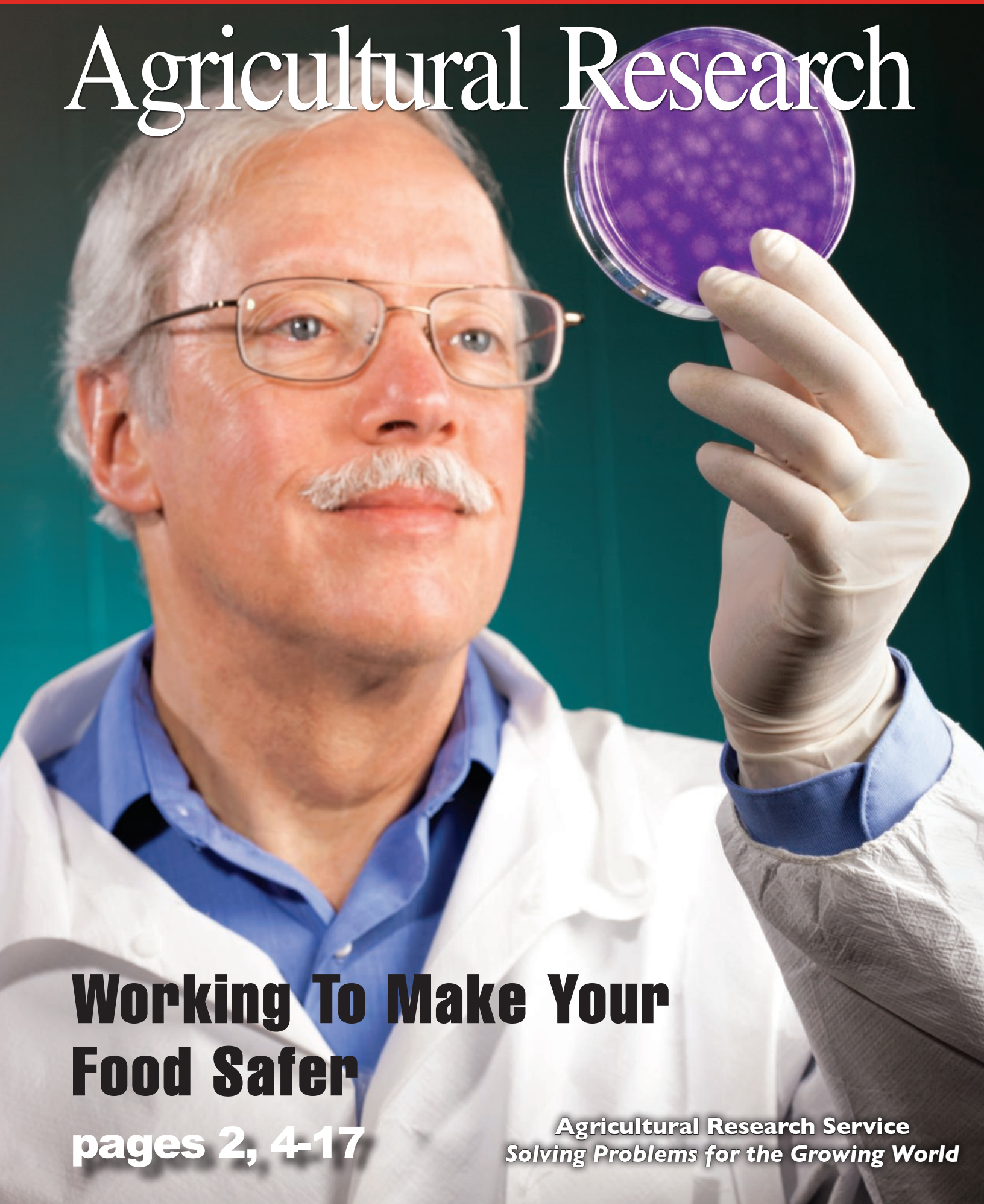
U.S. Department of Agriculture | Agricultural Research Service | April 2011

Agricultural Research



Working To Make Your Food Safer

pages 2, 4-17

Agricultural Research Service
Solving Problems for the Growing World

Food Safety Advances and Collaborations Here and Abroad

The safety of the food supply has become an increasingly visible global public health issue. Outbreaks of foodborne illness are seen as a major cause of morbidity, mortality, and economic burden. The cause of many outbreaks remains unresolved, and issues such as increased international trade, changes in eating habits, and increased travel abroad complicate investigations.

Persistent outbreaks that may directly affect public health, industry, and trade require the immediate attention of the nation's food safety team.

The Agricultural Research Service's national program on Food Safety provides, through research, the means to ensure that food and feed meet foreign and domestic regulatory requirements and are safe for consumers. The program's research, described in its 2011-2015 Strategic Action Plan (available as a pdf at tinyurl.com/FoodSafetyPlan) seeks ways to assess, control, or eliminate potentially harmful food contaminants, including both introduced and naturally occurring pathogenic bacteria, viruses, and parasites, as well as toxins and non-biological-based chemical contaminants, mycotoxins, and plant toxins.

Food safety research has changed during the past decade, moving past simple surveillance procedures to asking complex questions relative to public health. The food chain is a single entity, where each stage of production, processing, and distribution is part of a larger system. Consequently, the program is creative, considering alternate perspectives, exploiting new opportunities and technologies, and crossing conventional boundaries.

Safe food is not just a local issue; it's a global one. Therefore, ARS's efforts involve both national and international collaborations through formal and informal partnerships. Accomplishments and outcomes from research efforts benefit agencies both here (like the U.S. Department of Agriculture's Food Safety and Inspection Service, the U.S. Food and Drug Administration, and the U.S. Environmental Protection Agency) and abroad (the United Kingdom's Food Standards Agency, the European Food Safety Authority, and the World Health Organization), as well as commodity organizations, industry, and consumers.

During the past few years, there have been many accomplishments from the ARS Food Safety national program. A new method was developed to detect Shiga-toxin-producing non-O157:H7 enterohemorrhagic *E. coli,* which causes an illness in humans similar to that caused by *E. coli* O157:H7. And dioxin surveys have substantiated the safety of the U.S. meat and poultry supply here and abroad.

Other new technologies include a process imaging system for the USDA's Agricultural Marketing Service to detect small cracks, blood spots, and structural deformities in eggshells and a process for detecting ricin, staphylococcal enterotoxins, and botulinum neurotoxins in foods. These new technologies are superior to any others that were commercially available.

Another new technology, called "QuEChERS," was developed to monitor chemical residues in foods. The method was successfully validated for implementation in regulatory monitoring labs in the United States, the European Union, and other countries and is considered the gold standard for residue detection.

Other research endeavors include determining a baseline for the environmental prevalence of *E. coli* O157 and non-O157 *E. coli* in the Salinas Valley, providing the first epidemiological data in the area known as the "Salad Bowl of America." This was in collaboration with the University of California-Davis and the USDA Animal and Plant Health Inspection Service's Wildlife Services-California.

