U.S. Department of Agriculture | Agricultural Research Service | April 2012

Agricultural Research

Tuning In to Technology

Benefits of Biotech, Computational Tech, and Nanotech

FORUM

Innovations Through Biotechnology

"We need to put a premium on creating innovative solutions to address our current and future problems."—U.S. Department of Agriculture Secretary Tom Vilsack on priorities for the 2012 Farm Bill

This month in *Agricultural Research,* we highlight Agricultural Research Service projects grown from seeds planted on what were once the distant horizons of biotechnology—metagenomics, genomic selection, metabolomics, and more.

Each project represents a fusion of leading-edge science and technological innovation that is helping to shape ARS's response to the growing food, fiber, and fuel needs of a U.S. and world population forecasted to exceed 430 million and 9 billion individuals, respectively, by 2050.

Take, for example, the field of metagenomics, an exciting new discipline that involves sampling the DNA code, or genome sequences, from a community of microorganisms en masse. That community may have diverse origins—from a drop of ocean water to a pinch of soil or even a swab from the intestinal tract of a diseased farm animal.

ARS scientists Laszlo Zsak and Michael Day, both at the Southeast Poultry Research Laboratory in Athens, Georgia, and Brian Oakley and Bruce Seal, of the Richard B. Russell Research Center there, used the metagenomic approach to analyze RNA and DNA viruses associated with two major enteric diseases in poultry: poult enteritis complex and poult enteritis mortality syndrome (see "Metagenomics Offers Insight Into Poultry Diseases," page 18).

They found a new, unexplored universe of viruses, from common RNA viruses (astrovirus, reovirus, and rotaviruses) to an abundance of unexpected viruses (picobirnaviruses and caliciviruses).

One DNA virus genome sequence in particular has them excited. It is a previously undiscovered bacteriophage, named "phiCA82," that is related to viruses that infect and kill bacteria. Studies are under way to determine if phiCA82 and other new phages encode enzymes that can be used to destroy pathogenic bacteria and may provide an alternative to antibiotics for treating animals.

In plant breeding efforts, ARS scientist Jean-Luc Jannink at the Robert W. Holley Center for Agriculture and Health, in Ithaca, New York, is leading the charge for enhancing future crop designs with the use of "genomic selection"—the statistical analysis of genomewide marker data to predict the breeding value for selected progeny, including small- and large-effect trait genes (see "A New Approach to Molecular Plant Breeding," page 13).

Jannink and university collaborators showed that recent advances in genomic selection can dramatically increase genetic gains per unit of time and cost for wheat and barley. When combined with year-round nurseries and inexpensive, high-throughput genotyping platforms, genomic selection will enable breeders in the future to develop new varieties more quickly and efficiently.

In human nutrition, ARS scientist James Harnly, at the Beltsville [Maryland] Human Nutrition Research Center, responded to a critical need in the dietary supplements industry for validated analytical methods to identify diverse metabolites in supplements containing botanical materials.

The adaptation of metabolomics—high-resolution analysis of metabolites using mass spectrometry—to analyze dietary supplements has provided important new insight into the chemical composition and quality of these popular products (see "Digital Detectives Decipher Ingredients," page 6).

Finally, in food safety, ARS scientists created a new generation of advanced antimicrobials. At the Animal Biosciences and Biotechnology Laboratory in Beltsville, David Donovan showed that hybrid enzymes, produced by genes that combine activities from two or more phage enzymes, can target and destroy pathogenic bacteria in nature. Two such bacteria are major pathogens of dairy cattle—*Staphylococcus* spp. and *Streptococcus* spp., including methicillin-resistant *Staphylococcus aureus,* or MRSA. (This research is expected to be reported in an upcoming 2012 issue of *Agricultural Research*.)

Like the new generation of hybrid vehicles, hybrid antimicrobials combine the best features of their parent compounds—lytic activity and species specificity—thus reducing the probability that bacteria will develop resistance.

All projects highlighted in this issue of *Agricultural Research* would not be possible without the contributions from the innovative scientists and staff of ARS who are exploiting new scientific discoveries and technologies to develop solutions for agriculture's great challenges and priorities.

Jack Okamuro
National Program Leader
Crop Production and Protection
Beltsville, Maryland

Kay Simmons
Deputy Administrator
Crop Production and Protection
Beltsville, Maryland

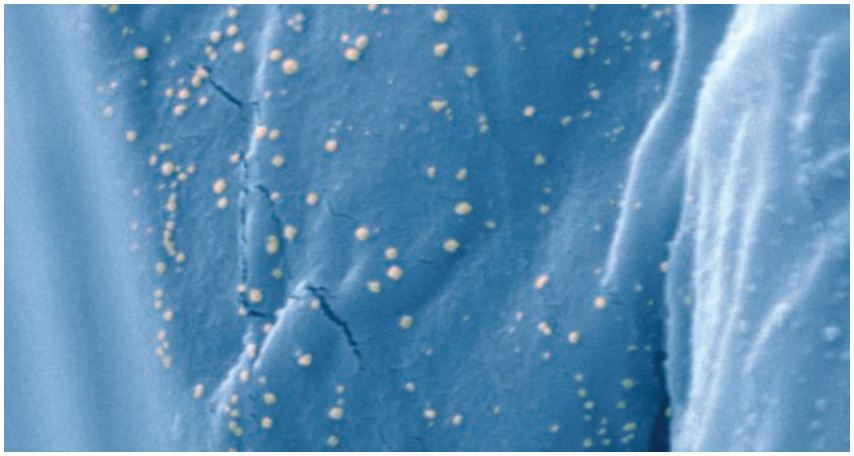
April 2012
Vol. 60, No. 4
ISSN 0002-161X

Agricultural Research

Solving Problems for the Growing World

Agricultural Research is published 10 times a year by the Agricultural Research Service, U.S. Department of Agriculture (USDA). The Secretary of Agriculture has determined that this periodical is necessary in the transaction of public business required by law.

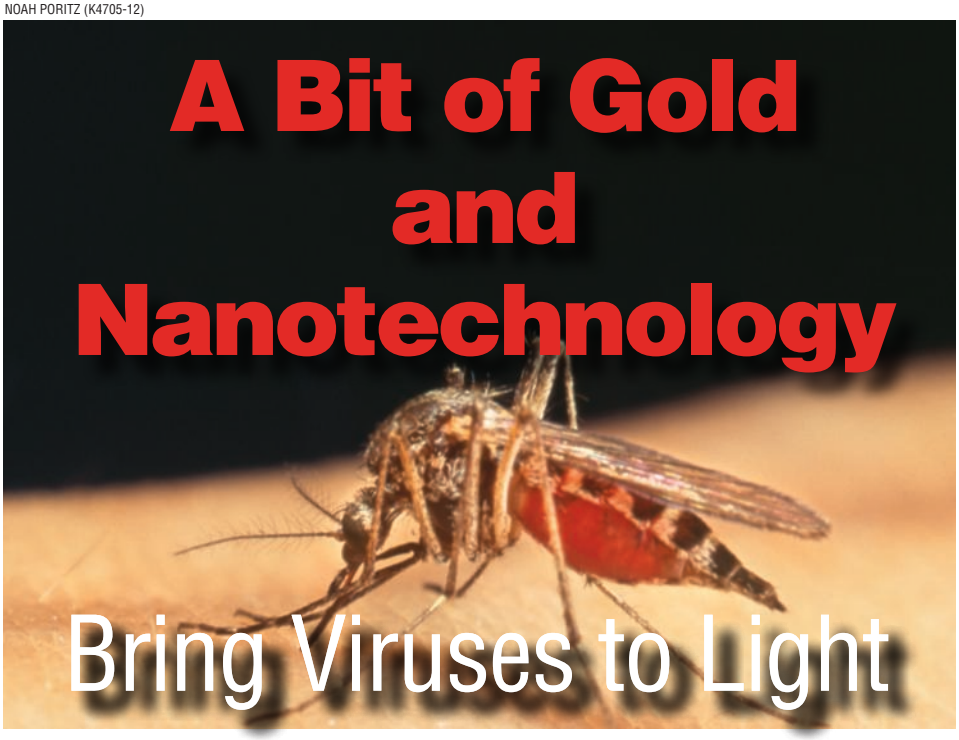
PHOTO BY SUNGHYUN NAM, COLORIZED BY STEPHEN AUSMUS (D2468-1)



Scanning electron microscope image of silver nanoparticles on the surface of cotton. The silver helps cotton fabric resist microbial growth. Magnification about 12,000x. Story begins on page 14.

Cover: At ARS's Food Composition and Methods Development Laboratory, in Beltsville, Maryland, postdoctoral associate Jianghao Sun prepares for HPLC/MS analysis of extracts from teas and supplements made from teas. The purpose is to evaluate the differences in the ingredients in the teas and supplements. Story begins on page 6. Photo by Peggy Greb. (D2473-2)

NOAH PORITZ (K4705-12)



A Bit of Gold and Nanotechnology Bring Viruses to Light

Mosquitoes like this female *Aedes (Ochlerotatus)* sp., sucking blood to get nourishment for her eggs, can transmit the viruses that cause West Nile fever and Rift Valley fever.

All that glitters is not gold, but it is in the case of nanotechnology that's being used to develop detection tools for viruses that affect animals and people.

Scientists at the Agricultural Research Service's Center for Grain and Animal Health Research (CGAHR) in Manhattan, Kansas, and the University of Wyoming are using surface-enhanced Raman scattering (SERS) with gold nanoparticles to design tests that rapidly identify the virus that causes West Nile fever.

The West Nile virus is spread by infected mosquitoes and can cause headaches, fever, and body aches. In some cases, it causes a more serious and sometimes fatal neuroinvasive disease—aseptic meningitis, encephalitis, or acute flaccid paralysis. Symptoms include disorientation, muscle weakness, and paralysis.

SERS technology is based on the concept that molecules have their own unique Raman scattering signal—wavelengths that can be detected with a spectroscope. When Raman spectroscopy is used, molecules give off Raman spectra that have sharp peaks, making them more distinguishable than with fluorescence, where molecules emit broad peaks. If a molecule is moved in close proximity to a metal like gold or silver, as with SERS technology, the signal is enhanced maybe up to a millionfold.

Using SERS, scientists designed a nucleic acid diagnostic assay that captures Raman dyes—fluorescent tags—and brings them close to the gold nanoparticles in a compound solution. They also developed an immunological assay that rapidly detects antibody responses.

"The advantage of SERS is that it amplifies the signal without requiring an enzymatic reaction or long incubation and can be done in a complex solution without multiple washing steps," says microbiologist William Wilson in the CGAHR Arthropod-Borne Animal Disease Research Unit. "The assay is easier and quicker in detecting West Nile virus."

In an initial study, researchers used gold nanoparticles and a Raman label of methylene blue dye to detect West Nile virus and develop a nucleic acid assay.

