human and animal health, says Dawson. "These studies indicate that pigs are a good species to further test concepts and principles that have been discovered by first using mice as a model, particularly for immune-response research."

PEGGY GREB (D3159-1)



Nutritionist Harry Dawson (left) checks the performance of the DNA sequencer, while molecular biologist Celine Chen prepares samples of pig macrophages (immune cells) for sequencing.

The First Swine Genome Assembly

If you overheard scientists talking about the genome sequence of an organism, you might think they were talking about software programs. The first "genome assembly" released for a given species gets a release date. Subsequent assemblies for the same species get a new name and a higher number.

The human genome version h37 was released in 2009. The mouse genome version m38 was released in 2011. And the first pig genome version, Sscrofa 10.2, was released in 2012 by an international coalition of researchers called the "Swine Genome Sequencing Consortium." The project was supported in part by a USDA grant and by Agricultural Research Service scientists headed by animal geneticist Gary Rohrer at the U.S. Meat Animal Research Center in Clay Center, Nebraska.

Obesity Research Goes to the Hogs

Also at the BHNRC, Solano-Aguilar has worked on a series of studies showing that the pig is instrumental as a model for human obesity-related research. She worked with Kati Hanhineva, of the University of Eastern Finland in Kuopio, to study metabolic changes that occur in pig tissues and biofluids after the pigs consumed a high-fat diet.

The researchers studied the Ossabaw pig because it has a greater tendency to deposit excess fat and develop obesity-related diseases when fed a high-calorie diet, compared to other pig breeds. The emphasis was on using juvenile pigs as a model for obesity in children. "This is an important area because it is generally difficult to evaluate obesity-related metabolic disturbances in children," says Solano-Aguilar.

The authors wanted to study diet-induced metabolic changes taking place in the tissues they collected from the pigs—liver, pancreas, brain, and intestine. And they wanted to compare whether the changes they found in the tissues were also present in the pig's urine and plasma—biofluids that are typically collected during human clinical studies.

The study pigs were fed either a maintenance diet or a high-fat diet. The researchers found changes in lipid metabolites in all analyzed host tissue samples from the pigs fed the high-fat diet. Some tissue-dependent changes were not reflected in the biofluids.

Using swine as a biomedical research model was useful for studying metabolic effects induced by a high-fat diet, says Joseph Urban, a coauthor at the BHNRC laboratory who initiated a multi-institute cooperative agreement with the Finnish scientists.

"Biofluids give us part of the picture," Urban says, "but being able to look at organ tissue helped us target changes that are indicative of both disease and poor response to diet." The study was published in the *Journal of Proteome Research* in 2013.—By **Rosalie Marion Bliss,** ARS.

This research is part of Human Nutrition (#107) and Animal Health (#103), two ARS national programs described at www.nps.ars.usda.gov.

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Controlling Odors and Greenhouse Gas Emissions From Stored Swine Manure

Storing and reusing swine manure for fertilizer makes good environmental and economic sense, but managing foul-smelling manure is probably one of the least favorite tasks on a producer's to-do list. Now, findings by Agricultural Research Service microbiologists Terry Whitehead and Mike Cotta may someday give farmers options for odor control that will protect livestock health and help restore harmony between rural producers and nearby residents.

Odor from swine manure contains hundreds of compounds, so it's difficult to find the specific ones that are the main culprits contributing to the bad smell. These compounds are made by microbes that break down undigested feed and other materials in the manure. Scientists have determined that one group of these microbes, sulfate-reducing bacteria, produces hydrogen sulfide. Hydrogen sulfide and other sulfur compounds make up about half of the offensive odorants from swine manure.

But manure generates more than just a distinctive odor. Bacterial activity in manure pits also generates methane and nitrous oxide, which are both greenhouse gases.

Research conducted by scientists elsewhere has indicated that tannins—compounds naturally present in tree leaves and other feed materials—can block bacterial activity in the guts of ruminant livestock such as dairy cows, which in turn lowers digestive efficiency and effectiveness. To Whitehead and Cotta, who are in the ARS Bioenergy Research Unit in Peoria, Illinois, this observation suggested that perhaps the tannins could be an effective agent for controlling the compounds that produce odor associated with manure.

The scientists conducted a laboratory study to evaluate the effectiveness of tannins from the quebracho tree, which have been used in other studies of ruminant digestion, in reducing the emission of odors and greenhouse gases from swine manure pits. The scientists collected swine manure from a local producer and incubated it under laboratory conditions that mimic on-farm conditions. This allowed them to test the effects of adding tannins to the manure by monitoring gas emissions. They also determined the levels of sulfate-reducing bacteria that typically flourish in that environment.