Our researchers are also conducting studies to better understand the long-term effects of antibiotic use and find alternatives to control foodborne pathogens. The ARS Food Safety national program involves extensive national and international collaborations and scientific exchanges with many sources. Our scientists work with the Center for Food Safety Engineering at Purdue University to develop new technology platforms for improving microbial and chemical hazard detection. We contribute to Combase, the international database resource on the behavior of pathogens in foods. We work with various international partners in the European Commission on integrated projects such as MycoRed, which aims to find strategies to reduce mycotoxins in feed and food. Work is also under way with the Shanghai Jiao-Tong University in China; the Academy of Finland; Teagasc and University College in Dublin, Ireland; the Institute of Chemical Technology in Prague, Czech Republic; the National Veterinary Institute in Oslo, Norway; the University of Tasmania in Tasmania, Australia; the International Institute of Tropical Agriculture in Nigeria, Africa; and the Institute of Food Research and the Food Standards Agency in the United Kingdom.

No single program can solve the food safety challenges and issues that confront us now or in the future. Multidisciplinary collaborations are necessary to integrate resources and develop strategies for solving specific problems. In this way, the research program as a whole is expected to substantially enhance the global safety of the food supply.

James A. Lindsay
ARS Senior National Program Leader
Food Safety
Beltsville, Maryland

Mary E. Torrence
ARS National Program Leader
Food Safety
Beltsville, Maryland

April 2011
Vol. 59, 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.

Tom Vilsack, Secretary
U.S. Department of Agriculture

Catherine E. Woteki, Under Secretary
Research, Education, and Economics

Edward B. Knipling, Administrator
Agricultural Research Service

Sandy Miller Hays, Director
Information Staff

Editor: Robert Sowers	(301) 504-1651
Associate Editor: Sue Kendall	(301) 504-1623
Art Director: BA Allen	(301) 504-1669
Photo Editor: Anita Daniels	(301) 504-1609
Staff Photographers:	
Peggy Greb	(301) 504-1620
Stephen Ausmus	(301) 504-1607

Most information in this magazine is public property and may be reprinted without permission (except where copyright is noted). Non-copyrighted articles and high-resolution digital photos are available at ars.usda.gov/ar.

Paid subscriptions are available from the U.S. Government Printing Office (Superintendent of Documents). See back cover for ordering information. Complimentary 1-year subscriptions are available directly from ARS to public libraries, schools, USDA employees, and the news media. Call (301) 504-1638 or e-mail armag@ars.usda.gov.

This magazine may report research involving pesticides. It does not contain recommendations for their use, nor does it imply that uses discussed herein have been registered. All uses of pesticides must be registered by appropriate state and/or federal agencies before they can be recommended.

Reference to any commercial product or service is made with the understanding that no discrimination is intended and no endorsement by USDA is implied.

The USDA prohibits discrimination in all its programs and activities on the basis of race, color, national origin, age, disability, and where applicable, sex, marital status, familial status, parental status, religion, sexual orientation, genetic information, political beliefs, reprisal, or because all or part of an individual's income is derived from any public assistance program. (Not all prohibited bases apply to all programs.) Persons with disabilities who require alternative means for communication of program information (Braille, large print, audiotape, etc.) should contact USDA's TARGET Center at (202) 720-2600 (voice and TDD). To file a complaint of discrimination, write to USDA, Director, Office of Civil Rights, 1400 Independence Avenue, S.W., Washington, D.C. 20250-9410, or call (800) 795-3272 (voice) or (202) 720-6382 (TDD). USDA is an equal opportunity provider and employer.

PEGGY GREB (D1448-2)

Stories on pages 2 and 4-17 emphasize some of the many ways ARS is working to keep food safe both here in the United States and abroad.

Cover: In seafood-focused food safety research, microbiologist Gary Richards evaluates a hepatitis A virus assay at the ARS laboratory at Delaware State University-Dover. See article beginning on page 16 for more about ARS's work with this virus. Photo by Peggy Greb. (D2171-1)

Pouncing on Food Pathogens: It Takes a Planet!

PEGGY GREB (D2161-1)

At the Plant Mycotoxin Research Unit in Albany, California, molecular biologist Jong Kim (left) and research leader Bruce Campbell inspect assays of natural compounds that can significantly improve the fungicidal activity of commercial antifungal agents.

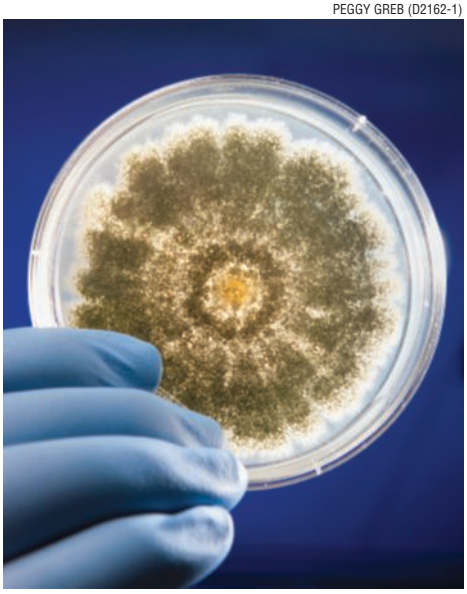
Fungi that produce chemicals harmful to people, animals, or plants respect no boundaries. *Aspergillus flavus,* the fungus that makes cancer-causing compounds known as "aflatoxins," is one such organism. This mold is a threat to the wholesomeness of popular tree nuts worldwide, mainly almonds, pistachios, and, to a lesser extent, walnuts.

Agricultural Research Service scientists in Albany, California, have teamed up with colleagues halfway around the globe—in Moscow—to explore new strategies for destroying *A. flavus*. Their anti-*Aspergillus* tactics might help quell other troublesome fungi, as well. That is why this collaboration with the Russian Research Institute of Phytopathology in Moscow encompasses not only *A. flavus* but also several other key fungal foes. Targeted microbes include, for instance, *Fusarium culmorum* and *Bipolaris sorokiana,* both of which can cause root rots and other problems, and *Alternaria alternata,* which causes leaf spot disease of some crops.

Research leader Bruce C. Campbell, who heads the ARS Plant Mycotoxin Research Unit at Albany, developed the international collaboration to quicken discovery of natural compounds that could work in concert with known fungicides. Such pairings would deliver a one-two punch, with the natural compound making the target fungi more vulnerable to the fungicide.

Studies at Albany, started in 2004 by Campbell and by ARS molecular biologist Jong H. Kim, provide strong evidence to support this intriguing concept. By reducing the amount of fungicides commonly used today, the strategy may prove to be less costly and more environmentally friendly than conventional approaches, Campbell says.