"We were having difficulty in getting a really clean signal," Wilson says. "We could demonstrate signals, but they weren't of the intensity that we would like." Eventually, however, the scientists were able to clean up the signals.

Confirming a Detection Tool

Gold nanoparticles have the ability to enhance light scatter, which can be taken advantage of in detecting virus-infected cells. This is the principle behind SERS that the researchers have used to develop an immunoassay and test its ability in detecting antibodies to West Nile virus. Gold nanoparticles were coated with a West Nile virus protein, and a malachite green-conjugated protein A/G was used as a Raman reporter. Protein A/G binds to immunoglobulin molecules (antibodies). When antibodies specific to viral proteins are present, the proteinA/G-antibody complex then binds to the viral protein-coated gold nanoparticles.

"Because the protein A/G was labeled with a Raman dye, that would bring the Raman dye in close proximity with the gold nanoparticles and we would get the enhancement from the Raman signal," Wilson says.

In the future, a pen-side diagnostic test for Rift Valley fever and other diseases of cattle may arise from SERS research.

SCOTT BAUER (K7612-17)

Nanotech

The results proved that gold nanoparticles in Raman scattering could be used as a detection tool in an antibody-based system. Still, researchers wanted to increase the signal's intensity and improve the quality.

"Instead of just using a gold nanoparticle, we used a magnetic particle, which has a capture probe," Wilson says. "We capture the West Nile target RNA or DNA and come in with a second probe that detects the second region of the West Nile target."

The second probe is a gold nanoparticle that already has the reporter molecule attached to it, he explains.

"We pull that into an area and focus the laser on it to see the signal," Wilson says. "So it's a way of enhancing our signal's intensity for the end solution hybridization."

Gold and magnetic nanoparticles are coated with capture antibodies or DNA in a dry reagent form to avoid having to process blood samples, says Patrick Johnson, assistant professor of chemical and petroleum engineering at the University of Wyoming.

"At most, you may have to remove just the cells from the blood, put the remaining fluid with the dry reagent of nanoparticles, mix it for a short period, and expose the sample to a strong magnet," he says. "You can then read the magnetic pellet with a laser."

If virus proteins are present, they will be detected through SERS spectroscopy, Johnson adds.

Taking It to the Field

Scientists are using a bench-top computer system in laboratories to analyze and report the signal.

"The goal is to bring laboratory-level analytical sensitivity to the field for portable care devices," Johnson says. "You will be able to take a blood sample, put it in a small vial, and read it with a handheld device."

Systems exist that could easily be adapted to field-type instruments, Wilson says. The device would be more like an advanced dipstick that could be used during a disease outbreak to rapidly determine areas where the outbreak is occurring.

KEITH WELLER (K4166-5)

ARS scientists are using surface-enhanced Raman scattering with gold nanoparticles to design tests for identifying viruses that cause West Nile fever and Rift Valley fever (RVF). RVF is spread by mosquitoes to humans and to sheep, cattle, and some other livestock in Africa and the Middle East.

"It would allow physicians to do quick tests at the bedside of patients, and veterinarians could potentially use it for pen-side tests at farms," Wilson says.

Researchers are using SERS with West Nile virus as well as Rift Valley fever virus, but the process could be adapted to other pathogens, Johnson says. Future studies will involve examining how this technology can be used to identify multiple disease agents in one test. Available pen-side diagnostic tests are generally agent specific.

"The sensitivity of this test is much greater than the current pen-side system," Wilson says. "It's potentially as good as amplification technology, such as PCR [polymerase chain reaction]. It's faster and also gives us the potential to easily do multiple-pathogen detection."—By **Sandra Avant,** ARS.

> "The assay is easier and quicker in detecting West Nile virus."
>
> —William Wilson

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

William Wilson is in the USDA-ARS Arthropod-Borne Animal Diseases Research Unit, Center for Grain and Animal Health Research, 1515 College Ave., Manhattan, KS 66502; (785) 537-5570, william.wilson@ars.usda.gov.

Television shows featuring crime scene investigators have been keeping viewers intrigued for years. But the Agricultural Research Service's intriguing "food composition investigators" are just as innovative at deciphering truth from fiction relating to ingredients of plant-based foods and dietary supplements. The researchers are at the ARS Food Composition and Methods Development Laboratory, which is headed by research leader James Harnly. The laboratory is part of the Beltsville [Maryland] Human Nutrition Research Center (BHNRC).

They're using new equipment and a metabolomics approach to discover compounds and to accurately identify ingredients in foods and supplements. They are also looking at chemical composition patterns to find differences between cultivars, growing years, and locations—knowledge important for consumers, farmers, and marketers.

Metabolomics is the analysis of all the intermediate and end products, or metabolites, of cell processes. These individual molecules are produced by active, living cells during the metabolic process. Lipids, fats, sugars, amino acids, and flavonoids, for example, are all metabolites. The plant metabolome furnishes nutrients necessary for human health.

Metabolomics requires bioinformatics tools for data analysis, visualization, and integration. The researchers are using newly procured equipment—high-resolution mass spectrometers (HRMS)—to conduct metabolomic studies. "High resolution" means the technology gives a high level of information. For example, with HRMS, glucose has a measured mass of 179.0556 atomic mass units (amu), compared with 179 amu as detected by a regular mass spectrometer. HRMS has the potential for identifying every metabolite in a sample.

Green Tea: Sip it or Supp it?

The U.S. Food and Drug Administration issued "current good manufacturing practices" that require all botanical ingredients in supplements to be identified. To answer the increased demands for valid analytical methods, Harnly chaired an expert review panel that established "Guidelines for Validation of Botanical Identification Methods" for AOAC International (Association of Analytical Communities)—a nonprofit association dedicated to excellence in analytical methods.

Pei Chen, a chemist with the ARS food-composition lab, headed a study of the differences between phytochemicals in green tea supplements and green tea leaves used for brewing beverages. Green tea-based dietary supplements have gained popularity in the U.S. market in recent years. But when it comes to sipping green tea versus taking the dietary supplement form, the jury is out as to the better choice relative to health. HRMS can be very useful in identifying the differences.

Chen and BHNRC colleagues Jianghao Sun and Long-Ze Lin analyzed "extractions" of 20 commercially available green tea dietary supplement products (12 tablets or capsules and 8 liquid samples) and 8 dry green tea leaf samples, and they compared the chemical constituents of the samples using an analytical technique called "HPLC/MS."

ARS scientists are using mass spectrometry of red grapefruit juice to determine differences in fruit grown conventionally versus organically.

PEGGY GREB (D2492-1)

Biotech

Brand names were not disclosed in the published study, but the researchers noted that most major dietary supplement manufacturers were represented.

The study demonstrated that flavonol glycosides were degraded and the compound catechin had oxidized during manufacturing and storage for many of the green tea supplement samples studied. They also found some additives in the supplements that were not listed on the labels.

The BHNRC team found a significant chemical variance across the range of green tea dietary supplements they sampled. They also noted that the daily intake amount recommended by the labels varied significantly. They concluded that although there are some good green tea dietary supplement products, there is no way for the consumer to know the quality from reading the labels. More importantly, the consumer may ingest other botanical extracts unintentionally, and the quality of those green tea products varies significantly. The claim that a green tea dietary supplement is a good alternative to tea leaves is questionable from a chemical-composition point of view, and the results "demonstrate the urgency of quality control for green tea dietary supplement products," wrote the authors. The 2011 study was published in the *Journal of AOAC International.*

Organic and Traditional Differences

Switching to fresh fruit, the BHNRC "Rio Red Grapefruit" study showed that MS fingerprinting, when combined with a pattern-recognition method called "ANOVA-PCA," could clearly establish that there are chemical differences between grapefruit samples in terms of growing year, harvest time, and farming method (conventional or organic).

For the study, Harnly and Chen studied samples of Rio Red grapefruit furnished by Gene Lester of the ARS Food Quality Laboratory, also in Beltsville. The grapefruits were grown using conventional and organic cultivation methods. They were harvested at three growing phases (early, mid, and late season) during 2005 and 2006. The juices were analyzed by mass spectrometry with no separation of the molecules. The overlapping mass spectra of all the molecules, or the "spectrometric fingerprint," is very complex and, like human fingerprints, is analyzed by looking at the overall pattern.

ANOVA-PCA was used to determine whether a distinction could be made between the two cultivation methods and the three harvest dates (growing phases) by analyzing the MS spectral fingerprints of the grapefruit juices. The analysis showed that the chemical patterns of the fingerprints were statistically different among the farming modes, growing years, and times of harvest, regardless of the MS method used.

The results are important in demonstrating that conventional and organic products have different chemical compositions, although it is unknown at this time if these differences have significance to consumers. The 2010 study was published in the *Journal of Agricultural and Food Chemistry.*

Let's Not Forget Herbals

American ginseng is one of the most commonly used herbal medicines in the world. But discriminating between ginsengs grown in different countries is difficult using traditional methods.

Chen headed a study involving MS fingerprints and pattern-recognition analysis methods to discriminate between American ginseng grown in the United States and that grown in China. They studied 15 American ginseng samples grown in Wisconsin and 25 samples grown in China. The MS fingerprints, representing the chemical compositions of the samples, made it possible to distinguish between samples grown in the two different locations. The 2011 study was published in *Analytical and Bioanalytical Chemistry.*—By **Rosalie Marion Bliss,** ARS.

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

James Harnly and Pei Chen are with the USDA-ARS Food Composition and Methods Development Laboratory, 10300 Baltimore Ave., Beltsville, MD 20705-2350; (301) 504-8569, ext. 261 [Harnly], (301) 504-8144, ext. 238 [Chen], james.harnly@ars.usda.gov, pei.chen@ars.usda.gov.

Chemist Pei Chen prepares extracts from teas and tea-based dietary supplements to study differences in phytochemicals (health-protecting compounds) in the two forms of tea.

PEGGY GREB (D2474-1)

SERS

High-Tech Tactic May Newly Expose Stealthy *Salmonella*

At laboratories of the future, even the smallest quantity of *Salmonella* bacteria may be easily detected with a technology known as "SERS," short for "surface-enhanced Raman scattering."

Agricultural engineer Bosoon Park, in the Agricultural Research Service's Quality and Safety Assessment Research Unit in Athens, Georgia, is leading exploratory studies of this analytical technique's potential for quick, easy, and reliable detection of *Salmonella* and other foodborne pathogens.

According to the U.S. Centers for Disease Control and Prevention, *Salmonella* causes more than 1 million cases of illness in this country every year.

If SERS proves successful for cornering *Salmonella,* the technique might be used at public health laboratories around the nation to rapidly identify pathogens responsible for outbreaks of foodborne illness. Tomorrow's foodmakers might opt to use SERS at their in-house quality-control labs to help ensure that their products are free of unsafe levels of this or other harmful bacteria.

For *Salmonella* testing, labs have many choices. They can opt for well-established technologies, such as monoclonal antibody assays, ELISA (enzyme-linked immunosorbent assay), or PCR (polymerase chain reaction) tests.

