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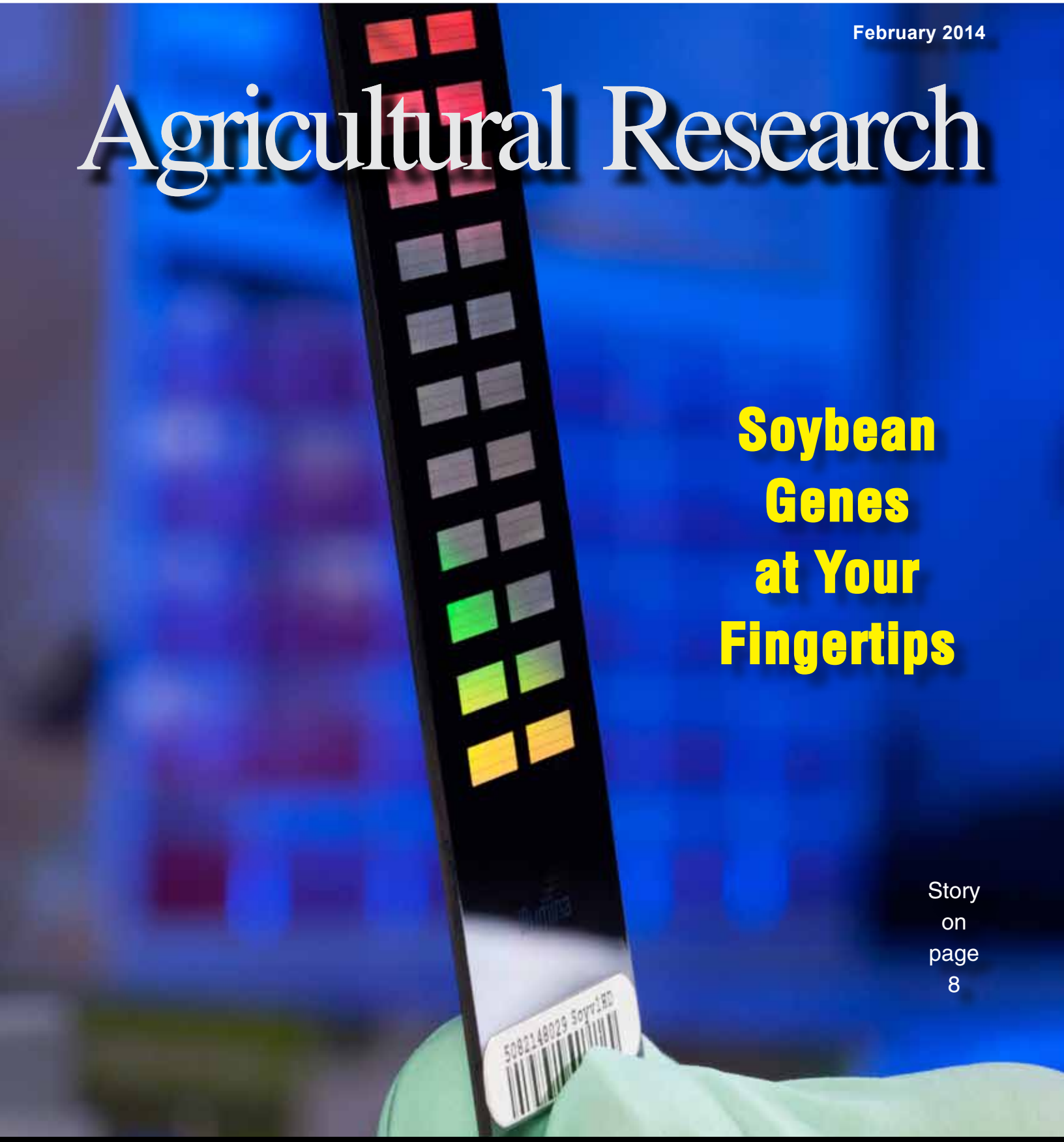

Soybean Genes at Your Fingertips

Story on page 8

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

FORUM

Building on the Basics About Soil Carbon

A few facts and figures about soil carbon:

•Plants pull carbon dioxide (CO_2) from the air during photosynthesis. Some of the carbon that becomes green plant material can make its way into the soil through the process of decay from soil microbes.

•There are around 720 million tons of carbon in our planet's terrestrial ecosystem, and around 352 million tons are in the form of organic soil carbon.

•Converting forests and grasslands to cropland and pasture over the last several hundred years has released 11 to 23 million tons of soil carbon into the atmosphere.

•U.S. farmers sequester about 11.2 million tons of CO_2 every year in the form of soil carbon by converting cropland back to grassland, increasing conservation tillage and continuous cropping, and improving organic fertilizer management.

Even better, strategies that increase CO_2 sequestration and protect soil carbon levels generally improve crop production efficiency as well, helping farmers meet production targets and protect air quality. So, depending on who you ask, the agronomic benefits from sequestering carbon in the soil might even trump the benefits of removing CO_2 from the atmosphere.

We've known for years that maintaining plant residues on the soil surface, adopting complex cropping systems that provide continuous ground cover, and applying carbon-rich amendments can add significant amounts of carbon to the soil. Yet we still had a lot of questions about soil carbon dynamics.

So in 2002, scientists from the Agricultural Research Service and outside collaborators established a national network of soil carbon research projects called "GRACEnet" (Greenhouse-Gas Reduction through Agricultural Carbon Enhancement Network). We developed this network to coordinate research projects at multiple ARS locations and determine the effects of management practices on soil carbon sequestration, trace gas emissions, and environmental quality. We also wanted to use this information to develop new management practices that reduce net greenhouse gas emissions and increase soil carbon sequestration.