PEGGY GREB (D2162-1)



Petri dish containing the fungus *Aspergillus flavus*. This common fungus is a concern because it produces carcinogenic aflatoxins, which can contaminate certain foods and cause aspergillosis, an invasive fungal disease.

Campbell, Kim, and their collaborators have published their findings in *Applied Microbiology and Biotechnology, Biochemical and Biophysical Research Communications, FEMS Microbiology Letters, Fungal Biology, Letters in Applied Microbiology, Mycopathologia, and World Mycotoxin Journal.*

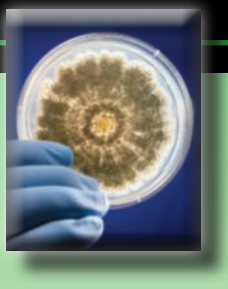
At the Moscow institute, scientists are rigorously testing the concept in studies coordinated by Vitaly Dzhavakhiya and Larisa Shcherbakova. In their experiments, the team has determined, for example, that a very small amount of thymol, a natural compound from thyme, when added to Folicur (tebuconazole), a commercial fungicide, "was about twice as effective in reducing growth of *A. alternata* than when the fungicide and thymol were applied singly," Shcherbakova reports.

In related work, the team is testing enzymes produced by beneficial, edible mushrooms, determining the enzymes' prowess as "biodestroyers" of aflatoxins. Several mushrooms in the *Phoma* genus appear to be promising sources of aflatoxin-degrading enzymes.

Both the U.S. and Russian teams are also curious to know how, precisely, the most promising natural compounds and enzymes succeed in disrupting the inner workings of harmful fungi. In particular they want to discover how the compounds and enzymes reduce a fungus's ability to grow, to defend itself against fungicides, and—in the case of certain *Aspergillus* species—to produce aflatoxins.

The project is one of many collaborations with the former Soviet Union that are administered by ARS's Beltsville, Maryland-based Office of International Research Programs. In fiscal year 2010, the U.S. Department of State provided about $1 million to fund these collaborations.

Other ARS international partnerships target other problematic microbes. Here's a quick look at two of those projects.



High-Tech Tactics To Detect Pathogen Sources on Fresh Produce

Before that crisp head of lettuce or juicy apple reaches your hands, it passes through a series of inspections to make sure it's good enough for you to eat. From color to shape to size, the produce is evaluated against a wide variety of criteria before it arrives in your local grocery store.

Number one on that list? Food safety, because the qualities you love about fresh produce won't matter one bit if you get sick. That's why biophysicist Moon Kim is working hard to develop new technologies that can help food safety inspectors detect harmful pathogens on the fruits and vegetables everyone enjoys.

Kim works with ARS agricultural engineer Kevin Chao, biomedical engineer Alan Lefcourt, and others at the Environmental Microbial and Food Safety Laboratory in Beltsville, Maryland. They have been recognized for their advances in food safety technology. The researchers first developed a high-speed, multispectral line-scan imaging system for use on poultry carcasses, which has been applied to identifying unwholesome birds and detecting traces of feces that could transmit harmful pathogens to humans. They are modifying the technology for use on fresh fruits and vegetables, which can also contain traces of feces from manure used to fertilize the soil. (Read more about this in "Machine's Eye View of Poultry and Produce," *Agricultural Research,* January 2007.)

Now, through a formal agreement with South Korea's Rural Development Administration, Kim and colleagues are collaborating on applications of this groundbreaking technology for use in South Korea. "Food safety and security is a global issue," explains Kim. "Ensuring that food supplies are free from pathogens and disease benefits everyone, worldwide."

For the past 4 years, ARS and Korean scientists have been collaborating to improve the sensing technology for fresh produce. They recently developed and patented a multitask imaging system capable of examining quality and safety attributes of apples. The new technology scans 3-4 apples per second, providing efficient and effective inspection of defects and fecal contamination. Details of this research have been published in *Sensing and Instrumentation for Food Quality and Safety*.

Kim and colleagues are currently looking at ways to improve the new technology, such as developing methods to examine the entire surface of a round object. With the researchers' continued dedication, consumers can rest assured that the food they eat will be safe and secure.

Foreign Beef: "Microbial Profiling" System Gets an OK from Scientists

When a side of beef is neatly carved into steaks and roasts, bits and pieces of meat trimmed from these familiar retail cuts are left over. In the meatpacking industry, they're known, not surprisingly, as "trim."

In the United States, there's a high demand for trim that can be used to make lean ground beef, perfect for burgers, meatloaf, meatballs, and other favorites. In fact, the U.S. demand for lean ground beef exceeds our domestic supply. That's why, in part, we import about 3 *billion* pounds of beef and veal every year.

Several years ago, questions were raised as to whether America's procedures for monitoring the safety of imported beef were adequate for detecting pathogens in trim. "Foodborne pathogens and their reported incidences aren't necessarily the same from one part of the world to the next," notes ARS microbiologist Joseph M. (Mick) Bosilevac.

An example: *Escherichia coli* O157:H7 is the leading species, or serotype, in *E. coli*-associated foodborne illness in the northern hemisphere. But in the southern hemisphere, other toxin-producing *E. coli* serotypes such as O111 have also been associated with outbreaks of foodborne illness.

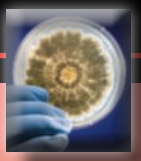
What's more, when imported beef and domestic beef are combined to make a lean ground beef product, "traceback" becomes much more complex. Traceback, in which sources of food contamination are, if possible, traced back to their point of origin, is a standard part of investigations that occur during and after major outbreaks of foodborne illness.

"The intent of our study was to find out whether U.S. microbiological profiling of imported beef trim adequately addresses the potential differences between foreign and domestic beef in terms of cleanliness and safety, or what we describe as 'hygienic status and pathogen presence,'" says Bosilevac.

For the study, Bosilevac and coresearchers examined 1,186 samples of beef trim from the United States and from Australia,

To determine the presence of *Salmonella, Listeria,* and non-O157:H7 *E. coli* in beef samples, technician Greg Smith (right) collects surface samples from boneless beef trim as microbiologist Mick Bosilevac prepares a sample for eventual analysis.