Park's team is evaluating the strengths and shortcomings of SERS in relation to these popular analytical methods and several newer ones as well, including atomic force microscopy and surface plasmon resonance.

According to Park, a SERS analysis is relatively simple to perform, an advantage when labs are swamped with samples from an outbreak of foodborne illness or a product recall.

With further research, SERS may offer superior detection capabilities, making it faster and easier to find very small quantities of bacteria in a complex, real-world background, such as a food or beverage sample. That's especially important, since it's apparently possible for as few as 10 *Salmonella* cells to cause illness, depending on the strain involved and other factors.

Nobel Laureate's Work is Basis of Today's SERS

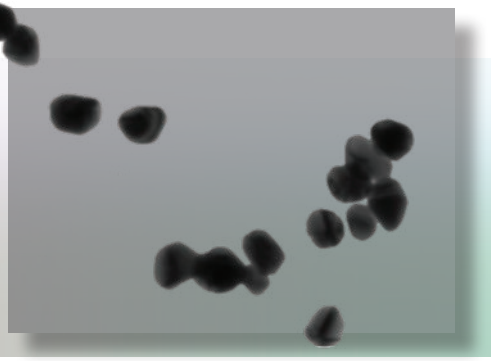
Developed over the past two decades or so, SERS has expanded the power and potential of Raman spectroscopy, its parent technology. Raman spectroscopy is based on discoveries made in the early 1900s by Sir Chandrasekhara V. Raman, who received the 1930 Nobel Prize in physics for his research.

In a SERS analysis, a specimen is placed on a surface, such as a stainless steel plate, silicon wafer, or glass slide, that has been "enhanced"—or changed from smooth to rough. For example, a glass slide or silicon wafer could be etched, to roughen it, or a small stainless steel plate could be coated with an extremely thin "nanolayer" of minuscule particles of a metal, such as silver, gold, or copper.

In some of its research, Park's team enhanced the surface of stainless steel plates by coating them with tiny spheres, made up of a biopolymer encapsulated with silver nanoparticles.

Rough surfaces and colloidal metals, such as silver, can enhance the scattering of light that occurs when a specimen, placed on this "nanosubstrate," is scanned with the Raman spectrometer's laser beam. The scattered light that comes back to the spectroscope forms a distinct spectral pattern

Transmission electron microscope image of biopolymer spheres coated (encapsulated) with silver nanoparticles, which might make an ideal surface (substrate) for SERS detection of foodborne pathogens in food and beverage samples.



JAYA SUNDARAM (D2477-1)

Biotech

JERRY HEITSCHMIDT (D2475-1)

Postdoctoral research associate Jaya Sundaram (left) and agricultural engineer Bosoon Park are determining whether SERS signatures can be used to reliably identify pathogenic foodborne bacteria.

known as a "Raman spectral signature," or a "Raman scattered signal."

"The idea of using a substrate of silver nanoparticles for Raman spectroscopy is not new," says Park. "But in SERS studies to detect foodborne pathogens, our use of a substrate of silver nanoparticles that encapsulate a biopolymer is novel, to the best of our knowledge."

Though SERS research with biological specimens is still in comparatively early stages, researchers expect to prove the concept that all molecules, such as those that make up *Salmonella,* have their own unique Raman spectral signature. In the future, the Raman signature of an unidentified biological specimen could be compared to known Raman signatures to find a match and thus identify the unknown specimen.

Park's team has already developed preliminary Raman spectral signatures for two common pathogenic kinds, or serotypes, of *S. enterica*—Enteritidis and Typhimurium—collected from raw chicken.

Silver-Encapsulated Spheres Tested as a Nanosubstrate

For this research, the group used a biopolymer encapsulated with silver as the nanosubstrate, and they worked with comparatively large concentrations of the serotypes (about 108 colony-forming units per milliliter of solution). The high concentrations simplified detection and showed, apparently for the first time, that SERS can differentiate the two *Salmonella* serotypes by their Raman signatures.

Plans call for developing a database, or library, of Raman spectral signatures of these *Salmonella* serotypes and other major foodborne pathogens at various concentrations. Ideally, the signatures would be posted on the Internet for public access by researchers and analytical labs worldwide.

In follow-up studies, Park intends to use less-concentrated samples of *Salmonella* serotypes to more rigorously challenge the technology's ability to detect and quantify the pathogen. These tests may ultimately reveal SERS's sensitivity, or detection limit—the smallest number of *Salmonella* cells, in a given concentration of solution, that can be accurately and reliably detected with a given combination of variables. Those variables might include the types of SERS instruments, sample preparation method, sample nanosubstrate, power and duration of the laser beam scanning, and more.

In an earlier experiment, using a different silver-based substrate, the team showed that SERS can differentiate live *Salmonella* cells from dead ones. That's significant. It could help quality-control labs evaluate the performance of today's food-sterilization methods, such as high-pressure processing, irradiation, and thermal processing. In addition, reliable differentiation can speed and simplify the work of researchers who are developing and testing new techniques to keep food safe to eat.

"SERS is one of several candidate technologies we are currently testing," says Park. "If we find that it continues to offer major advantages over other options, our goal would be to develop a SERS-based system that wouldn't require extensive training and would use affordable SERS instruments."

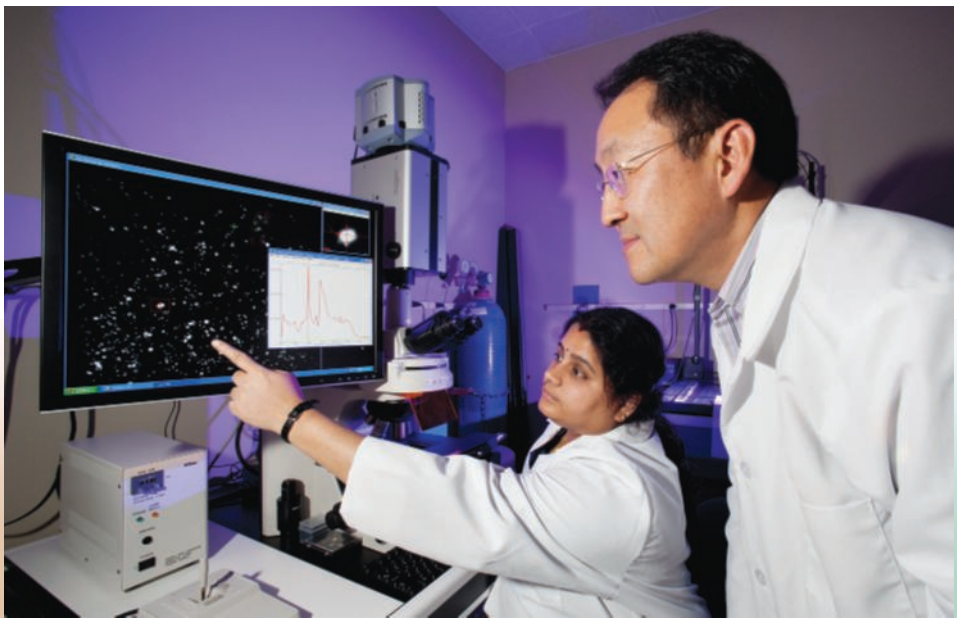
Park has presented some of these findings at an SPIE (Society of Photo-Optical Instrumentation Engineers) conference in Orlando, Florida. His collaborators include Yao-Wen Huang and Yiping Zhao of the University of Georgia-Athens; Arthur Hinton, Jr., Kurt C. Lawrence, Jaya Sundaram, William R. Windham, and Seung Chul Yoon, all with ARS at Athens; and Yongkuk Kwon of South Korea's Animal, Plant, and Fisheries Quarantine and Inspection Agency.

The team's studies may do much to keep us safe from some of the world's worst foodborne pathogens.—By **Marcia Wood,** ARS.

This research supports the USDA priority of food safety and is part of Food Safety, an ARS national program (#108) described at www.nps.ars.usda.gov.

Bosoon Park is in the USDA-ARS Quality and Safety Assessment Research Unit, Richard B. Russell Research Center, 950 College Station Rd., Athens, GA 30605; (706) 546-3396, bosoon.park@ars.usda.gov.

Bosoon Park (right) and Jaya Sundaram examine a hyperspectral microscope image of an experimental substrate developed for SERS food-safety analyses.



JERRY HEITSCHMIDT (D2476-1)

What's in Your Blood?

The Ongoing Hunt for Metabolites

At any given time, blood circulating through your body carries thousands of small molecules known as "metabolites." Medical and nutrition researchers are eager to discover more about these compounds—amino acids, sugars, fats, and more—that are formed in and by our bodies.

Metabolites are of interest because the presence and concentrations of some of them can provide meaningful profiles, sometimes referred to as "metabolic signatures" or "fingerprints."

In the future, for instance, your metabolic fingerprint—detected in a small sample of blood taken for your annual physical examination—might prove to be a reliable indicator of your health and a predictor of certain diseases. This information could then be used to create personalized recommendations about what you should eat for optimal health.

In another futuristic scenario, scientists specializing in the field of nutritional metabolomics might use metabolic fingerprinting to assess whether experimental diet- and physical activity-based strategies can prevent the onset of disorders such as type 2 diabetes or cardiovascular disease.

Medical and nutrition researchers who are investigating serum metabolites for these and other promising applications have a remarkable new resource for their studies.

And it's only a mouse click away.

It is the Serum Metabolome database (www.serummetabolome.ca), a comprehensive, first-ever public catalog of 4,229 metabolites in human serum.

A team of 24 researchers in Canada and the United States—including Agricultural Research Service investigators John W. Newman and Theresa L. Pedersen—participated in this international project. Newman, a chemist, and Pedersen, a support scientist, are with the ARS Western Human Nutrition Research Center in Davis, California. Newman is also an associate adjunct professor of nutrition at the University of California-Davis, where the center is located.

The ARS team and scientists at five other centers each used different analytical techniques, or "platforms," to examine blood samples from healthy adult volunteers and from adults with cardiovascular disease.

Several Significant Firsts

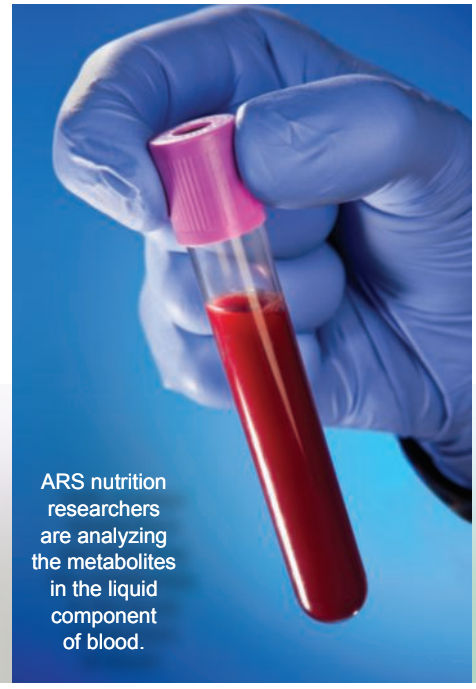
Most of the metabolites documented in the database are not new to science. Nonetheless, the database represents several important firsts.