Since GRACEnet was established, researchers have published more than 250 papers that have helped to fill in the blanks about how land-based agricultural practices affect greenhouse gas emissions and soil carbon dynamics. In 2012, many of GRACEnet's research highlights were published in a book called "Managing Agricultural Greenhouse Gases: Coordinated Agricultural Research through GRACEnet to Address our Changing Climate."

The book was edited by three ARS scientists—Mark Liebig, Alan Franzluebbers, and Ron Follett. Over100 ARS scientists contributed to it, as did collaborators from other government agencies, universities, and private industry.

Much of the content is about soil carbon, including how to measure it, how it is lost, and how it can be protected and replaced.

What have we found so far?

In the eastern United States, adopting no-till crop production can typically sequester around 450 pounds of carbon per acre per year.

In central U.S. agroecosystems, complex crop rotations can increase carbon soil sequestration.

In western U.S. dryland systems, soil carbon accumulates slowly, because the lower biomass yields mean that plants are adding less carbon to the soil. But adopting no-till management in this region increases the accumulation of surface crop residue and organic carbon.

Farmers working on intensely irrigated systems in the western United States could increase soil carbon levels by improving grazing regimes, fertilization practices, and irrigation management.

Rangelands typically have short periods of high carbon inputs to the soil during the growing season, followed by long periods of soil carbon balance or small losses for the rest of the year.

Improved pasture management—including plant composition, stocking rate, and stocking methods—could significantly increase soil carbon sequestration rates.

Overzealous harvesting of biomass feedstock—for instance, removing much or all of the postharvest corn stover from a field—may exacerbate a decline in soil carbon and water quality and accelerate soil erosion. Producers can compensate for these potential soil carbon losses by reducing or eliminating tillage, adding cover crops, and including or expanding the presence of perennial crops.

Changing from row crops to perennial "energy grasses," such as switchgrass, can sequester 440 to 670 pounds of carbon per acre per year.

The story on page 4 of this issue features the results of some long-term ARS studies of soil carbon dynamics in annual and perennial crops. The findings underscore how deeply we need to dig into the soil—and how long we need to track changes in the soil—to fully understand the many factors that promote soil carbon retention and loss.

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Natural Resources and Sustainable Agricultural Systems
Beltsville, Maryland

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PEGGY GREB (D2732-1)

Soil scientists Brian Wienhold (left) and Gary Varvel compare corncob residue in various stages of decomposition in a no-till field in Lincoln, Nebraska. In this study and several others, ARS researchers were surprised to learn how much carbon corn and switchgrass sequester in the soil between 12 inches and 5 feet deep. Story begins on page 4.

Cover: When soybeans were domesticated thousands of years ago, genes for important traits may have been lost. The genetic information contained on this glass slide, known as a "beadchip," may help scientists find useful genes so that they can be bred into cultivated soybeans. Story begins on page 8. Photo by Peggy Greb. (D3104-1)

A Surprising Supply

STEPHEN AUSMUS (D1540-2)

Soil scientist Ron Follett (right) examines a map of ARS soil carbon research sites across the United States while physical science technician Ed Buenger conducts mass spectrophotometer analysis of soil samples for carbon and nitrogen.

For years, many agronomists believed that significant levels of soil carbon only accumulated near the soil surface. So when four Agricultural Research Service scientists submitted a research paper claiming that large amounts of soil carbon were sequestered as deeply as 5 feet in the soil profile—and by annual as well as perennial crops—they had some trouble getting their paper through the review process.

The study was a 9-year project that evaluated the effects of nitrogen fertilizer and harvest treatments on soil organic carbon sequestration in switchgrass and no-till maize crops managed for biofeedstock production.

"Soil organic carbon sequestration has a major impact on the long-term sustainability of bioenergy crop production because it can significantly affect soil fertility and greenhouse gas emissions," says ARS geneticist Ken Vogel (retired). "So using accurate sequestration rates is essential in developing life-cycle analyses that assess the long-term environmental costs and benefits of biofuel crop production."

Vogel, soil scientists Ron Follett (retired) and Gary Varvel, and agronomist Rob Mitchell conducted their study on marginally productive fields similar to the croplands that would be suitable for commercial switchgrass production. Mitchell and Varvel are with ARS research units in Lincoln, Nebraska. Follett was with ARS in Fort Collins, Colorado.

The team established large plots that could accommodate field-scale equipment and took baseline soil samples to a depth of 5 feet before the first crops were cultivated. These baseline samples showed that soil organic carbon levels varied within the first foot of the subsoil by as much as around 18 tons per acre, while soil carbon levels 5 feet below the soil surface varied by as much as almost 90 tons per acre.

Soil Carbon Stores: Annual vs. Perennial

To explore this phenomenon, the scientists then planted two switchgrass cultivars and no-till maize and applied nitrogen fertilizers at three different rates ranging from 54 pounds per acre to around 160 pounds per acre. Nitrogen fertilizers support biomass production, and the scientists wanted to see if the production of more plant biomass resulted in sequestration of more carbon in the soil. Some switchgrass plots were also maintained without any nitrogen amendments.

Postharvest crop residue, or "stover"—which also contributes to soil carbon—was not removed on half of the no-till maize fields; on other fields, half of the stover was removed. After the crops were established, the researchers resampled soils in the production fields at 3-year intervals.