Seven days after the researchers added quebracho tannins to the manure, they found that hydrogen sulfide and methane production had been reduced more than 90 percent and that production continued to dwindle for another 3 weeks. Sulfate-reducing bacteria populations also significantly declined, by 70 to 90 percent, in the tannin-enriched mix.

Larger-scale testing at swine facilities is needed to determine whether the tannins have similar rates of efficacy in commercial swine production facilities. If these findings are replicated in field-scale studies, quebracho tannins could be used in manure pits to reduce the activity of sulfate-reducing bacteria and the amounts of hydrogen sulfide and methane. The benefits of this approach would be twofold: Producers would have a cost-effective way of mitigating odors and greenhouse gas emissions, and when the manure is eventually spread onto the fields as fertilizer, the added tannins would not pose a risk to the environment.

Whitehead and Cotta published their results in the December 2012 issue of *Applied Microbiology and Biotechnology*.—By **Ann Perry,** ARS.

KEITH WELLER (K8771-17)

In a swine facility near Peoria, Illinois, microbiologist Terry Whitehead collects fresh manure samples to use in tests to identify bacteria that may be involved in odor production.

This research is part of Agricultural and Industrial Byproducts, an ARS national program (#214) described at www.nps.ars.usda.gov.

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New Class of Animal Deworming Agent

A team of researchers has demonstrated that when a bacteria-derived protein was fed to worm-infected swine, the infection was nearly completely eliminated.

Intestinal parasitic roundworms are host-species specific and are found in pigs, cats, dogs, humans, and other animals. A roundworm specific to humans, *Ascaris lumbricoides,* is rarely seen in developed countries. Still, about 1 billion people are infected with this species of worm worldwide, usually via some contact with feces. When *A. lumbricoides* infects children, nutrient deficiency, respiratory distress, stunted growth, and immune defects occur. In the extreme, infection can cause life-threatening gut blockage. Worms can also migrate into the bile ducts and the permeable membrane that covers the abdominal organs.

The parasitic roundworm that commonly infects pigs is *Ascaris suum,* which is so genetically similar to *A. lumbricoides* that some evidence suggests they are the same species. *A. suum* infection in pigs is considered a good model for *A. lumbricoides* infection in humans because of its similar migration through the body and to the intestines.

The research team included microbiologist Joseph Urban and his colleagues at the Agricultural Research Service's Beltsville [Maryland] Human Nutrition Research Center and Raffi Aroian and Yan Hu at University of California-San Diego.

In its experiments, the team used a crystal protein called "Cry5B," provided by Aroian's group, which is derived from the soil bacterium *Bacillus thuringiensis.* Cry5B protein is considered nontoxic to vertebrates and mammals, but it has been used in the past as an insecticide. *A. suum* genetically expresses receptors for Cry5B.

Previously, Cry5B had been shown by Aroian and colleagues to be toxic to hookworms. In laboratory tests, Cry5B triggered activation of stress-response pathways in *Ascaris* larvae and adults similar to that observed with other worms.

"Feeding two moderate doses of Cry5B to pigs resulted in nearly complete elimination of intestinal *A. suum* infection, and all intestinal roundworm larvae were damaged or destroyed," says Urban. "The dosage we provided in this study is comparable to the dose range used in existing commercial antiparasitic drugs."

There is a need for more practical delivery systems for antiparasitic drug treatments, according to the scientists. Cry5B holds potential for use where worm resistance is becoming a problem, especially among ruminant livestock. The University of California researchers have filed a patent application on the protein expression, and further cooperative research with ARS is being planned.

"These results show the potential of Cry5B to treat *Ascaris* infections in pigs and other livestock and to work effectively in the human gastrointestinal tract," says Urban.

More information can be found in the article published in the June 2013 issue of *PLOS: Neglected Tropical Diseases*.—By **Rosalie Marion Bliss,** ARS.

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

Joseph Urban is with the USDA-ARS Diet, Genomics, and Immunology Laboratory, Beltsville Human Nutrition Research Center, 10300 Baltimore Ave., Beltsville, MD 20705-2350; joe.urban@ars.usda.gov, (301) 504-5528, ext. 267.

STEPHEN AUSMUS (D3150-1)

Animal caretaker Andy Covell (left) and microbiologist Joseph Urban orally inoculate a piglet in a study to eliminate infection from the parasitic roundworm *Ascaris suum.*

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

Corvallis, Oregon
3 research units ■ 95 employees

Yakima Agricultural Research Laboratory, Wapato, Washington
1 research unit ■ 44 employees

Roman L. Hruska U.S. Meat Animal Research Center, Clay Center, Nebraska
4 research units ■ 118 employees

National Center for Agricultural Utilization Research, Peoria, Illinois
7 research units ■ 254 employees

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

Madison, Wisconsin
3 research units ■ 102 employees

Henry A. Wallace Beltsville Agricultural Research Center, Beltsville, Maryland
27 research units ■ 806 employees