To begin with, it documents an estimated 84 percent of human serum metabolites. As such, it "represents the most comprehensive coverage of serum metabolites ever offered, in one convenient, reliable resource," says Newman.

What's more, the database pulls together information on serum metabolites not only from the participating labs, but also from an impressive array of sources in the scientific literature—more than 2,000 in all. Careful review of these sources led to the addition of nearly 700 metabolites to the database, augmenting those detected and quantified in the laboratory analyses.

The sources were gleaned from the project's computer-assisted data mining of some 19 million scientific abstracts.

An additional first: The database is the most extensive electronically searchable compendium available today of the human



ARS nutrition researchers are analyzing the metabolites in the liquid component of blood.

PEGGY GREB (D2478-3)

Biotech

serum metabolome. This search capability greatly speeds and simplifies its use by researchers worldwide.

The database lists the detected metabolites and, if known, their association with diseases. The quantities, or concentrations, of metabolites are presented as ranges that encompass the values determined during the study and values from the curated literature.

Analytical Methods: How Do They Compare?

The project "presented an invaluable opportunity to learn more about the strengths and shortcomings of various analytical platforms by comparing the metabolite coverage and values obtained from each," says Newman.

Five different analytical approaches were used in the project. Newman's team worked with two: UPLC-ESI-MS/MS (ultra-performance liquid chromatography-electrospray ionization-tandem mass spectroscopy) and GC-MS (gas chromatography-mass spectroscopy).

His lab's analyses focused on lipid metabolites involved in regulating biological processes. These metabolites are formed by the body from the fats and oils in foods such as nuts, dairy products, meats, and fish. According to Newman, regulatory lipid metabolites "are numerous, and they play important roles in tissue growth and repair, blood clotting, inflammation, pain perception, and appetite regulation. They are critical to our health.

"Our targeted analyses of these metabolites provided a swath of coverage that the other platforms used in this study didn't capture," he says. "In particular, more than 80 percent of the metabolites found on our UPLC-ESI-MS/MS platform were not detected by the other techniques."

He says that the study "provides a strong starting point for determining the best future combinations of various analytical platforms to achieve maximum coverage of the serum metabolome. We know that some metabolites can be measured on multiple platforms and that different platforms have different advantages. Some are less expensive, while others are more sensitive, for example. We think our comparisons of results from different approaches will help researchers select analytical tools in the future."

Regulatory lipid metabolites "are numerous, and they play important roles in tissue growth and repair, blood clotting, inflammation, pain perception, and appetite regulation. They are critical to our health."

—John W. Newman

Newman and his team are international authorities on nutritional metabolomics and the use and improvement of advanced technologies to detect and accurately quantify lipids in blood, tissue, food, and other materials. His group is interested in determining how the kinds and concentrations of lipids in our bodies are influenced by our eating habits, physical activity patterns, and genetic and environmental factors and the relation of these lipids to obesity and its adverse effects on health. The serum metabolome is, not surprisingly, a perfect tool for these investigations.

A peer-reviewed article, published in 2011 in *PloS One,* documents the serum metabolome project. The research was led by David S. Wishart, who is with both the University of Alberta and Canada's National Institute for Nanotechnology.

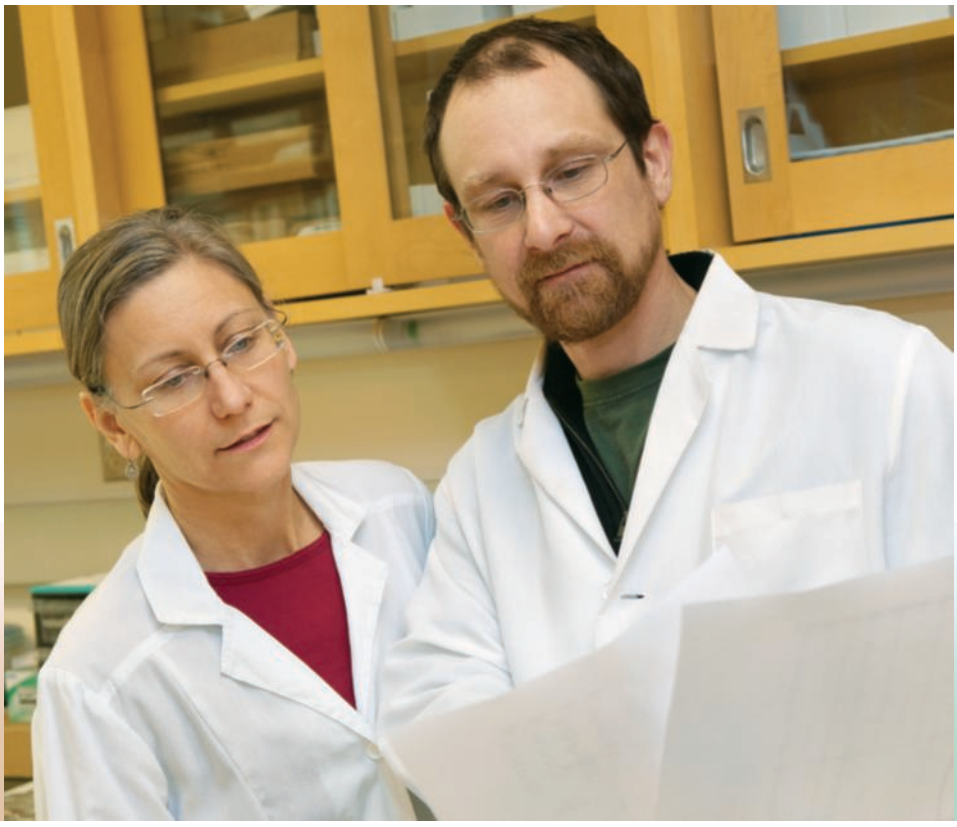
Funding was provided primarily by the Canadian Institutes for Health Research, Alberta Advanced Education and Technology, Genome Alberta, Genome BC, the Alberta Ingenuity Fund, and the University of Alberta, but also by ARS, the U.S. Department of Veterans Affairs, and the National Institutes of Health.

The endeavor is part of the ambitious Human Metabolome Project, also led by Wishart, which aims to document all of the metabolites in our fluids and tissue.—By **Marcia Wood,** ARS.

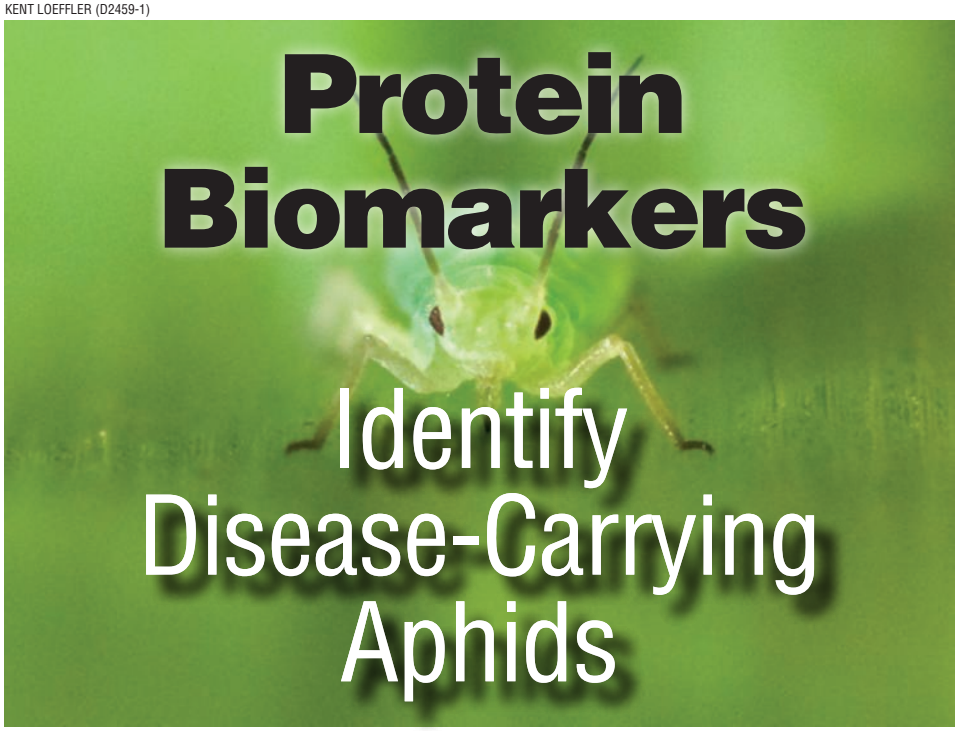
This research is part of Human Nutrition, an ARS national program (#107) described at www.nps.ars.usda.gov.

John W. Newman is with the USDA-ARS Western Human Nutrition Research Center, 430 W. Health Sciences Dr., University of California, Davis, CA 95616; (530) 752-1009, john.newman@ars.usda.gov.

Researchers John Newman and Theresa Pedersen review a summary of lipid metabolites in blood. Our bodies form these metabolites from fats and oils in foods such as nuts, dairy products, meats, and fish.



KENT LOEFFLER (D2459-1)



Protein Biomarkers Identify Disease-Carrying Aphids

Close-up of greenbug aphid, *Schizaphis graminum,* showing the piercing-sucking mouthparts it uses to feed and inject virus into plants.

Aphids can transmit viruses that cause crop diseases and reduce the quality and quantity of fresh foods. Spraying insecticides can control aphids and reduce the spread of viruses, but spraying is expensive and can harm the environment. Additionally, not all aphids transmit viruses. So a key question for growers is knowing when and what to spray to control viral diseases.

Agricultural Research Service scientists Michelle Cilia and Stewart Gray, in the Biological Integrated Pest Management Unit at the Robert W. Holley Center for Agriculture and Health in Ithaca, New York, have found a way to distinguish aphids that spread viruses from those that don't—by studying the aphid's proteins.

They knew from previous work that for aphids to pick up and transmit viruses, the virus must be able to interact with specific aphid proteins that direct movement of the virus through the insect and back into a plant during feeding. In laboratory studies of greenbug aphids, they discovered that the laboratory-raised insects' ability to transmit yellow dwarf viruses could be predicted by the presence or absence of nine different biomarker proteins in the insect cells.

To see if their lab findings would prove true in the field, they analyzed greenbug aphids collected from cereal crops and noncultivated grasses around the United States by ARS colleagues John Burd and Melissa Burrows, of the Wheat, Peanut, and Other Field Crops Research Unit in Stillwater, Oklahoma.