What was their biggest surprise? In the no-till maize field, soil organic carbon levels increased over time at all depths, with

# of Deep Soil Carbon

all nitrogen treatments, and with either type of postharvest stover management. Almost all increases were statistically significant. Maize grain yields were greatest from fields that had been amended with 107 pounds of nitrogen per acre and where no stover had been removed, a management strategy that resulted in an average annual increase in soil carbon that exceeded 0.9 tons per acre.

The researchers were equally surprised that more than 50 percent of the soil organic carbon was found at depths between 1 foot and 5 feet below the soil surface. This region of the soil profile, which typically has not been sampled by other researchers investigating carbon sequestration levels, is below the tillage zone and is therefore more stable over time.

In the switchgrass plots, the researchers also observed impressive increases of soil carbon sequestration throughout the soil profile. Sequestration rates increased as nitrogen fertilization rates increased, and almost all increases of soil carbon were statistically significant.

As they observed with the no-till maize plots, more than 50 percent of the soil carbon was found between 1 and 5 feet below the soil surface. The average annual increase of soil organic carbon throughout the first 5 feet of subsoil also exceeded 0.9 tons per acre per year, which was equivalent to 3.25 tons of carbon dioxide per acre per year.

"We had not expected to find these stores of deep soil carbon, even though we always knew plant roots reached this deep, because we didn't realize how much the activity around roots can affect soil carbon budgets," Follett says. "Most studies only sample soils for carbon to a depth of 18 inches."

Because of their findings, the team concluded that calculating soil carbon sequestration rates for bioenergy crops is not a one-size-fits-all proposition. Crop selection, soil differences, environmental conditions, and management practices affect sequestration rates differently from one region to another. As a result, bioenergy crop production models will probably need some major adjustments.

"Our work suggests that carbon sequestration rates used in current life-cycle analysis models for bioenergy crops are probably resulting in underestimates of how much carbon is being sequestered in the soil," says Vogel. "It also highlights how

In a biomass energy evaluation test, agronomist Rob Mitchell evaluates midsummer growth of various switchgrass strains.

PEGGY GREB (D2612-1)

nitrogen amendments and other management decisions do matter when it comes to corn and carbon sequestration—and that annual crops can make important contributions to soil carbon."

The paper was accepted by *Bioenergy Research* and published in 2012. But even though its results were so surprising, two other ARS studies had highlighted similar dynamics.

Annual Crops With Long-Lasting Effects

In 2011, results from a related long-term soil carbon study conducted by Varvel and his ARS colleague Wally Wilhelm (deceased) were published in *Soil & Tillage Research.* The researchers had studied soil carbon levels in fields established in 1980 for three different nonirrigated cropping systems—continuous corn, continuous soybean, and a soybean/corn rotation—that were managed with six different tillage systems.

In 1999, as part of the study, Varvel and Wilhelm collected soil samples from these fields at several intervals to a depth of 5 feet. They found that tillage management and crop selection independently affected soil nitrogen and carbon levels and that the highest levels of nitrogen and carbon had accumulated in the continuous-corn cropping system under no-till management. But as with the later study, the biggest surprise was how much nitrogen and carbon accumulated in the soil profile between 12 inches and 5 feet in all the cropping and tillage systems.

"When we collected these samples, many soil scientists believed that annual field crops don't sequester carbon in conventional tillage systems, so the results were a shock," says Varvel. "But conducting a long-term study allowed us to observe what happens with soil carbon sequestration once a management system is established and the year-to-year variations diminish." He also noted that identifying these deeper pools of carbon and nitrogen can help growers more effectively select tillage management that helps retain these nutrients in the soil.

These findings aligned with results from an 8-year study, published in 2013, that Follett conducted on carbon sequestration in no-till and conventional-till irrigated continuous corn systems near Fort Collins. He and Fort Collins soil scientist Ardell Halvorson found that no-till management resulted in higher levels of soil carbon than did conventional tillage and that those levels didn't change much over the 8 years.

The team measured carbon levels in the soil to a depth of 4 feet and found that the amount of longstanding soil carbon lost after conventional tillage was greater than the amount of carbon added to the soil from stover biomass. These results highlighted the need to learn more about older soil carbon pools deep in the soil profile and whether these carbon levels decline as a result of soil microbial activity, irrigation, or other processes.

"Some of the soil carbon in these soils is thousands of years old and is very stable, so its disappearance was a surprise," says Follett, who published the results in *Soil Science Society of America Journal.* "Regular irrigation of the typically semi-arid soil could be one of the factors that resulted in the carbon loss, but we would need to conduct additional studies to determine that."

Follett notes that the soil microbial groups in these environments still need to be identified, as do the environmental shifts that allow these microbes to more easily access the carbon for their own use. He also shares Varvel's belief that these findings underscore how farmers can use no-till management to conserve soil carbon deep in the soil profile—and the value of long-term studies for understanding soil carbon dynamics.

"It takes time for new management systems to have any effect on soil carbon. Identifying these effects can require long-term studies, sampling deeper within the soil profile, and using advanced measurement techniques," Follett says. "We're looking for little changes in a great big pool."—By **Ann Perry,** ARS.

This research is part of Pasture, Forage, and Rangeland Systems (#215), Bioenergy (#213), and Climate Change, Soils, and Emissions (#212), three ARS national programs described at www.nps.ars.usda.gov.

To reach scientists mentioned in this article, contact Ann Perry, USDA-ARS Information Staff, 5601 Sunnyside Ave., Beltsville, MD 20705-5128; (301) 504-1628, ann.perry@ars.usda.gov.