STEPHEN AUSMUS (D2159-11)



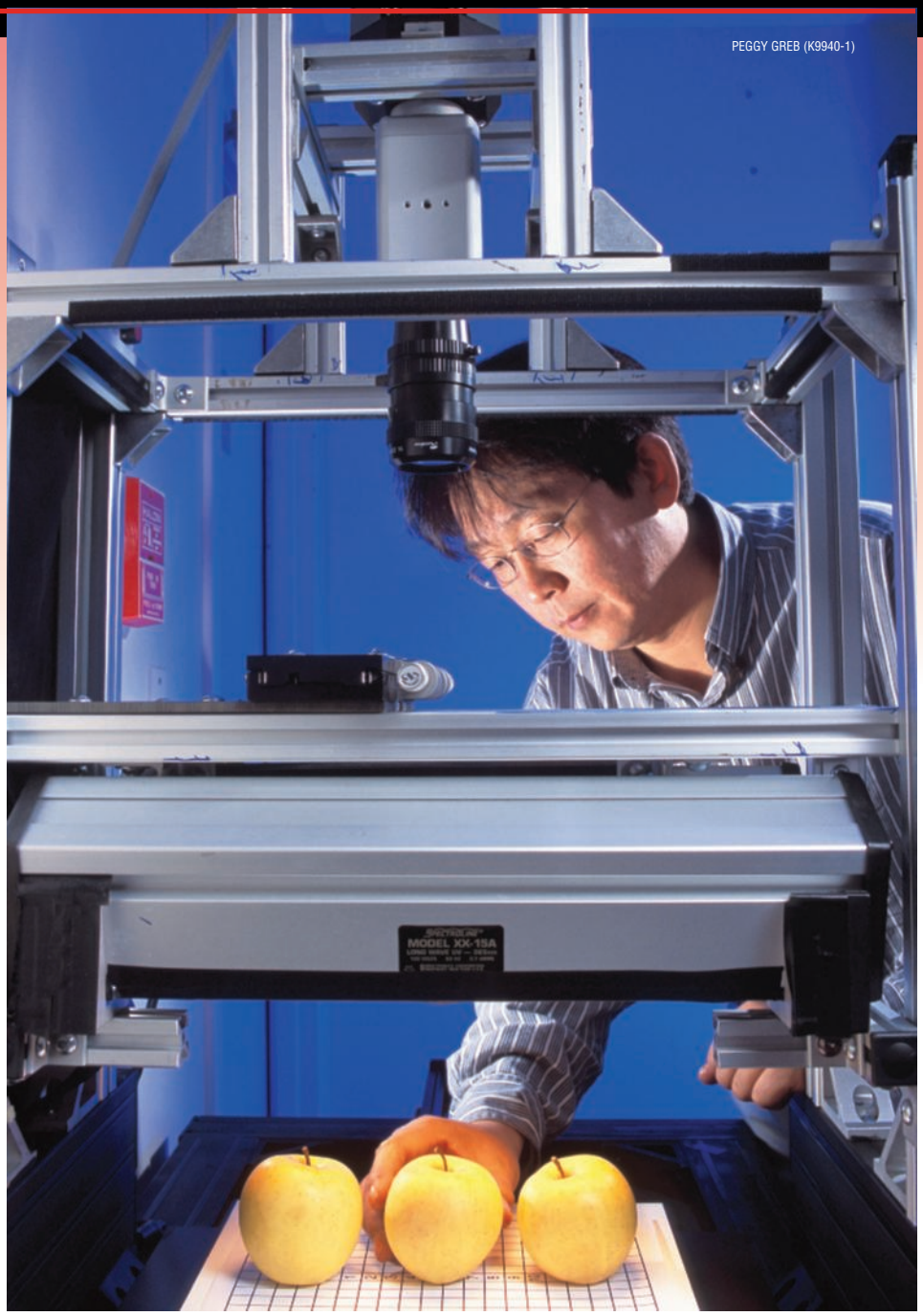
New Zealand, and Uruguay—three nations that provide more than half of America's beef imports. The researchers looked for contaminants such as aerobic bacteria, *Staphylococcus aureus, Campylobacter, Salmonella, Listeria,* and *E. coli*—specifically the close relatives of *E. coli* O157:H7 that can cause severe foodborne illness.

"Our results indicate that the pathogen-monitoring procedures used in the United States today are adequate for evaluating the safety of imported beef trim," says Bosilevac. He's based at the ARS Roman L. Hruska U.S. Meat Animal Research Center (USMARC) at Clay Center, Nebraska.

Bosilevac and coinvestigators Michael N. Guerini, Dayna M. Brichta-Harhay, and Terrance M. Arthur at Clay Center; and Mohammad Koohmaraie, formerly with USMARC, documented the research in an article that appeared in a 2007 issue of the *Journal of Food Protection.*

The study led to an informal, ongoing collaboration in which Bosilevac and research leader Tommy L. Wheeler have presented information about USMARC's leading-edge technologies for detecting and identifying foodborne pathogens to colleagues at several of Uruguay's national laboratories and at the Instituto Nacional de Investigación Agropecuaria, the Uruguayan counterpart of the U.S. Department of Agriculture. Food safety specialists from Uruguay have also come to USMARC to see this science in action.

The beef-trim research was funded in part by the Beef Checkoff, a producer-financed program of beef-related promotion and research.—By **Marcia Wood,** ARS, and **Stephanie Yao,** formerly with ARS.



This hyperspectral imaging system, being used by biophysicist Moon Kim, takes pictures at different wavelengths simultaneously. Three-dimensional images are created from the process, and researchers can then choose the wavelengths best suited for spotting fecal contamination or cuts and bruises in agricultural products.

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

Bruce C. Campbell and Jong H. Kim are in the Plant Mycotoxin Research Unit, USDA-ARS Western Regional Research Center, 800 Buchanan St., Albany, CA 94710; (510) 559 5846 [Campbell], (510) 559-5841 [Kim], bruce.campbell@ars.usda.gov, jongheon.kim@ars.usda.gov.

Moon Kim, Kevin Chao, and Alan Lefcourt are with the USDA-ARS Environmental Microbial and Food Safety Laboratory, 10300 Baltimore Ave., Beltsville, MD 20705; (301) 504-8450 ext. 245 [Kim]; (301) 504-8450 ext. 260 [Chao]; (301) 504-8450 ext. 258 [Lefcourt], moon.kim@ars.usda.gov, kevin.chao@ars.usda.gov, alan.lefcourt@ars.usda.gov.

Joseph M. (Mick) Bosilevac and Tommy L. Wheeler are in the Meat Safety and Quality Research Unit, USDA-ARS Roman L. Hruska U.S. Meat Animal Research Center, Spur 18D, Clay Center, NE 68933; (402) 762-4225 [Bosilevac], (402) 762-4221 [Wheeler], mick.bosilevac@ars.usda.gov, tommy.wheeler@ars.usda.gov.

ARS Scientists Study the Ins and Outs of *E. coli* Contamination

Hundreds of strains of *Escherichia coli* exist, including strains in the human gut that are essential to digestion. Only a few types, like *E. coli* O157:H7, cause foodborne illness. But food safety experts know it's possible for some of these pathogenic strains to survive in the environment and contaminate leafy greens that are grown in contaminated soil.

Although levels of *E. coli* microbes can potentially be controlled on the outsides of raw produce, there is concern that plant roots could take in the pathogens along with nutrients and water. This could allow the bacterium to infiltrate the plant's internal vascular system and increase the incidence of foodborne illness.