The Ithaca researchers found the field-collected aphids consistently transmitted yellow dwarf virus only when they carried most, if not all, of the nine key biomarker proteins. "The aphid does not need all nine to spread the yellow dwarf virus, but some are essential," Cilia says.

The discovery in the lab was published in the March 2011 issue of the *Journal of Virology,* and the study confirming the biomarkers in the field was reported in *Proteomics* in June 2011.

The findings mark the first time that protein biomarkers have been linked to an insect's ability to transmit viruses, and the discovery is expected to lead to development of a test to identify potential disease vectors.

Cilia and Gray are also collaborating on an effort to test whether the biomarker proteins can predict disease-vectoring ability in other insects. Collaborators include Michael MacCoss and Michael Bereman at the University of Washington; Alvin Simmons at ARS's U.S. Vegetable Laboratory in Charleston, South Carolina; and Lava Kumar and Rachid Hanna at the International Institute of Tropical Agriculture in Africa. The project is being funded by the National Science Foundation Basic Research to Enable Agricultural Development program through Cornell University, with support from the Bill & Melinda Gates Foundation.—By **Dennis O'Brien,** ARS.

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

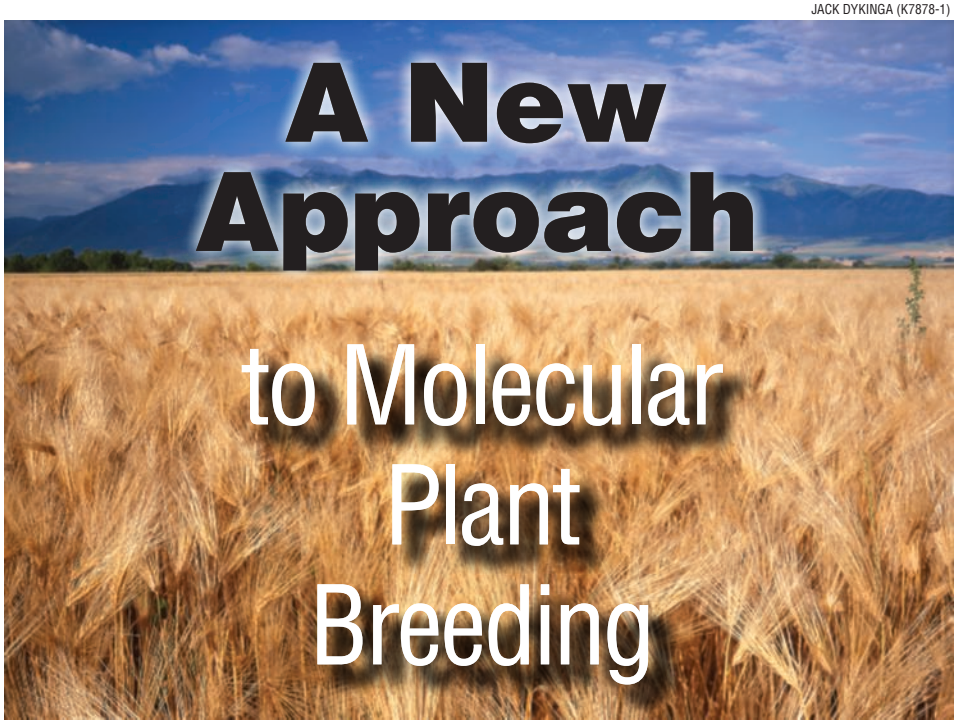
To reach scientists featured in this article, contact Dennis O'Brien, USDA-ARS Information Staff, 5601 Sunnyside Ave., Beltsville, MD 20705-5129; (301) 504-1624, dennis.obrien@ars.usda.gov.

Left: Plant pathologist Stewart Gray and molecular biologist Michelle Cilia examine greenhouse plants for virus symptoms. Right: Greenbug aphid feeding on an oat leaf infected with yellow dwarf disease.

KENT LOEFFLER (D2460-1)

KENT LOEFFLER (D2458-1)

JACK DYKINGA (K7878-1)



A New Approach to Molecular Plant Breeding

To grow wheat and evaluate it for traits in the field takes 5 to 9 years. Using genomic data, ARS scientists can do it in about 6 months.

An ARS scientist in Ithaca, New York, is using a new statistical approach to help speed the development of improved varieties of crops.

Plant breeders constantly strive to breed new varieties that yield more, resist emerging pests and pathogens, tolerate heat and drought, and grow in marginal soils and environments. Increasingly, molecular tools are used to speed those efforts. By identifying genes associated with desirable traits, scientists don't have to wait for time-consuming field observations.

"To grow wheat and evaluate it for traits in the field takes 5 to 9 years. Using genomic data, we can do it in about 6 months," says Jean-Luc Jannink, who is in the ARS Plant, Soil, and Nutrition Research Unit at the Robert W. Holley Center for Agriculture and Health on the Cornell University campus, in Ithaca.

Though molecular tools speed up the process, they also require analyzing massive amounts of data. Jannink has demonstrated how new statistical approaches should help plant breeders handle all that data. Analysis of the data enables "genomic selection" (GS) of untested breeding lines, using predictions about which will perform best. Dairy cattle breeders have begun to use GS, and with Jannink's help, it will catch on among plant breeders.

Even with modern molecular tools, breeders face challenging traits like drought tolerance and high yield, which result from the combined action of multiple genes, each having a small effect. These genes, called "quantitative trait loci" (QTLs), are stretches of DNA that affect an observable trait. The conventional marker-assisted selection (MAS) approach has limited power when a trait is affected by small-effect genes. The sheer volume of data involved forces MAS to exclude some small-effect QTLs when individual plants are selected for further breeding.

Jannink's approach exploits more genetic data by including all of the small-effect QTLs and estimating the effects of all of the known genetic markers in a plant population. In a recent study, Jannink and his colleagues constructed statistical models, using both GS and MAS approaches, and compared how well they could predict the values of 13 agronomic traits in crosses made from a "training population" assembled for the study. The accuracy of the models was measured by comparing their predictions with field observations of 374 lines of wheat, characterized by Mark Sorrells, a collaborator and wheat breeder at Cornell University. In their study, Jannink and colleagues were able to use all the available data, including all of the trait data, along with the genomewide marker data that captured the genetic values of the wheat lines.

The results, published in the March 2011 issue of *The Plant Genome,* showed that the GS approach was more accurate at predicting trait values than the MAS method was. Jannink reported similar success in another study, this time of oats, published in June 2011 in *The Plant Genome.* The work is expected to speed up molecular breeding efforts and might prove extremely useful, given the rapid pace of advances in DNA technology, Jannink says.—By **Dennis O'Brien,** ARS.

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

Jean-Luc Jannink is with the USDA-ARS Robert W. Holley Center for Agriculture and Health, 538 Tower Road, Cornell University, Ithaca, NY 14853; (607) 255-5266, jeanluc.jannink@ars.usda.gov.

Wheat seed heads.

DOUG WILSON (K3945-7)

(D2467-1)

Cotton Gets Nanotech and Biotech Treatment in New Orleans

Scientists at the Agricultural Research Service's Cotton Chemistry and Utilization Research Unit (CCUR) in New Orleans, Louisiana, have a long history of research successes leading to advances in the use, manufacturing, and quality of cotton fiber.

For example, groundbreaking studies led by chemist Ruth Benerito at the Cotton Chemical Reactions Laboratory (CCUR's predecessor), starting in the 1950s, gave rise to easy-care, permanent-press clothing and other consumer-friendly improvements that helped cotton better compete with synthetic fibers, like polyester and nylon.

New challenges and consumer demands have since emerged, but the ARS lab's tradition of excellence and innovation in research continues.

Under the leadership of Brian Condon, CCUR researchers today are leveraging the latest developments in nanotechnology to bring cotton into the 21st century. Foreseeable applications range from the purely functional—like better shrink resistance—to the truly futuristic, such as fabrics made of cotton-optical fiber blends that can change color.

Flame-Retardant Coating

In one ongoing project, Condon and CCUR chemist SeChin Chang are collaborating with Texas A&M University (TAMU) scientists to evaluate a first-of-its-kind, environmentally friendly flame retardant for cotton apparel and durable goods.

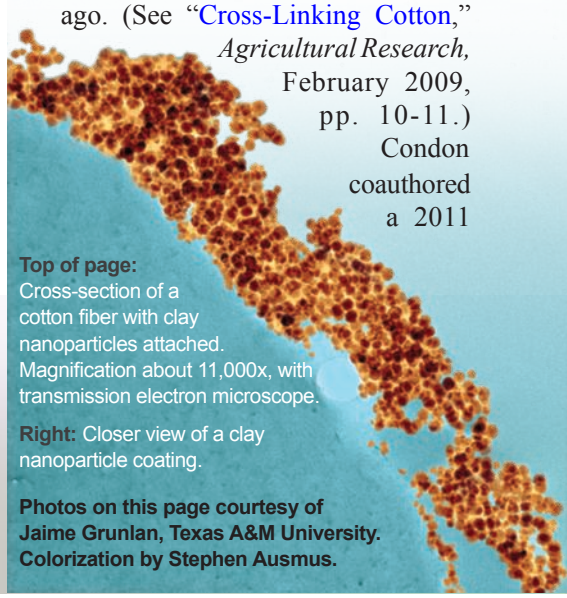
Halogenated flame retardants have been among the most widely used chemical treatments for cotton. But there's been a push to find alternatives that are not only more benign, but that also avoid imparting the same stiffness to fabric characteristic of some chemical treatments. For these and other reasons, "the textiles industry would like to move away from using halogenated flame retardants," says Condon.

Made of water-soluble polymers, nanoscale clay particles, and other "green" ingredients, the ARS-TAMU flame retardant is applied as a nanocoating that reacts to open flame by rapidly forming a swollen, charred surface layer. This process, known as "intumescence," stops the flame from reaching underlying or adjacent fibers.

A team led by Jaime Grunlan at TAMU's Department of Mechanical Engineering, in College Station, Texas, originally developed the intumescent nanocoating using a layer-by-layer assembly. In this procedure, alternating layers of positively and negatively charged ingredients, including clay particles 50-100 nanometers wide, are deposited onto the surface of a desired material. The result is a striated nanocoating that, when viewed under a scanning electron or other high-powered microscope, resembles the stacked layers of a brick wall.

Condon's interest was piqued after listening to Grunlan discuss his team's research at a recent American Chemical Society meeting, and he approached the TAMU professor about potential benefits to cotton. That conversation, in turn, led to a cooperative research project enabling Condon and Chang to evaluate the nanocoating at CCUR.

Treating cotton for flame resistance isn't a recent concept, adds Condon, whose lab is part of the ARS Southern Regional Research Center in New Orleans. In fact, some of the most successful early treatments were born of research conducted by Benerito and colleagues there several decades ago. (See "Cross-Linking Cotton," *Agricultural Research,* February 2009, pp. 10-11.) Condon coauthored a 2011



Top of page: Cross-section of a cotton fiber with clay nanoparticles attached. Magnification about 11,000x, with transmission electron microscope.