Soil scientist Ardell Halvorson (foreground) and technician Mary Smith prepare field samples for gas chromatograph analysis of carbon dioxide and methane.

STEPHEN AUSMUS (D1533-6)

Potatoes Show Promise for Meeting Climate Change Challenges

New research shows that potatoes—often cultivated as a rainfed crop with little or no irrigation—are still the go-to tuber when times get tough.

Agricultural Research Service agricultural engineer David Fleisher and colleagues wanted to measure how potato plants would respond to elevated atmospheric carbon dioxide (CO_2) levels and the increasingly erratic rainfall patterns expected to result from global climate change. So the team conducted two outdoor-chamber studies to evaluate effects of short-term drought cycles at current and elevated CO_2 levels. Fleisher and his research partners—plant physiologist Richard Sicher, soil scientist Dennis Timlin, research leader V.R. Reddy, and research associate Jinyoung Barnaby—all work at the ARS Crop Systems and Global Change Laboratory in Beltsville, Maryland.

The studies were conducted using soil-plant-atmosphere research chambers that provided precise control over CO_2 levels, air temperature, irrigation, and humidity. The chambers contained sensors that monitored air, soil, and canopy temperatures; relative humidity; and sunlight above and below the canopy.

In both studies, 11-day drought cycles were applied before tuber formation began and around 10 days after tuber formation began. The first study ran from May 28 to August 10, and the second study ran from August 11 to October 20. Having two different study periods allowed the scientists to evaluate how variations in sunlight during the drought periods affected plant response.

The researchers observed significant differences in plant response, which they attributed to the variation in sunlight. With all other growth factors being equal, the plants in the first study—when there was more sunlight—had a 30- to 200-percent increase in total potato production, depending on CO_2 levels and water availability.

STEPHEN AUSMUS (D1637-22)

Agricultural engineer David Fleisher studies water-stressed potato plants in a soil-plant-atmosphere research chamber that controls carbon dioxide and irrigation levels. Results from the study reveal how climate change affects potato plant growth.

The team also noted that the cyclic droughts resulted in lower levels of dry-matter and leaf-area production. They concluded that drought stress before tuber formation probably enhanced the future delivery of carbon, water, and plant nutrients to the tubers instead of to the stems or leaves—and that this response increased under elevated CO_2 levels. Averaged across all drought treatments, tuber yield from plants growing under elevated CO_2 levels was as much as 60 percent greater than that from plants growing under current CO_2 levels.

"We found that, except for the most severe droughts, tuber yields under elevated CO_2 levels exceed tuber yields under current CO_2 levels," Fleisher says. "This could be in part because plant water-use efficiency can increase under elevated CO_2 levels."

Fleisher emphasizes that this investigation was a chamber study and not a field study. But the results suggest that growers could adapt to changing atmospheric CO_2 levels by ensuring that potato plants are adequately irrigated during crucial pre-tuber development stages. He also thinks data from the study could be used to test crop models and other tools used to assess drought management strategies under elevated CO_2 conditions.

Results from this study were published in 2013 in *Agricultural and Forest Meteorology*.—By **Ann Perry,** ARS.

This research is part of Climate Change, Soils, and Emissions, an ARS national program (#212) described at www.nps.ars.usda.gov.

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New Tool for Improving Soybeans

For all that was gained when soybeans were domesticated thousands of years ago, scientists believe that something was lost: valuable genes left out of today's cultivated varieties that could make them more productive and better able to resist the pests and diseases that are constant threats. Perry Cregan, Qijian Song, and Charles Quigley, who are with the Agricultural Research Service's Soybean Genomics Laboratory in Beltsville, Maryland, have developed a tool to help in the search for regions of the soybean genome where those useful genes could still be found.

The tool is a glass chip with a difficult name: the SoySNP50K iSelect SNP BeadChip. It's about 3 inches long and has an etched surface that holds thousands of DNA markers, which can be deciphered by computers and used by scientists to characterize the genomes of large numbers of soybean plants.

If researchers at ARS and elsewhere scan enough soybean plants, they should be able to discover previously unknown genes associated with important characteristics. The chip will speed up those searches. Genetic information that once took days or weeks to collect can be gathered in about 20 minutes, Quigley says.

To create the chip, the group analyzed and compared the DNA of six cultivated and two wild soybean plants to identify single nucleotide polymorphisms (SNPs), a type of molecular marker often used by scientists working with DNA. They compared SNPs from the eight soybean plants with sequences of a cultivated soybean variety, Williams 82, and came up with thousands of candidate SNPs to use as signposts when comparing the genes of different soybean plants at thousands of positions along their chromosomes.

Ultimately, they distilled the candidates down to 60,800 SNPs and sent them to Illumina, Inc., the California technology firm that used the data to create the beadchip. Another team of ARS scientists worked with Illumina several years ago to create a chip for identifying genetic markers in cattle.

PEGGY GREB (D3104-1)

This SoySNP50K iSelect SNP beadchip has 24 etched rectangles, which hold hundreds of thousands of microbeads, allowing detection of more than 50,000 bits of genetic information from a soybean DNA sample.

The researchers have already used the soybean chip to profile 96 wild and 96 cultivated soybean varieties by comparing SNP alleles, or variant forms, at each of their 52,000 positions on the genome, as registered on the chip. The information allowed them to identify regions of the soybean's genome that played a key role in the plant's domestication. Results were published in a paper in *PLOS One* in January 2013.