Agricultural Research Service microbiologist Manan Sharma, postdoctoral research associate David Ingram, food technologist Jitu Patel, and microbiologist Patricia Millner all work at the agency's Environmental Microbial and Food Safety Laboratory in Beltsville, Maryland. The team wanted to find out the odds for possible internal contamination via a plant's root system.

The researchers modified several strains of *E. coli* to contain a gene for fluorescence, which allowed them to track the pathogen's journey in spinach from field to harvest. Some of the modified bacteria they developed were highly pathogenic strains of *E. coli* O157:H7, and others were nonpathogenic. These strains were developed in collaboration with researchers at the University of Maryland School of Medicine.

They placed the fluorescence gene at a specific location within the chromosome structure of the bacterium where it would not interfere with any essential metabolic functions or stress responses of the cells. This strategy made the cells more likely to survive and fluoresce under stressful conditions within the plant, which in turn gave the scientists a higher level of confidence about their observations.

First the team confirmed that the pathogenic *E. coli* could survive in the soil for up to 28 days at different levels. They also observed that the fluorescent *E. coli* cells had been able to migrate into the roots of spinach plants.

E. coli, magnified about 7,000x.

PHOTO BY ERIC ERBE, COLORIZATION BY CHRISTOPHER POOLEY (K11077-1)

The researchers also examined baby spinach plants over the course of 28 days after germination to see whether any of the *E. coli* strains were taken up past the roots and into the plant's interior structures. For this part of the study, they grew baby spinach in pasteurized soil and hydroponic media.

Sharma and his colleagues found that at day 28, there was no evidence that *E. coli* had become "internalized" in leaves or shoots of baby spinach plants grown in soil. They did detect *E. coli* in hydroponically grown spinach samples analyzed 14 and 21 days after the plants had germinated, but they observed only sporadic, very low levels of bacterial survival in shoot tissue after 28 days.

Sharma believes these findings confirm that although *E. coli*—including the highly pathogenic strains—can survive in soils, it is highly unlikely that foodborne illness would result from the bacterium becoming internalized through roots in leafy produce.

"I think this study goes a long way in answering the question of how leafy greens can be contaminated by *E. coli* during production," Sharma says. "In addition, it gave us an opportunity to develop strains of *E. coli* for studying these types of phenomena in the future."—By **Ann Perry,** ARS.

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

To reach scientists mentioned in this story, contact Ann Perry, USDA-ARS Information Staff, 5601 Sunnyside Ave., Beltsville, MD 20705-5129; (301) 504-1628, ann.perry@ars.usda.gov. ✻

Using a specialized microscope, microbiologist Manan Sharma (center) and student Sean Ferguson (left) observe whether fluorescent *E.coli* cells are internalized into roots of baby spinach plants. Right: microbiologist David Ingram prepares spinach tissue for observation.

STEPHEN AUSMUS (D2151-14)

Preharvest Food Safety Keeping

STEPHEN AUSMUS (D2152-5)

Left to right: Visiting Spanish veterinarian scientist Sandra Diaz and physiologist Annie Donoghue examine Petri dishes for pathogens like *Campylobacter, Salmonella,* and *E. coli,* while postdoctoral fellow Ixchel Reyes-Herrera and University of Arkansas microbiologist Pamela Blore prepare plates to study the efficacy of natural compounds against pathogenic gastrointestinal bacteria from poultry.

The food you eat every day travels a long way from farm to fork, and dangers—in the form of foodborne pathogens or other contaminants—lurk along that road, waiting to hitch a ride on a lettuce leaf or a piece of beef or chicken. Making sure our food is safe to eat is of paramount importance to Agricultural Research Service scientists across the country. ARS research on food safety is multifaceted and wide ranging. The following touches on some of the agency's research on *Escherichia coli, Salmonella,* and *Campylobacter* and chemical residues in meat.

Lesser Known "Big Six" *E. coli* Targeted in Gene-Based Research

While *E. coli* O157:H7 is perhaps the best known of the *E. coli* species that cause foodborne illness, its lesser known relatives are increasingly of concern. Food safety regulators, public health officials, and food producers in the United States and abroad want to know more about these less-studied pathogens.

That's why ARS scientists such as Pina M. Fratamico and other ARS, university, and corporate coresearchers are developing new techniques to quickly and reliably identify these microbes. The scientists are sorting out who's who among these related pathogens by uncovering telltale clues in the microbes' genetic makeup.

The gene-focused approaches to rapid, reliable, and reproducible detection and identification are paving the way to science-based assays. With further work, the assays might be presented as user-friendly test kits for use by regulatory agencies and others. Foodmakers, for example, might be able to use such kits for in-house quality control, while public health labs might rely on them when processing specimens from patients hospitalized with a foodborne illness. Too, the assays might be used in research to develop a clearer picture of the prevalence of these microbes in food, people, animals, and the environment.

In the past few years, a half-dozen of these emerging *E. coli* species (also called "serogroups") have come to be known among food safety specialists as "the Big Six," namely, *E. coli* O26, O45, O103, O111, O121, and O145.

"These *E. coli* serogroups can produce one or more kinds of Shiga toxin—the compounds that can make us ill," says Fratamico. "We know that some strains belonging to these six serogroups have the potential to cause outbreaks of foodborne illness.

"We also want to develop the PCR-based laboratory assays into field-ready test kits so that we can better understand the prevalence of these strains in food. In addition, we want to determine whether they cause more illness than O157:H7 does, and if so, why.

"These species are virtually indistinguishable from other *E. coli* strains, including nonharmful *E. coli,* when you use conventional culture methods to grow the microbes in the laboratory," says Fratamico. She is a microbiologist and research leader of the ARS Molecular Characterization of Foodborne Pathogens Research Unit at the agency's Eastern Regional Research Center in Wyndmoor, Pennsylvania.

Along with ARS and university collaborators, Fratamico has already developed gene-based PCR (polymerase chain reaction) assays for each of the Big Six. All of these assays are based on unique forms of two genes, *wzx* and *wzy,* that occur in serogroup-specific forms in these microbes. The assays can be performed with either of three widely used PCR options—conventional, real-time, or multiplex. The assays also allow detection of two Shiga toxin genes, *stx1* and *stx2,* so that users can determine whether or not the strain they are scrutinizing is harmful.

Fratamico and coresearchers Connie E. Briggs, Yanhong Liu, Chin-Yi Chen, Xianghe Yan, and Terence P. Strobaugh, Jr., at Wyndmoor; Chitrita DebRoy, Michael A. Davis, and Elisabeth Roberts of Pennsylvania State University-University Park; and Takahisa Miyamoto of Kyushu University, Hakozaki, Japan, are collaborating on this work. Their findings appeared in the following journals: *Applied and Environmental Microbiology, Canadian Journal of Microbiology, Foodborne Pathogens and Disease, Journal of Clinical Microbiology,* and *Molecular and Cellular Probes.*

Pathogens and Chemical Residues Out of Beef and Poultry

STEPHEN AUSMUS (D1465-7)

Microbiologist Jim Wells is investigating the relationship between WDGS-based feed and the incidence and persistence of *E.coli* O157:H7 in cattle manure and on their hides. Here, Wells processes bovine fecal samples for microbial analysis while microbiologist Elaine Berry plates the processed samples for *E. coli* tests.