Right: Closer view of a clay nanoparticle coating.

Photos on this page courtesy of Jaime Grunlan, Texas A&M University. Colorization by Stephen Ausmus.

(D2466-1)

Nanotech

ACS Nano paper on the potential of intumescent coatings together with Chang, Grunlan and his TAMU team, and Alexander Morgan of the University of Dayton Research Institute in Ohio.

Early trials of the nanocoating using standard flame-resistance tests are promising. In one case, 95 percent of treated cotton fabric remained intact after exposure to flame, whereas the untreated fabric used for comparison was completely destroyed

"What we're investigating now is how well it will perform after repeated launderings of treated fabric," says Condon. "After all, the coating contains clay, and that's something detergents are made to remove."

Even if the coating does eventually wash out and the treated fabric loses its flame resistance, the nanotech approach could still be used to protect textiles and durable goods that aren't frequently washed, such as upholstery, mattress pads, box spring covers, automotive interiors, and firefighter coats.

Tapping Silver's Antimicrobial Properties

On another nanotech front, Condon and CCUR engineer Sunghyun Nam are investigating a way to inhibit microbial growth in cotton using silver particles ranging in size from about 2 to 6 nanometers. Silver nanoparticles have been used as an antimicrobial agent in many products, including clothes, plastic food containers, and medical textiles. The methods of producing them, however, have mostly relied on the use of toxic agents and organic solvents.

Condon's team has explored an alternative approach using an environmentally friendly agent, polyethylene glycol, and water as a solvent to generate silver nanoparticles of the desired size. Condon and Nam reported on this method in the *Journal of Nanoparticle Research* together with engineer Dharnidhar Parikh, formerly with CCUR. Also under investigation is a new method by which the nanoparticles form directly on cotton fiber, eliminating handling and storage of the particles before application. "This is a leg up for cotton over the synthetics," which have not been amenable to silver nanoparticle treatment, says Condon.

From Nanotech to Biotech

The CCUR scientists have also been busy on the biotech front.

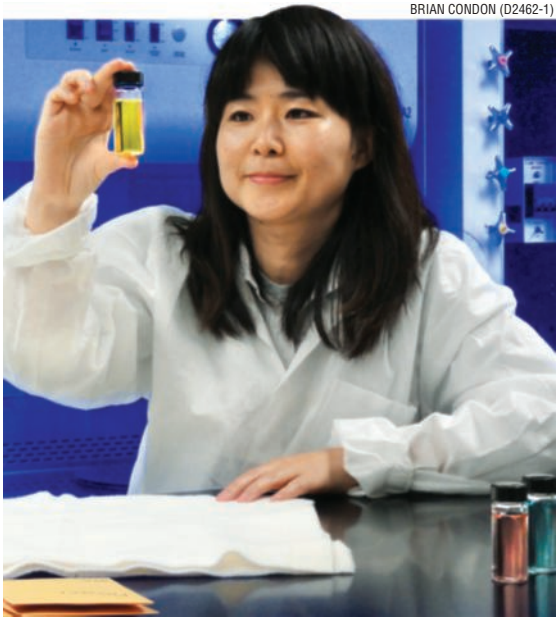
In one project, CCUR chemist Vince Edwards, together with Condon, devised a treatment for impregnating nonwoven cotton fabrics with lysozyme, an enzyme that slices open the cell walls of microorganisms, killing them—including microbes that cause infection or odor. That same cell-slicing capacity may also be used for biodefense applications that deactivate nerve agents—essentially by chewing them up, or "hydrolyzing" them, he adds. By adding the lysozyme to cottons, the resulting nonwovens could have these bactericidal and detoxifying properties.

In another biotech project, Condon and CCUR chemist Michael Easson are experimenting with equipment that generates an ultrasonic field of mechanical energy (similar to that used to clean jewelry) to improve the biobased processing of raw, or "greige," cotton using enzymes, like cellulase. Chemical processing agents are now used to strip away waxes, pectins, fats, and other fiber components that can hinder subsequent dying procedures and diminish the quality of finished cotton products. But the waste these "wet chemistry" methods generate is a concern.

"We're interested in enzymes as an environmentally friendly alternative, and we found that subjecting a solution of the enzymes to a field of ultrasonic energy can speed up their reaction rates," says Condon.

In experiments with lint fibers, for example, use of ultrasonic energy increased the bioprocessing efficiency of cellulase by 22 percent. The same concept can also work to improve the conversion of cotton sugars into biofuels—a potential value-added market for 2 million tons of U.S. cotton gin waste generated annually.

BRIAN CONDON (D2462-1)



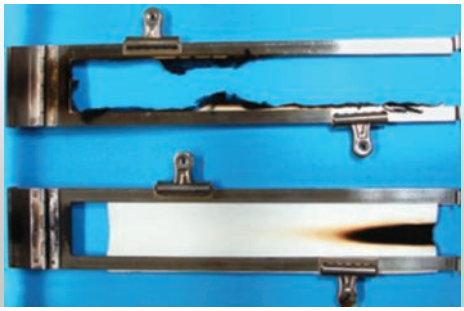
Chemist Sunghyun Nam examines suspensions of silver nanoparticles. Fabrics can be treated with these nanoparticles to add antimicrobial qualities.

Besides publishing their findings in scientific journals, Condon's team is actively seeking commercial partners to help usher these advances into the marketplace—all with an eye towards assuring the viability of America's $25 billion cotton industry at a time of increasing production costs, dwindling resources, and global competition.—By **Jan Suszkiw,** ARS.

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

Brian Condon is in the USDA-ARS Cotton Chemistry Research Unit, Southern Regional Research Center, 1100 Robert E. Lee Blvd., New Orleans, LA 70124; (504) 286-4540, brian.condon@ars.usda.gov.

Following flame exposure, the untreated fabric (top) is completely destroyed, but the fabric treated with clay nanoparticles is intact.



SeCHIN CHANG (D2465-1)

Biotech

PEGGY GREB (D1518-1)

Barcoding Insects To Control Them

Adults of the native carabid beetle *Lebia grandis* are voracious predators of Colorado potato beetle eggs and larvae.

Mention barcodes and it often brings to mind the sales tags and scanners found in supermarkets and other stores. But Agricultural Research Service scientists are using "DNA barcodes" in their search for ways to control and monitor insects that pose the greatest threats to crops as diverse as wheat, barley, and potatoes.

In DNA barcoding, scientists sequence a designated part of an organism's genome and produce a barcode from it for a systematic comparison with the sequenced DNA of other closely related species. DNA barcodes are being developed on a wide range of plants and animals as part of a global effort to catalog the diversity of life on Earth.

At the Invasive Insect Biocontrol and Behavior Laboratory in Beltsville, Maryland, entomologist Matthew Greenstone is using DNA barcodes in an unconventional way: to identify insect predators best equipped to control the Colorado potato beetle. The Colorado potato beetle is the single most damaging insect pest of potatoes in the eastern United States. It also damages tomatoes and peppers and is known for developing resistance to any pesticides used to control it.

Greenstone is trying to find the insects that are the beetle's worst nightmare. Numerous studies have analyzed the gut contents of predator insects to evaluate their ability to control pests. But predators eat and digest prey at different rates, so simple gut analysis is insufficient for accurately comparing predator effectiveness, Greenstone says. He has fine-tuned that approach and used barcoding to come up with a way to factor in how quickly different predatory insects actually digest the Colorado potato beetle.

"Scientists often use barcoding to distinguish one closely related species from another. We're using it to identify prey in the gut of an insect predator, and in a sense, that's an atypical use," says Greenstone. Greenstone and his colleagues collected four insects that previous studies showed were the most common potato beetle predators. They fed them laboratory-raised potato beetles and looked at the digestion rates of each of the four insects to determine the Colorado potato beetle's DNA "half life"—defined as the point at which at least some DNA of the potato beetle could still be found in half of the fed individuals of each predator species. They used the potato beetle's barcoded DNA to detect it in the predators' guts.

The results, published in the journal *Entomologia Experimentalis et Applicata,* show the importance of taking digestion rates into account when considering different insect predators as biocontrol agents. They may also provide guidance to growers on the most effective control strategies for combating a voracious pest.

"Different pesticides have different effects on different predators, and not all predators are equally susceptible to all insecticides. Based on what you learn, you might delay spraying insecticides, rule out the use of insecticides that harm your most important biocontrol agents, or limit spraying to certain times, depending on the predator's habits," Greenstone says.

ARS researchers are also using barcoding to understand and track the threat of various biotypes of Russian wheat aphid, an insect about the size of a sesame seed, that is a major worldwide pest of wheat, barley, and other cereals. Since it appeared in 1986 in Texas, it has cost U.S. wheat growers alone about $200 million each year.

Gary Puterka, an entomologist in the ARS Wheat, Peanut, and Other Field Crops

Russian wheat aphid adult next to its young. This aphid gives live birth to young that are identical clones of itself during the asexual phase of its lifecycle.

GARY PUTERKA (D2491-1)

Biotech

Research Unit in Stillwater, Oklahoma, periodically surveys Russian wheat aphid populations across eight western states to provide guidance to wheat growers on infestation levels and on the range of biotypes so they can decide whether to implement control measures. He works closely with ARS entomologist Kevin Shufran, who is also in Stillwater, on efforts to control the aphid.

Until recently, the Russian wheat aphid, in North America, was believed to reproduce strictly by asexual cloning, which made it particularly susceptible to resistance mechanisms bred into wheat and barley crops. But Puterka's surveys have turned up evidence showing that it is reproducing sexually.

If the Russian wheat aphid is sexually reproducing, the resulting genetic recombination would produce new "bioytpes" that will be better equipped to counter resistant crops by giving each new generation a varied genetic tool kit, increasing the likelihood that offspring will be able to overcome the plant's resistance mechanisms, feed on it, and survive to reproduce resistant offspring. Sexual reproduction could also broaden the pest's range, enabling it to lay eggs as cooler weather approaches, like other alpine aphids do, and survive harsh winters.

Researchers found a new biotype of Russian wheat aphid in Colorado in 2003 that spread so quickly and caused so much damage—overcoming a new resistance gene that breeders had recently developed for wheat growers—that its success was seen as a new introduction or evidence that the pest had "gone sexual." Subsequent surveys have turned up additional evidence of new biotypes, an evolving threat.

Puterka's survey showed that the Colorado biotype now makes up 90 percent of the population in eight major wheat-producing states. Another study by Puterka found a small, localized population of Russian wheat aphids in a Colorado field that were the offspring of male and female parents. This population of nymphs had a signature trait—missing antenna segments—that was evidence they hatched from eggs and were the result of sexual reproduction. Thirty-nine new biotypes were detected when these nymphs were increased and screened.

"These populations are continually shifting in terms of different biotypes, and as natural selection dictates, the biotypes that are the fittest are the ones that will survive and dominate," says Puterka.