They also used the chip to analyze the 18,484 cultivated soybean accessions and 1,168 wild soybean accessions in the USDA Soybean Germplasm Collection at Urbana, Illinois, and submitted the data to SoyBase, the USDA-ARS soybean genetics and genomics database, so it can be accessed by breeders and geneticists. The work received support from the grower-funded United Soybean Board Checkoff.—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.

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Geneticist Qijian Song (left), research leader Perry Cregan (center), and plant geneticist Charles Quigley observe the scanning of an Illumina BeadChip to complete the genetic analysis of soybean DNA samples from each of 24 soybeans with more than 50,000 DNA markers.

PEGGY GREB (D3103-1)

STEPHEN AUSMUS (D1417-21)

Test Strip Quickly Detects Botulism-Causing Toxins

This lateral flow device can provide positive identification of multiple forms of botulinum toxin in less than 20 minutes.

When someone mentions botulism, most people probably associate this nerve-damaging disease with eating food that was improperly cooked or incorrectly stored. That mode of natural contamination is still of concern. But botulism has also become a homeland security focus, because bioterrorists could use the toxins that cause botulism to make everyday foods and beverages deadly.

The toxins are produced by a common soil-dwelling bacterium, *Clostridium botulinum,* and several of its close relatives.

Now, a handy test strip that Agricultural Research Service molecular biologist Robert Hnasko and colleagues have developed may give homeland security and food safety officials a powerful tool to use against the toxins. When put to work as the basis of a field-ready test kit, the strip, which measures about a quarter-inch wide by 2.25 inches long, "can provide results in less than 20 minutes," says Hnasko. He is with ARS's Western Regional Research Center in Albany, California.

The test is well suited for rapid, preliminary screening in emergency situations, such as a bioterrorist threat or an outbreak of foodborne botulism in which the culprit food has not yet been pinpointed.

"In a crisis, emergency medical technicians or other first-responders could use the test to find out the two things they most need to know: Does the sample they're screening contain botulinum toxin and, if it does, is there enough toxin in it to kill someone?" Hnasko explains.

The strip fits snugly into a holder (technically a "lateral flow device") like those in home pregnancy test kits. Only a small amount of prepared sample is needed, and the results, shown on a color display, are easy to see and understand.

"You don't need any expensive lab equipment or specialized training to run this test," Hnasko says. "It's cheaper, faster, and easier to use than standard lab-based assays. If the situation calls for it, you can use those other tests for confirmation."

The strip is equipped with laboratory-built proteins, known as "monoclonal antibodies," that bind exclusively to A or B type (serotype) botulinum toxins. Together, these types are responsible for more than 80 percent of all cases of foodborne botulism in the United States.

ARS biologist Larry Stanker, also based at Albany, led the experiments that yielded the antibodies.

Using monoclonal antibodies in a lateral flow device to detect botulinum toxins isn't new. However, the test that Hnasko and co-investigators developed is likely the first of its kind that can concurrently detect—and differentiate—the A and B serotypes.

Studies documented in 2012 in the *Journal of Immunological Methods* demonstrated the strip's strong performance in detecting A and B toxins that the researchers had deliberately added to samples of milk, apple juice, and orange juice.

Hnasko and Stanker collaborated in the experiments with microbiologist Jeff McGarvey and technician Alice Lin, both at Albany, and with former Albany research associate Katie Ching.

The scientists are continuing to seek collaborations with test-kit developers and manufacturers to expand the test strip's food, medical, and homeland security applications.—By **Marcia Wood,** ARS.

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

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In Albany, California, molecular biologist Robert Hnasko (left) and biologist Larry Stanker examine newly produced lateral flow devices that can identify botulinum toxin serotypes.

ALICE LIN (D3098-1)

VAN-DEN TRUONG (D3099-1)

Taking Purées to a New Level

A brownie with about "half-the-usual" calories is making taste-testers purr. The secret ingredient: butternut squash, which has a naturally high sugar content and velvety texture. When added to chocolate, butternut squash purée is a nearly unidentifiable ingredient called a "replacer," which helps the confection compete with high-fat counterparts taste-wise.

"You can cut back on sugar, fat, and oils—which are more expensive and less nutritious—when you use naturally sweet vegetable purée," says Bill Heafy, "and you get a better nutrition label."

Heafy is operations sales manager at Yamco LLC, based in Snowhill, North Carolina. In 2007, Yamco licensed a unique process for making and packaging nutritious sweetpotato purée that needs no refrigeration while sealed. The process, which provides purée that is used in a variety of finished food products, was jointly patented by collaborators with the Agricultural Research Service on behalf of the U.S. Department of Agriculture, North Carolina State University (NC State) in Raleigh, and Industrial Microwave Systems, LLC, in Morrisville, North Carolina. Team members were awarded the Food Technology Industrial Achievement Award by the Institute of Food Technologists based in Chicago, Illinois, and a Technology Transfer Award by USDA-ARS.

ARS food scientist Van-Den Truong, with the Raleigh-based Food Science Research Unit, and collaborators tested the product extensively at an NC State pilot plant. Truong's collaborators include NC State food engineers Josip Simunovic, Gary Cartwright, K.P. Sandeep, and Ken Swartzel, and former graduate students Pablo Coronel, Prabhat Kumar, and Laurie Steed. The purée exhibits ideal nutrient and color retention with little flavor loss and is shelf-stable at room temperature prior to opening.