Of course, microbes like *E. coli* are present in most mammals' digestive tracts. Some ARS researchers are looking into how particular feeds can influence the levels of *E. coli* O157:H7, a particularly problematic strain.

Less Feed Supplement May Mean Less *E. coli*

When corn is converted to ethanol, leftovers from the biorefining process include what are known as "wet distiller's grains with solubles" (WDGS). Typically, they are yellow and have a texture somewhat like that of wet corn meal.

Since 2007, WDGS have been the subject of an array of studies at the ARS Roman L. Hruska U.S. Meat Animal Research Center (USMARC) in Clay Center, Nebraska. The investigations are revealing more details about the pros and cons of adding WDGS to cattle feed. (See "Evaluating an Ethanol Byproduct as a Potential Cattle Feed Ingredient," *Agricultural Research,* September 2009.)

"WDGS are rich in protein and are also a source of energy and minerals," says microbiologist James E. Wells at USMARC. He has led studies to investigate the relation between WDGS-based feed and the incidence and persistence of *E. coli* O157:H7 in cattle manure and on their hides.

Cattle are a natural reservoir for the microbe, which is apparently harmless to them but, of course, can be pathogenic to humans. In addition, *E. coli* in manure can newly infect or reinfect animals in pastures and feedlots. What's more, *E. coli* on hides can contaminate carcasses at the packinghouse.

In early experiments with 608 steers, Wells and coinvestigators at Clay Center provided the animals with either a corn-based feed (corn grain and silage) or a 40-percent WDGS feed during the finishing stage, that is, the last 16 weeks before harvest.

The team's analyses showed that the incidence and prevalence of *E. coli* O157:H7 in manure and the incidence on hides was significantly higher for the WDGS-fed cattle than their corn-fed counterparts.

"The differences may be due to changes within the animal's digestive system, such as an increase in gastrointestinal pH, possibly caused by eating the WDGS," says Wells. "But other factors may also have played a role."

The study, one of the largest and most detailed of its kind, was made possible in part by USMARC's well-equipped labs, large research herd—representing many leading cattle breeds—and extensive network of research pens and other facilities that simplify collection of specimens.

Wells, along with research leader Tommy L. Wheeler, Steven D. Shackelford, Elaine D. Berry, Norasak Kalchayanand, and other colleagues at Clay Center, published some of these findings in a 2009 article in the *Journal of Food Protection.* The research was funded in part by the Beef Checkoff, a promotion and research program financed by U.S. beef producers.

Additional studies are planned. "We're still not entirely certain why feeding 40 percent WDGS resulted in higher levels of *E. coli* in cattle manure," says Wells. "There are economic and performance benefits to feeding this ethanol coproduct, so we need to find ways to reduce the *E. coli* O157:H7 effect before we make recommendations about WDGS to producers."

Another tactic to control *E. coli* may come in the way of vaccines for cattle.

New Vaccines: Can They Quell *E. coli* O157: H7 in Cattle?

Though much remains to be discovered about sometimes-deadly *E. coli* O157:H7, most experts readily agree that cows—whether dairy or beef—are a major reservoir of this foodborne pathogen. With that in mind, it's easy to understand why a team of ARS scientists,

Animal caretaker Wally McDonner provides feed supplemented with a yeast extract to Japanese quail to test the feed's efficacy against *Salmonella* and *Campylobacter.*

STEPHEN AUSMUS (D2155-14)

STEPHEN AUSMUS (D2154-4)

Microbiologist Gerry Huff (left) inoculates chicken embryos in tests to determine the virulence of bacteria as technician Dana Bassi uses a digital egg monitor to determine embryo viability.

led by microbiologist Vijay K. Sharma, is creating vaccines designed to undermine the pathogen's undisputed success in colonizing cattle intestines.

The microbe can grow in the bovine digestive tract without causing any apparent harm to the animal. In humans, of course, it's a different story: In us, foodborne *E. coli* O157:H7 can cause severe gastroenteritis, bloody diarrhea, and sometimes life-threatening hemolytic uremic syndrome.

Sharma and ARS microbiologists Thomas A. Casey and Evelyn A. Dean-Nystrom (retired) have developed two experimental vaccines that show promise for disrupting colonization. By so doing, the vaccines would also reduce long-term shedding of the microbe into the animals' manure.

STEPHEN AUSMUS (D2156-1)

In efforts to develop new techniques to quickly and reliably identify pathogenic *E. coli* serogroups, microbiologist Pina Fratamico (left) and molecular biologist Yanhong Liu (center) view real-time PCR results from study samples as microbiologist Lori Bagi loads a thermal cycler with more samples for testing.

Shedding is a normal part of any persistent colonization, according to Sharma, and is vital to the pathogen's spread from one animal to the next and throughout the environment.

Manure-borne *E. coli* O157:H7 poses several hazards. On the ranch or at the feedlot, the microbe can travel, via rainfall, into drinking water or into irrigation water that may later contaminate vegetables or other fresh produce. At the packinghouse, *E. coli* O157:H7 in manure that is stuck to cattle hides or carcasses may end up contaminating equipment or meat.

Sharma and Casey, based at the ARS National Animal Disease Center (NADC) in Ames, Iowa, have tested the vaccines in preliminary experiments with 24 healthy calves. Their research included giving some of the animals a placebo or either of the vaccines, both of which were modified strains of heat-killed *E. coli* O157:H7. In tests of their immunity, calves were exposed to live *E. coli* O157:H7. Among the results: Fifty percent of the calves that received either of the two experimental vaccines stopped shedding *E. coli* O157:H7 within 1 to 2 days after being exposed to the live pathogen. What's more, blood tests taken 28 days after the first vaccination showed that blood levels of antibodies—immune system proteins—against certain *E. coli* O157:H7 colonization proteins were significantly higher in calves immunized with either of the test vaccines.