But the extent of the threat posed by the Russian wheat aphid largely remains a mystery, and the field surveys are time consuming. For accurate results, Puterka must make clone colonies from each adult collected and screen them by exposing them to nine different types of resistant germplasm to confirm their biotype.

The most efficient way to determine whether Russian wheat aphids are sexually reproducing would be to find their eggs. When the females reproduce asexually, they give birth to live females, but they lay eggs when they reproduce sexually. The problem is that the eggs of all aphid species look alike, so the scientists cannot distinguish Russian wheat aphid eggs from other aphids' eggs.

Shufran and Puterka have developed a process that uses DNA barcoding to tell the different aphid eggs apart. To establish

PEGGY GREB (D2486-1)

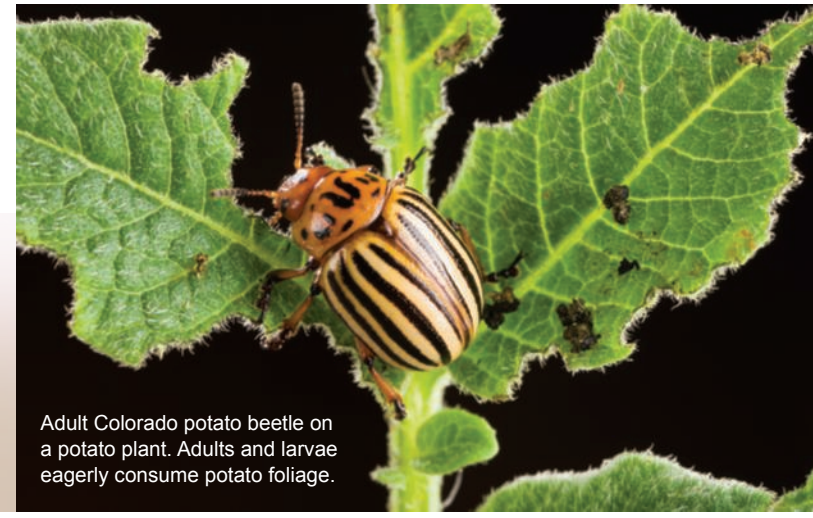
Entomologist Matt Greenstone examines DNA analysis results of Colorado potato beetle removed from the gut of an insect predator collected from the field. He is looking for evidence that the predator mainly consumes Colorado potato beetle.

that it would work, they extracted DNA from the eggs of 10 previously identified species of aphids, including several of the Russian wheat aphid's closest relatives. They sequenced the first 640 base pairs of a gene known as *"CO1."* In a blind test, Shufran compared DNA from eggs provided by Puterka, who masked the identities of the different species. With help from various aphid genetic databases, Shufran was able to correctly distinguish the different aphid species by comparing their *CO1* sequences. Results were published in *Annals of the Entomological Society of America*.

With the new tool, Puterka and Shufran will be able to identify Russian wheat aphid eggs for the first time and can better track the biotypic diversity of an aphid that poses a major threat to wheat and other crops.—By **Dennis O'Brien,** ARS.

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

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



Adult Colorado potato beetle on a potato plant. Adults and larvae eagerly consume potato foliage.

PEGGY GREB (D1515-1)

SCOTT BAUER (K7038-1)

Metagenomics Offers Insight Into Poultry Diseases

Less than 10 years ago, the world marveled at the completion of the human genome project, which involved traditional technology to identify all the genes in a single organism—the human. Today, a more powerful technology is being used to detect thousands of organisms in an entire community.

Unlike traditional gene sequencing, the new molecular technique—metagenomics—eliminates the need to cultivate and isolate individual microbial species. Scientists can apply genomic analysis to mixed communities of microbes instead of to just one organism.

For example, researchers examining viral enteric (intestinal) diseases in poultry can take intestinal samples from different poultry flocks. The material can be processed to sequence all the viral nucleic acid—RNA and DNA—in the sample and then analyzed as a single genome.

Learning more about how genes interact is extremely important in the battle against enteric diseases for scientists at the Agricultural Research Service Southeast Poultry Research Laboratory (SEPRL) in Athens, Georgia. Disorders like poult enteritis mortality syndrome, poult enteritis complex, and runting-stunting syndrome cause diarrhea in birds, resulting in decreased weight, mortality, and increased production costs.

Research has revealed that several viruses may be responsible for enteric diseases, yet a single causative agent has not been identified. Metagenomics research may help solve that mystery.

Scientists at SEPRL are using metagenomics to uncover vast amounts of known and previously unknown viruses in poultry. They have discovered and sequenced the complete genome of a new bacteriophage (phage) that might have future antimicrobial applications, described for the first time the complete genome of new chicken and turkey parvoviruses, and developed a PCR (polymerase chain reaction) test to detect these novel parvoviruses in commercial poultry flocks.

Biotech

Unlocking a Treasure Trove

With help from industry producers and veterinarians, microbiologist Michael Day and research leader Laszlo Zsak, in SEPRL's Endemic Poultry Viral Diseases Research Unit, collected intestinal samples from five different turkey flocks affected by enteric disease. To identify and characterize viruses using metagenomics, they prepared intestinal homogenates from the samples. The homogenates were filtered to remove larger constituents, like bacteria, and leave the smaller particles, like viruses. Metagenomics techniques were then used to sequence nucleic acid of all the RNA viruses present in the samples.

"I was expecting to find RNA sequences from viruses that had not been described before in the poultry gut," Day says. "It turned out that there were quite a number of viruses in that particular sample."

A comparison to similar viruses in computer databases showed that the intestinal virus metagenome contained thousands of pieces of nucleic acid representing many groups of known and unknown turkey viruses. Common avian viruses such as astrovirus, reovirus, and rotavirus were confirmed. Many RNA viruses, like members of the Picornaviridae family, were also detected.

An unexpected discovery was an abundance of previously unknown turkey viruses, such as picobirnavirus, a small,

In studies of intestinal samples from turkeys with enteric diseases, ARS scientists have discovered a new virus that may have future antimicrobial applications.

SCOTT BAUER (K7043-16)

double-stranded RNA virus implicated in enteric disease in other agricultural animals, Day says. A calicivirus—the kind associated with human enteric diseases—was also identified in poultry.

Prospects of a Novel Phage

"Because metagenomics is so powerful, we generated and continued to analyze additional data from these samples and discovered a new bacteriophage," Zsak says. "Until now, no one had described this kind of phage in turkey enteric samples."

The virus, called "phiCA82," belongs to a group known as "microphages" and is the type of virus that naturally kills bacteria, Zsak says. Phages are important because they can potentially be used as alternatives to antibiotics and as weapons against multi-drug-resistant pathogens.

Zsak and Day found a short sequence of the phage DNA and designed a technique to sequence its entire genome. Colleagues Brian Oakley and Bruce Seal, both microbiologists in the Poultry Microbiological Safety Research Unit of the ARS Richard B. Russell Agricultural Research Center, also in Athens, helped analyze the data. One task was to find out whether the new phage was related to similar viruses.

"That's a question you would have with the discovery of any new kind of organism," Oakley says.

Oakley downloaded all publicly available viral genome sequences and used bioinformatics—the application of computer science and information technology to the field of biology—to compare the newly discovered genome to previously discovered ones. The comparisons revealed that the new genome was unique.

"Future studies need to be completed to find out if phages like this actually kill the bacteria they infect," Zsak says. "Once we can identify this mechanism, we can design identical ways to attack and kill these pathogens."

Phages infect bacteria and then replicate, Seal explains. They do this by digesting the cell walls of bacteria.

"We are interested in being able to clone the gene that expresses enzymes that digest the cell wall," Seal says. "If we can express those enzymes in an organism generally recognized as safe, like yeast for example, we can put them in feed to help reduce certain types of bacteria that cause disease."

Chipping Away at Chicken Viruses

In earlier studies, Zsak and Day used metagenomics to identify and analyze the genome of a novel chicken parvovirus, ChPV ABU-P1.

"This was the first in-depth characterization and analysis of the full-length genome sequence of the chicken parvovirus," Day says. "Comparisons were made to other members of the Parvovirinae subfamily that infect mammals and birds."

Scientists also developed a PCR assay to detect the virus in turkeys and chickens and used the test to examine enteric samples collected from U.S. commercial turkey and chicken flocks across different regions.

"PCR proved to be highly sensitive and specific in detecting parvoviruses in both clinical samples from infected birds and field samples from turkeys and chickens with enteric diseases," Zsak says.

Advantages of a Community Approach

The overall goal is to use metagenomics technology to develop and update diagnostic tools, identify effective new treatments, and improve management practices to help control costly animal and plant diseases, Day says.

The beauty of metagenomics is that viruses do not have to be isolated or identified. Small pieces of nucleic acid can be sequenced from samples taken from mixed communities—a process that allows scientists to discover new enzymes and proteins and look for genetic markers for disease-resistant traits or genes with possible antimicrobial applications.

"We need some way to understand a community and interrogate the nucleic acids in that community to see who's there and what they're doing," Oakley says. "Are there pathogenic bugs in there? Are there genes associated with pathogenesis? Metagenomics does that."—By **Sandra Avant,** ARS.

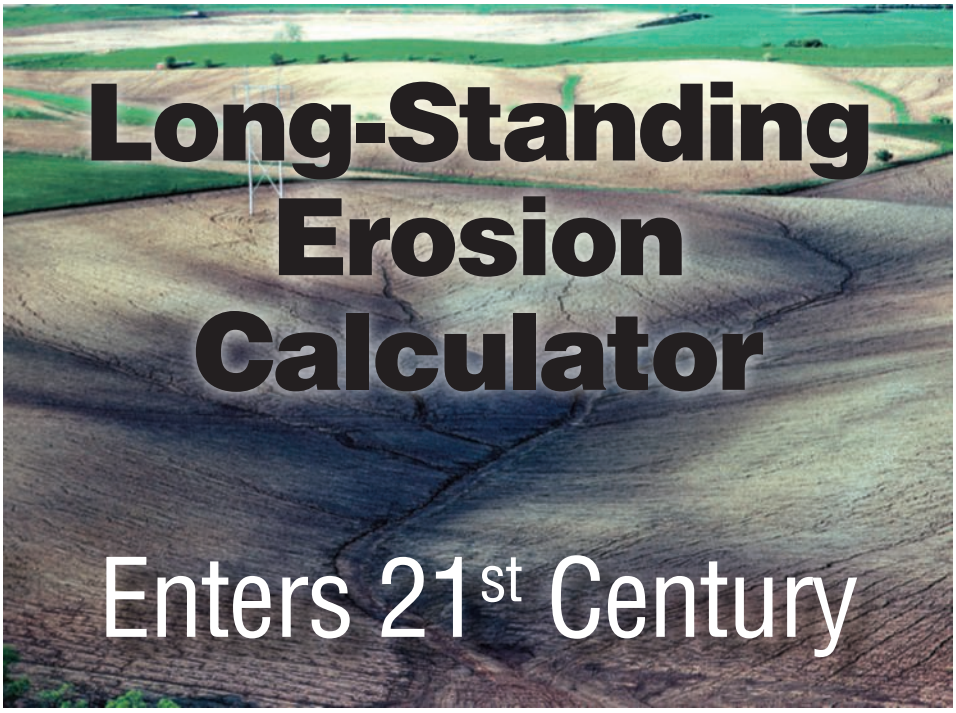
This research is part of Animal Health (#103) and Food Safety (#108), two ARS national programs described at www.nps.ars.usda.gov.