Responding to Market Demand

In 2009, Yamco began using the unique purée-making process to make pumpkin and butternut squash purée as well. "As a functional food, these purées are stellar because they can be used as a replacer, extender, and an ingredient," says Heafy. The purées are found in bakery goods, such as cookies and pies, soups, baby foods, nutraceuticals, and beverages, including beer. About 20 to 30 percent of Yamco's sweetpotato, pumpkin, and butternut squash purées, for example, are now sold to breweries.

In 2013, Yamco further amped up versatility for commercial buyers by offering 40-ounce (2.5-pound) and 60-ounce (3.75-pound) packages. Thanks to these new packaging options, the patented purée technology can now be used to process different vegetables for a wider range of customers.

"The capability to use the technology to make different kinds of vegetable purées has always been there," says Truong. "The success of the sweetpotato purée has driven market demand, and Yamco is responding to those demands."

Yamco is now making broccoli, carrot, and spinach purées, which can be used in entrees, soups, sides, and desserts. "We are selling these purées to schools and other foodservice operations, as well as restaurants, bakeries, baby-food companies, and more," says Bobby Ham, president of Yamco.

Another advancement is that the purée technology has gone international, with patents issued in Australia, Canada, China, and New Zealand in the past few years. In addition, the U.S. Agency for International Development—which works

ARS and North Carolina State University studied several varieties of purple-fleshed sweetpotatoes (left) and discovered that they have phytochemical nutrients, called anthocyanins, in amounts comparable to those found in grapes, plums, sweet cherries, eggplant, and red radishes.

to distribute commodity staples to people at risk of malnutrition in 100 developing countries—approved a proposal to use shelf-stable purée. "We are working with consultants to meet the special nutritional, packaging, and shipping requirement of the emergency feeding programs," says Ham.

Purple-Fleshed Sweetpotatoes Show Promise

Sweetpotato cultivars with deep-purple flesh have been developed to meet a growing demand among health food markets. Genotypes for the original purple-fleshed sweetpotatoes were developed by NC State breeders Craig Yencho and Ken Pecota, who are also ARS cooperators. In addition to basic nutrients, there are bioactive phytochemicals—specifically anthocyanins—in these cultivars that give the flesh its purple color. The role of these plant chemicals in terms of human health has been investigated, and a body of evidence suggests that they could be nutritionally beneficial.

Truong and colleagues have converted purple-fleshed sweetpotatoes into frozen and shelf-stable purées for food applications. Now, Truong has made improvements to the method for extracting anthocyanins from these richly colored varieties, and he has used the method to measure the levels of anthocyanins in various purple-fleshed sweetpotato genotypes.

Using pressurized liquid extraction, the team showed that the anthocyanin content of the purple-fleshed sweetpotatoes they studied was comparable to that found in commodities such as grapes, plums, sweet cherries, eggplant, and red radishes.

"The results showed us that many of these genotypes fall in the middle of the spectrum of relatively high-anthocynanin-content fruits and vegetables," says Truong. "The amount of anthocyanin levels in purple-fleshed sweetpotatoes can potentially be increased through breeding and selection of specific genotypes."

The study was published in the *Journal of Food Composition and Analysis* in 2012.

Truong and colleagues also measured phytochemical retention before and after processing purple-fleshed sweetpotatoes into purée. "Overall, we found good phytochemical retention among the purées that were made using the patented microwave processing method and the shelf-stable packaging systems," says Truong. "These purées can be used in beverages, soups, baby foods, bakery products, frozen foods, and ice cream." The studies were published in the *Journal of Food Science* in 2008.

VAN-DEN TRUONG (D3101-1)

ARS, North Carolina State University, and Industrial Microwave Systems LLC developed a process to make and package sweetpotato purées using a continuous-flow microwave technology. Yamco, an industry collaborator, commercialized the process.

Truong and colleagues have also conducted a study to evaluate the effect of steam cooking on anthocyanin pigments. Previously, Truong had identified cyanidin and peonidin as the major anthocyanins in extracts from purple-fleshed sweetpotatoes. The researchers have now analyzed raw and steamed samples from three different purple-fleshed sweetpotato varieties. They identified 17 anthocyanins and found that steam cooking had no significant effect on total anthocyanin content. Cyanidin and peonidin, which contribute to the blue and red hues of the purple sweetpotatoes, were easily measured by breaking down the anthocyanin extracts and using an analytical technique called "high performance liquid chromatography." This study was published in the *Journal of Agricultural and Food Chemistry* in 2010.

These studies show that purple-fleshed sweetpotatoes hold potential for processing into high-anthocyanin purée that can be used in a variety of foods.—By **Rosalie Marion Bliss,** 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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Sweetpotato genotypes with varying flesh color from the North Carolina State University Sweetpotato Breeding Program. ARS and the university are cooperators in the development of sweetpotato purées from various genotypes.

VAN-DEN TRUONG (D3100-1)

Obesity During Pregnancy

Scientists Explore Impacts on Baby's Bone Health

PEGGY GREB (D2377-1)

In studies of the relation of maternal obesity to fetal bone development, principal investigator Jin-Ran Chen uses a next-generation DNA sequencer to investigate changes in gene methylation.

No one knows precisely how Mom's obesity, during her pregnancy, might affect baby's chances of developing strong, healthy bones. But ARS-funded studies at the Arkansas Children's Nutrition Center in Little Rock may provide clues.

Jin-Ran Chen, M.D., a principal investigator in the center's Skeletal Development Laboratory, has shown that bone development of the unborn young of mother lab rats (dams) fed high-fat rations—to induce obesity—was "significantly impaired," in contrast to the bones of the fetal young of dams that were given lower fat rations.