Vaccines Are Minus One or Two *E. coli* Genes

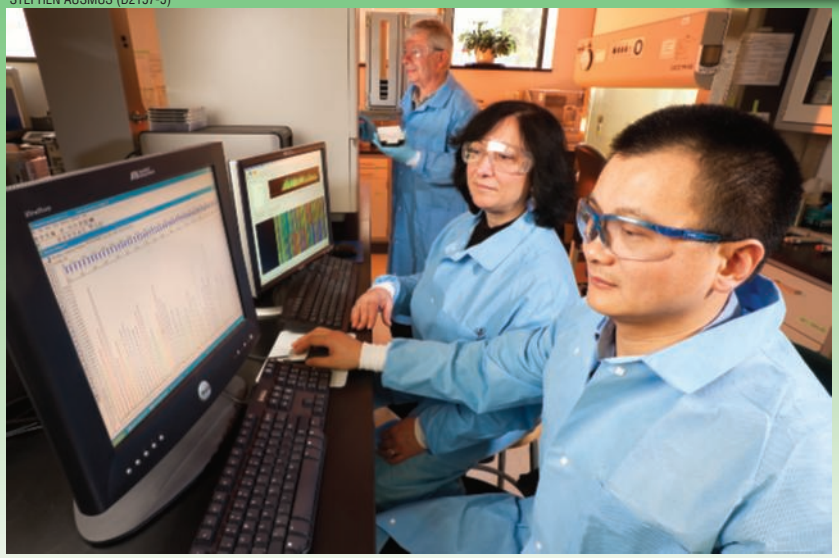
In creating the vaccines, the scientists deleted either a single *E. coli* O157:H7 gene of interest, *hha,* or two genes, *hha* and *sepB*. These genes affect the ability of the pathogen to produce and secrete proteins known as "LEE," short for "locus of enterocyte effacement." These proteins have an important job: They help *E. coli* stick to intestinal cells.

"LEE-promoted adherence to intestinal cells," says Sharma, "is a prerequisite for successful *E. coli* colonization of cattle, persistence in their intestines, and shedding of the microbe in manure."

What happens when the *hha* or *hha* and *sepB* genes are missing?

"We've shown that exposing calves to heat-killed *E. coli* O157:H7 that's missing one or both of these

STEPHEN AUSMUS (D2157-5)



Molecular biologist David Needleman (in the background) loads DNA to be sequenced in an automated DNA sequencer as microbiologist Pina Fratamico (center) and computational biologist Xianghe Yan view sequence data from *E. coli* O145.

genes causes the animals to create a large amount of antibodies against several important LEE proteins," Sharma reports.

His investigations into *hha*'s role in *E. coli* O157:H7 date back more than a decade. His team was the first to isolate and clone *hha* from *E. coli* O157:H7. Now, Sharma and coinvestigators are the first to select *hha* and the *hha-sepB* combination as the basis for experimental vaccines designed to protect cattle from *E. coli* O157:H7 colonization.

Their early studies appeared in the *Journal of Bacteriology* in 2004 and the Federation of European Microbiological Societies' *FEMS Microbiology Letters* in 2005.

The idea of vaccinating cattle against *E. coli* O157:H7 isn't new. Some commercial vaccines have already been developed, for example. But America's cattle are not, at present, routinely vaccinated against the microbe. That may change, especially if effective, affordable, easy-to-prepare and easy-to-use vaccines become readily available. Such vaccines could make food safer for us and could reduce the costs and consequences of outbreaks of foodborne illness traced back to *E. coli* O157:H7 contamination. With further research and testing, the *hha*- and *hha-sepB*-based vaccines may prove ideal for providing such protection.

Organic Poultry's Special Needs

To conduct research that may be beneficial to the organic industry, ARS has a new state-of-the-art organic poultry research facility that was developed collaboratively between an ARS unit in Fayetteville, Arkansas, and the University of Arkansas. The facility not only meets the livestock requirements of the U.S. Department of Agriculture National Organic Program (NOP), but also the animal welfare recommendations for poultry by the National Organic Standards Board and the Organic Poultry Guidance Document of the Accredited Certifiers Association.

In the United States, organic poultry production has increased almost 20 percent annually since the establishment of the NOP in 2002. This program accredits private businesses, organizations, and state agencies to certify producers and handlers of agricultural products according to NOP regulations. (The Fayetteville farm was certified under Nature's International Certification Services.) Organic poultry farms can only use compounds on the national list of substances allowed for organic production. Their use of antibiotics and other drugs and pesticides available to conventional poultry producers is restricted or prohibited. Alternatives to antibiotics are also needed for conventional poultry production, since regulations for antibiotic use are being tightened in response to the prevalence of antibiotic resistance in pathogens.

Microbiologist Gerry Huff at ARS's Poultry Production and Product Safety Research Unit (PPPSRU) in Fayetteville has investigated yeast extracts as alternatives to antibiotics for controlling disease-causing bacteria in turkey poults. Details of the study can be found in a paper published in 2010 in *Poultry Science*.

"Organic, natural remedies and preventatives are particularly needed for organic poultry production," says Huff. "Our lab has been studying

STEPHEN AUSMUS (D2143-5)

Technician Dee Kucera (foreground) harvests *E. coli* O157:H7 isolates from agar plates as technician Shannon Ostdiek (left) plates samples for *E. coli* O157:H7 isolation and microbiologist Jim Wells uses a robot to enrich *E. coli* O157:H7 from samples.

the effects of yeast extract as an immune stimulant and alternative to antibiotics in conventional turkeys. Initial studies suggest that dietary yeast extract has good potential as a nonantibiotic alternative for decreasing pathogens in organic turkey production. We need a larger study to confirm its efficacy. But it is expensive to work with turkeys—they eat a whole lot—so we are now using yeast extract in Japanese quail studies to test its efficacy against *Salmonella* and *Campylobacter*. We're using quail as a model system to evaluate natural treatments that will be beneficial for chicken and turkey production."

Huff's current study, in collaboration with Irene Wesley at NADC, involves 800 Japanese quail—a number they couldn't do with turkeys. Yeast extracts help boost the immune system's ability to kill bacteria, but there is a downside.

"Yeast ramps up certain aspects of the immune response, but this can decrease body weight in some individuals," says Huff. "Weight gain is suppressed because the energy normally used for growth is redirected toward the immune system. We need to balance the two effects of adding yeast extracts to turkey feed."

PPPSRU research leader Annie Donoghue is looking at an integrated systems approach to reducing *Salmonella* and *Campylobacter* in organic and all-natural poultry.

Because drugs are not permitted in organic production, mortality may be higher than in conventional poultry operations. "Food safety concerns with *Salmonella* and *Campylobacter* are high-priority areas for organic poultry producers, and strategies that promote gut health, limit disease, and prevent foodborne infections are needed," says Donoghue. Working collaboratively with professors Kumar Venkitanarayanan at the University of Connecticut and Dan Donoghue at the University of Arkansas, she found that caprylic acid, naturally found in milk and coconut oil, has efficacy against these foodborne pathogens when fed to poultry.

These studies were published in *Poultry Science* (January 2009) and the *Journal of Food Protection* (April 2009).—By **Sharon Durham** and **Marcia Wood,** ARS.

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

To reach scientists mentioned in this article, contact Sharon Durham, USDA-ARS Information Staff, 5601 Sunnyside Ave., Beltsville, MD 20705-5129; (301) 504-1611, sharon.durham@ars.usda.gov.