To reach scientists mentioned in this article, contact Sandra Avant, USDA-ARS Information Staff, 5601 Sunnyside Ave., Beltsville, MD 20705-5129; (301) 504-1627, sandra.avant@ars.usda.gov.

Microbiologist Michael Day examines the validation results of a new molecular diagnostic assay for a turkey picobirnavirus. Day used a metagenomic approach to detect the novel picobirnavirus RNA in turkeys experiencing enteric (intestinal) disease.

LAURA FERGUSON (D2470-1)

LYNN BETTS (NRCSIA99126)



Long-Standing Erosion Calculator Enters 21st Century

The core function of RUSLE2 is to estimate soil eroded by the impact of raindrops and by the flow of runoff water across land disturbed by plowing and tilling. Erosion caused by concentrated ephemeral gully flow in topographic swales is not currently included in RUSLE2 predictions but will be in the future.

Seth Dabney is busy tweaking a soon-to-be-unveiled update of the Revised Universal Soil Loss Equation, Version 2 (RUSLE2), which moves the original equation ever further from its origins in the age of slide rules to the era of computing.

Dabney is research leader of the Watershed Physical Processes Research Unit, at the Agricultural Research Service's National Sedimentation Laboratory in Oxford, Mississippi.

RUSLE2 has retained the integrity of the original Universal Soil Loss Equation (USLE)—in greatly expanded form—and integrated an updated database with a computer model that reflects both the latest in computer technology and scientific discoveries about erosion processes. It is unlikely that there is a more powerful, proven, practical computer model than RUSLE2. Its ability to predict daily erosion related to any human activity anywhere in the nation, based on a host of conditions, through a combination of simulation model, vast database, and scientific knowledge, makes it an excellent example of computational science and technology.

But RUSLE2 remains first and last what it was always designed to be: a management tool that allows conservationists to make better management decisions.

This state-of-the-art technology traces its heritage to the paper-and-pencil version of USLE, which has been the standard way to estimate soil erosion on farms for more than four decades. It was first developed in the late 1950s, before calculators, let alone computers. In fact, at one point the equation came with plastic slide rules custom made for it. The USLE is now recognized as one of the most significant developments in soil and water conservation history, worldwide.

Standing the Test of Time

The USLE began as a fairly simple equation that gave its answer in tons of soil lost per acre per year by multiplying five factors, with the numbers for each factor derived from paper tables of figures for different parts of the country and different soils. Proof of the worth of USLE's humble pencil-and-paper calculation is that it is still embedded as the heart—and anchor—of the new, sophisticated model that houses it, although the tables of factors no longer exist.

Its core function is to estimate soil eroded by the impact of raindrops and by the flow of runoff water across land disturbed by plowing and tilling. But functions have been added over the years, such as estimating how much plant residue can be removed from crop and pasture lands for ethanol production without exposing the soil to excessive erosion.

And the equation continues to add capabilities. It is now used to estimate erosion wherever land is disturbed, whether by farming or ranching, pasture replanting, or nonfarm activities such as construction, mining, or clearcutting and road building in forests.

Since the USLE moved into widespread use in the 1960s and 1970s, every conservation plan written for farmers, ranchers, and others by the USDA Natural Resources Conservation Service (NRCS) has been based on soil-erosion calculations derived from this equation. It is used by all 3,000 NRCS field offices as well as the agency's state and area offices.

Ken Renard, a hydraulic engineer who came to ARS in 1957 and has been a leader in the development of the USLE and RUSLE even in retirement, says that as "research made a lot of progress in more realistically describing erosion from farming operations," the need to update USLE technology became clear.

Computational Tech

The result was RUSLE1, which started moving into use in 1992; it was the first wholesale reworking of the erosion-predicting technique using digital computer technology.

The Improvements Continue

Spearheaded by now-retired ARS agricultural engineer George Foster, RUSLE2 is actually a combination of observation- and process-based science, incorporating its original roots in collected field data with the latest in computer models that can simulate processes such as erosion, says Giulio Ferruzzi, a conservation agronomist at the NRCS West National Technology Support Center in Portland, Oregon. Ferruzzi forwards all of the agency's requests for changes to the RUSLE2 to Dabney and Daniel Yoder, a professor at the University of Tennessee at Knoxville.

At the time RUSLE1 moved into use, the task of improving its computer programs fell to Yoder, who, while a graduate student, worked for ARS as an agricultural engineer at the National Soil Erosion Laboratory in West Lafayette, Indiana, at Purdue University, the birthplace of the equation.

Now, 20 years later, Yoder is still improving the RUSLE2 version, first developed in 2001. RUSLE2 brought the equation into the 21st century, making it a much more powerful tool, yet much more user-friendly, with graphics, including icons for things like bulldozers and tractors.

Yoder says, "I take Dabney's science ideas and translate them into computer code. But the process is give-and-take between me, Dabney and his ARS colleagues, and NRCS." He says their recent work on improvements to RUSLE2 involved better prediction of crop residue.

Ferruzzi adds that Dabney for the first time made it possible for NRCS to help ranchers more accurately learn the effects of cattle grazing on erosion. The update about to be unveiled simulates the natural life and death of pasture plants as well as the effects of the different eating habits of grazing animals. Ferruzzi explains that when cows are let into a pasture for a short time, they tend to eat everything in sight by "mob grazing." But when cows are put in a pasture for a season, they have time to be more selective, eating only the freshest growth. The model Dabney is working on takes that into account.

A New Way To View Erosion: But Ephemeral Gullies Still Elude

Dabney also added a way to model the erosive flow of water on an entire hill slope as one block, from top to bottom, missing—for now—only the topographic swales where water runs intermittently when it rains heavily and forms ephemeral gullies. The current version also predicts where the eroded soil will collect after it leaves the hillside, something particularly important to construction planners.

Renard says that ephemeral gullies often cause the most erosion on farms. They're particularly insidious because they are obliterated by tillage, but reappear during a wet spell the next season. Renard says that although RUSLE2 is still missing these gullies, Dabney is moving in the direction of solving that.

Ferruzzi says NRCS can use this new modeling technique to pinpoint where permanent gullies might form and suggest grassed waterways or other conservation measures there to prevent gully formation.

A Crown Jewel

Yoder calls the extensive NRCS database a "crown jewel of RUSLE2." Federally supported data collection began with 10 experiment stations in 1929, located strategically to represent as many crops and climate and management conditions as possible. NRCS has since built it up to include every state in the nation plus the territory of Puerto Rico and the Pacific protectorates.

Ephemeral gullies like this one in a central Iowa field often cause much erosion on farms. These gullies seem to disappear after tillage but then reappear during rain events.

LYNN BETTS (NRCSIA99140)

ARS NATIONAL SEDIMENTATION LABORATORY (D2464-1)

Spearheaded by now-retired ARS engineer George Foster, seen here in the late 1960s or early 1970s, RUSLE2 is a combination of observation- and process-based science, incorporating its original roots in extensive field data into a state-of-the-art computer-based conservation tool.

Dabney agrees, saying that the NRCS database is exhaustive, with 30,000 combinations of vegetation, farming and ranching operations, and residue types. "NRCS developed this database at regional centers in Nebraska, Oregon, South Carolina, and Texas, and it has been in use for 10 years now," Dabney says. NRCS has also developed maps for RUSLE2 that divide the country into crop management zones. The maps disregard political boundaries, instead delineating zones based on common crops and management practices.

Dabney says that it is this very database that keeps RUSLE2 from coming untethered from reality, as pure process models do when they operate outside of known territory. RUSLE2 can operate accurately anywhere in the United States—and probably the world—because it is grounded by the vast database collected from field measurements.

Dave Lightle, former NRCS national database manager for RUSLE2, worked for 10 years to produce the database, with the different combinations of management scenarios broken down by the crop management zones. The scenarios replace USLE's key crop management factor. "RUSLE2 now creates these scenarios. It goes way beyond USLE and RUSLE1," says Lightle, who continues to work part-time on RUSLE2 in retirement. Linda Scheffe, at the NRCS National Soil Survey Center in Lincoln, Nebraska, is the current RUSLE2 database manager.

RUSLE2 can operate accurately anywhere in the United States—and probably the world—because it is grounded by the vast database collected from field measurements.

The database contains 20 key soil properties that affect soil erosion for all soils in the United States. It also contains the climate for all counties, the precipitation ranges across the country, and hundreds of tillage options. RUSLE2 provides daily information, unlike USLE, which provided information on a yearly basis.

Lightle says that this database is the most sought-after feature of RUSLE and is being used in other models. Currently, users have to download soil data from the NRCS National Soil Information System, the agency's official soil database. But Lightle says that in the near future RUSLE2 will be live on the Internet, enabling access to that data without downloading.—By **Don Comis,** formerly with ARS.

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

To reach scientists mentioned in this story, contact Robert Sowers, USDA-ARS Information Staff, 5601 Sunnyside Ave., Beltsville, MD 20705-5129; (301) 504-1651, robert.sowers@ars.usda.gov. ✻

The Agricultural Research Service has labs all over the country.

Locations Featured in This Magazine Issue

Locations listed west to east below.

Davis, California
3 research units ■ 114 employees

Stillwater, Oklahoma
2 research units ■ 32 employees

Center for Grain and Animal Health Research, Manhattan, Kansas
5 research units ■ 129 employees

Southern Regional Research Center, New Orleans, Louisiana
7 research units ■ 181 employees

Oxford, Mississippi
3 research units ■ 99 employees

West Lafayette, Indiana
3 research units ■ 72 employees

Athens, Georgia
9 research units ■ 195 employees

U.S. Vegetable Laboratory, Charleston, South Carolina
1 research unit ■ 39 employees

Robert W. Holley Center for Agriculture and Health, Ithaca, New York
3 research units ■ 62 employees

Henry A. Wallace Beltsville Agricultural Research Center, Beltsville, Maryland
27 research units ■ 890 employees

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

U.S. Department of Agriculture
Agricultural Research Magazine
5601 Sunnyside Ave.
Beltsville, MD 20705-5129

PRST STD
Postage and Fees Paid
U.S. Department of Agriculture
Permit No. G-95

Official Business

Please return the mailing label
from this magazine:

To stop mailing _____ ☐
To change your address _____ ☐