Analysis of fetal bone cells from the skull and vertebrae suggests that changes in the functioning of a gene, *HoxA10,* may help explain this difference in early bone formation. Studies by scientists elsewhere have already established that the gene is important to bone formation and growth. But Chen's investigation is apparently the first to suggest that obesity, induced by the high-fat regimen, may turn off (downregulate) this gene, thus suppressing robust bone development.

Chen and team found that *HoxA10* was downregulated as a result of high levels of DNA methylation, a biochemical process also referred to as "gene methylation." This hypermethylation "essentially turned off this critical gene," Chen explains.

But that's not all. DNA methylation is already known to be what scientists describe as an "epigenetically regulated mechanism," in which the structure or sequence of a gene is not changed, but the way the gene functions is.

Epigenetic changes in a gene's function can be permanent, meaning that the impairment in bone development the scientists observed may last throughout life.

If the results seen in the rats hold true for humans, elevated methylation of *HoxA10* may increase baby's risk of developing bone disease, such as osteoporosis, later in life, Chen says.

Right now, an estimated 10 million Americans have osteoporosis, and another 18 million are at risk of developing this degenerative disease. Osteoporosis reduces both the mass (amount) and the density of bone, thus increasing the risk of hip, wrist, and spine fractures.

In followup studies, Chen will track bone health in offspring of obese and "lean" dams to learn more about the consequences of the mothers' nutrition regimens. "Everything we've learned so far," says Chen, "suggests that it's critical to start early in making sure a mother's nutrition benefits her developing child's bone health."

Chen and colleagues Tom Badger, Mike Blackburn, Ping Kang, Oxana Lazarenko, Martin Ronis, Kartik Shankar, and Jian Zhang, all with the nutrition center and with the University of Arkansas for Medical Sciences, documented their findings in a peer-reviewed scientific article published in 2012 in the Federation of American Societies for Experimental Biology's *FASEB Journal*. The nutrition center is a partnership between ARS, the university, and Arkansas Children's Hospital, also in Little Rock.—By **Marcia Wood,** ARS.

This research is part of Human Nutrition, an ARS national program (#107) described at www.nps.ars.usda.gov.

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Aspirin-Like Compound Primes Plant Defense Against Pathogens

Willow trees are well-known sources of salicylic acid, and for thousands of years, humans have extracted the compound from the tree's bark to alleviate minor pain, fever, and inflammation.

Now, salicylic acid may also offer relief to crop plants by priming their defenses against a microbial menace known as "potato purple top phytoplasma." Outbreaks of the cell-wall-less bacterium in the fertile Columbia Basin region of the Pacific Northwest in 2002 and subsequent years inflicted severe yield and quality losses on potato crops. The Agricultural Research Service identified an insect accomplice—the beet leafhopper, which transmits the phytoplasma to plants while feeding.

Carefully timed insecticide applications can deter such feeding. But once infected, a plant cannot be cured. Now, a promising lead has emerged. An ARS-University of Maryland team has found evidence that pretreating tomato plants, a relative of potato, with salicylic acid can prevent phytoplasma infections or at least diminish their severity.

Treating crops with salicylic acid to help them fend off bacteria, fungi, and viruses isn't new, but there are no published studies demonstrating its potential in preventing diseases caused by phytoplasmas.

Wei Wu, a visiting scientist, investigated salicylic acid's effects, together with molecular biologist Yan Zhao and others at ARS's Molecular Plant Pathology Laboratory in Beltsville, Maryland. "This work reached new frontiers by demonstrating that plants could be beneficially treated even before they become infected and by quantifying gene activity underlying salicylic acid's preventive role," according to Robert E. Davis, the lab's research leader.

For the study, published in the July 2012 *Annals of Applied Biology,* the team applied two salicylic acid treatments to potted tomato seedlings. The first application was via a spray solution 4 weeks after the seedlings were planted. The second was via a root drench 2 days before phytoplasma-infected scions were grafted onto the plants' stems to induce disease. A control group of plants was not treated.

In addition to visually inspecting the plants for disease symptoms, the team analyzed leaf samples for the phytoplasma's unique DNA fingerprint, which turned up in 94 percent of samples from untreated plants but in only 47 percent of treated ones. Moreover, symptoms in the treated group were far milder than in untreated plants. In fact, analysis of mildly infected treated plants revealed phytoplasma levels 300 times below those of untreated plants, meaning that the salicylic acid treatment must have suppressed pathogen multiplication. Significantly, the remaining 53 percent of treated plants were symptom- and pathogen-free 40 days after exposure to the infected scions.

Researchers credit salicylic acid with triggering "systemic acquired resistance," a state of general readiness against microbial or insect attack. Using quantitative polymerase chain reaction procedures, the team also identified three regulatory defense genes whose activity was higher in treated plants than in untreated ones.

Why salicylic acid had this effect isn't known. Other questions remain as well, including how treated plants will fare under field conditions. Nonetheless, such investigations could set the stage for providing growers of potato, tomato, and other susceptible crops some insurance against phytoplasmas in outbreak-prone regions.—By **Jan Suszkiw,** ARS.

This research is part of Plant Diseases, an ARS national program (#303) described at www.nps.ars.usda.gov.

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Below: ARS molecular biologist Yan Zhao (left) observes as visiting scientist Wei Wu pretreats a tomato plant with salicylic acid to test its effectiveness against phytoplasma bacterial infections.
Right: Close-up of tomato leaves sprayed with the pretreatment.

PEGGY GREB (D3105-1)

PEGGY GREB (D3106-1)

Myelin Matters

to piglet movement, reflexes, and coordination

When piglets are born, they need a certain set of skills to be able to thrive. Good coordination and quick reflexes help piglets avoid being accidentally crushed by their mother, which is a primary cause of piglet preweaning mortality.

As the number of piglets born in a litter increases, producers have seen a reduction in piglet birth weight and a rise in the death rate. An estimated 5 to 10 percent of piglets are stillborn, and another 10 to 20 percent do not survive to weaning. The key to solving some of these inefficiencies in swine production may lie within the piglet's brain.

At the Agricultural Research Service's Roman L. Hruska U.S. Meat Animal Research Center in Clay Center, Nebraska, scientists have shown that in low-birth-weight piglets, the brain is closer to normal size than other vital organs, suggesting its importance in survival. They're now investigating other aspects of brain development that may also affect survival.

A team led by Jeffrey Vallet, physiologist and research leader of the center's Reproduction Research Unit, is looking at the production of myelin—an insulating layer that forms around the nerves and is essential for proper functioning of the nervous system. In addition, the group, which includes physiologists Jeremy Miles and Lea Rempel, is examining supplements like creatine to determine the effects on stillbirth, preweaning survival, and myelin production.

STEPHEN AUSMUS (D3097-1)

Creatine plays a significant role in energy metabolism that has been shown to have an effect on the survival of newborn piglets until they're weaned.

Coordination and Reflexes Count

Myelin has broad effects on the coordination of movement and the speed of reflexes, which allows animals to move quickly and easily. Therefore, production of myelin—"myelination"—might affect the piglets' ability to stay alive.

"For example, when humans are born, their brains are not very well myelinated," Vallet says. "Babies can't control their arms and legs at first, but movements and other responses become steadier as they grow older."

On the other hand, when piglets are born, most of their myelin production has already taken place, he adds. "Newborn piglets need to be able to get up, move, and do everything they can to stay away from dangerous situations in their environment, or they're not going to survive very long."

A previous study showed that the brains of low-birth-weight piglets were undermyelinated, Vallet says. However, this work was not specific to myelin. It measured cholesterol—a major component

PEGGY GREB (D2656-1)

At Clay Center, Nebraska, physiologists Jeff Vallet and Lea Rempel examine a piglet. Their work includes looking at supplements like creatine to determine the effects on piglet stillbirth, preweaning survival, and myelin production.

of myelin but also a component of other parts of the brain.

Vallet and colleagues measured proteins and phospholipids that are basic components of myelin. They compared myelin content from three specific regions—the cerebellum, brain stem, and spinal cord—between the largest and smallest pig fetuses during the sow's late pregnancy. The cerebellum controls coordination, while the brain stem and spinal cord are involved in reflexes.

Scientists found no differences between the groups in spinal cord myelination, which might be due to the fact that the spinal cord myelinates first, Vallet says. "It seems likely there are mechanisms present in the spinal cord that maintain myelin in that tissue."

The most significant difference was discovered in the brain stem, where myelination was less in the smaller pig fetuses. An examination of the cerebellum showed similar results. Together, these findings suggest that reduced myelin may contribute to poor survival of low-birth-weight piglets.

"There are a lot of reasons why low-birth-weight pigs don't do very well," Vallet says. "This might be one factor we can improve on if we could figure out a way to reduce myelin deficiency."

A Dose of Creatine

Other research has shown that certain essential fats must come from the diet and that myelin's proteins interact with zinc as part of myelin formation. This suggested that essential fats or zinc might improve myelination. In a subsequent study, funded by the National Pork Board, Vallet added omega-3 fatty acids and zinc to the diets of pigs, but he found that neither supplement had an effect on myelination.

Scientists then looked at creatine, a compound found in muscle tissue. Creatine plays a significant role in energy metabolism, which has been shown to have a major effect on the survival of newborn piglets until they're weaned. Creatine is involved in maintaining energy supplies for muscle contraction and is used to improve the performance of human athletes during strenuous, highly repetitive activities.

"Studies show that the birth process, stillbirth, and preweaning mortality are related, but few strategies or treatments are available to reduce delays in the birth process," Vallet says. "We thought that creatine might affect both the birth process of the sow and energy metabolism in the piglet as well as myelination."

Pigs received either 20 grams of creatine or no treatment from day 110 of pregnancy until they gave birth. Video cameras were used to monitor the birth process—recording the amount of time between the births of piglets, the number of stillbirths, and preweaning mortality. A subset of piglets was measured for changes in brain myelination to determine whether creatine intake by the mother altered myelination in her piglets.

"Although the birth process was not affected by creatine, the number of low-birth-weight piglets crushed by their mother was reduced," Vallet says. "Also, the amounts of several phospholipids in myelin were increased by creatine treatment, particularly in the brain stem, suggesting that improved myelination may contribute to the ability of the piglet to avoid the sow when necessary."—By **Sandra Avant,** ARS.

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

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Piglets need to be able to move quickly to avoid dangerous situations in their environment. Myelin formation affects the coordination of movement and speed of reflexes that allow animals to move quickly and easily.

STEPHEN AUSMUS (D3097-3)

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

Albany, California
9 research units ■ 189 employees

Fort Collins, Colorado
7 research units ■ 139 employees

Roman L. Hruska U.S. Meat Animal Research Center, Clay Center, Nebraska
4 research units ■ 114 employees

Lincoln, Nebraska
2 research units ■ 69 employees

Little Rock, Arkansas
4 ARS employees; ARS funds cooperative research at this location.

Raleigh, North Carolina
4 research units ■ 63 employees

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