ARS and FSIS Take On Chemical Residues

Chemical residues of any kind are of concern in food-producing animals. Steven Lehotay and Marilyn Schneider at the ARS Eastern Regional Research Center (ERRC) in Wyndmoor, Pennsylvania, and colleagues with the USDA Food Safety and Inspection Service (FSIS) in St. Louis, Missouri, are developing both field-based and laboratory-based testing methods to detect veterinary drug residues in cattle.

For screening, the ERRC team and the FSIS Midwestern Laboratory compared the three major in-plant tests in use: the fast antibiotic-screening test (FAST), which was used by FSIS at the time of the study, and recently developed commercial tests called "PremiTest" and "KIS Test" (kidney inhibition swab). All three tests were evaluated in both kidney exudate and blood serum, and the new commercial tests were more effective and faster than FAST. These findings were used by FSIS to help them choose KIS to replace FAST for monitoring antibiotics in kidney tissues from cattle at slaughter establishments.

STEPHEN AUSMUS (D2160-9)

Chemist Steven Lehotay prepares samples for analysis to determine the presence of veterinary drug residues from kidney extracts.

The ARS team used their own instrument-based method to test for 121 drug residues at a time from more than 200 samples from culled dairy cows collected from a slaughter establishment. FSIS is working with the ERRC group to implement the new approach at the FSIS laboratories.

Chemist Janice Huwe, in the Animal Metabolism-Agricultural Chemicals Research Unit in Fargo, North Dakota, teams up with FSIS in an ongoing effort to find out whether unwanted chemicals are in meat animals. Huwe and her colleagues survey domestic food-producing animals from federally inspected slaughterhouses across the country for the presence of chemicals like dioxin and PCBs—toxic environmental pollutants—and PBDEs, flame-retardant chemicals used in electronics, clothing, and household goods.

And there is good news. A comparison of data from the two collection years of 2002 and 2008 showed declining trends for all the pollutants—decreases of up to 25 percent in beef, chicken, and turkey. Pork levels showed no change but remained at levels that were nearly undetectable. PBDE pollutants were reduced by more than 50 percent in each food category. This is most likely because PBDEs were removed from production in the United States in 2004.—By **Rosalie Marion Bliss,** ARS.

ARS National Research Program for Food Safety

The Agricultural Research Service's national research program on Food Safety (#108) seeks ways to control or eliminate potentially harmful food contaminants at every step of the food production and processing continuum. Food contaminants include both introduced and naturally occurring pathogenic bacteria, viruses, and parasites; toxins and nonbiological-based chemical contaminants; and mycotoxins and plant toxins.

The food safety program's aim is to provide scientific solutions to problems, leading to enhanced technology for producers and manufacturers, and to provide scientific information for development of regulations or guidelines by regulatory agencies so that consumers will have a secure, affordable, and safe food supply.

The overall vision of the program is to support public health. Since food safety and food security are global issues, ARS's research program involves both national and international collaborations through formal and informal partnerships.

The 2011-2015 ARS Strategic Action Plan for Food Safety emphasizes the following six major interrelated research areas:

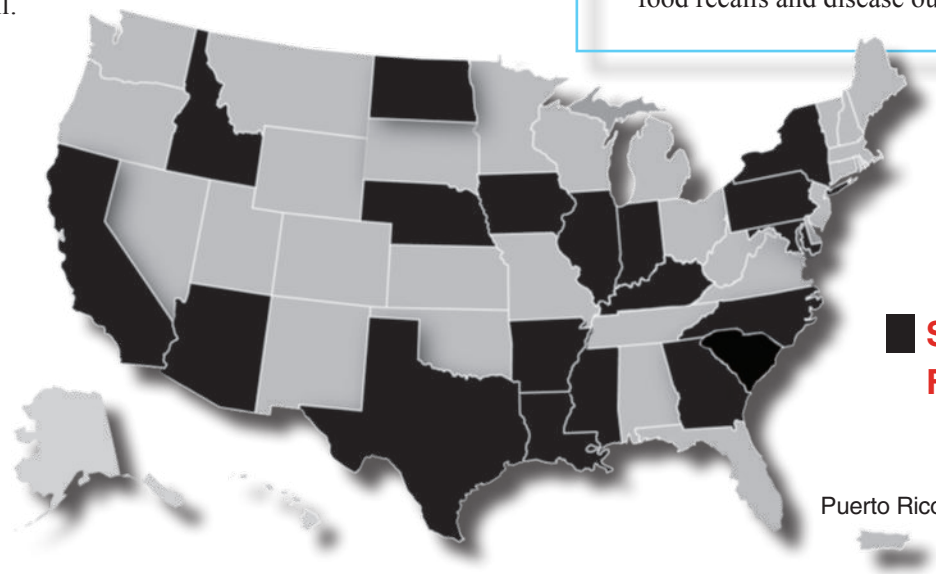
- Population studies, which identify and characterize the movement, structure, and dynamics of populations throughout the entire food safety continuum.
- Systems biology, which involves a unique integrative approach to understanding the basic genetic components of pathogens and their expression and directly relates this information to the microorganism's biology.
- Technology development to detect and characterize contaminants entering food through raw materials or during processing, with the aim of avoiding or preventing the need for processing interventions or recall.
- Technology development for reduction and control of foodborne pathogens or other zoonotic organisms and chemical contaminants.
- Predictive microbiology, which describes the behavior of microorganisms in the food environment, an integral part of microbial risk assessment used to support food safety measures.
- Technology development and scientific data for regulation and control of veterinary drugs, residues, heavy metals, persistent organic pollutants, and biological toxins derived from bacteria, fungi, and plants.

More information on national program #108 can be found at www.nps.ars.usda.gov.

Food Safety Research Information Office
fsrio.nal.usda.gov

The Food Safety Research Information Office (FSRIO) at the National Agricultural Library was established by congressional mandate as a comprehensive source of food safety research information. Among the most important features of FSRIO is a unique database of more than 5,100 research activities, including ongoing projects funded by U.S. and international government agencies as well as private organizations. For researchers, FSRIO's Research Projects Database offers an efficient insight into research that is currently under way but does not yet have published results. It serves as a valuable adjunct to literature searches.

Other features of the FSRIO website include regularly updated technical reviews on food pathogens and other hot topics, such as food biotechnology, and a database of food safety training materials for consumers and professionals. There are also links to resources on sanitation and safety standards, emergency preparedness, and other topics as well as the latest news stories on food recalls and disease outbreaks.



States Where ARS Does Food Safety Research

Leafy Greens: Keeping Salad Favorites Safe To Eat

PEGGY GREB (D2164-1)

Research associate Jennifer Kyle prepares lettuce juice samples for a fluorescence assay used to measure oxidative compounds.

In the 20 years or so since packaged salad mixes first began showing up in supermarkets nationwide, we've made them a produce-section favorite. It's no wonder. These bagged mixes—washed, cut, and ready to enjoy—offer convenience, selection, and quality, and perhaps best of all, they free us from the chore of washing and chopping, slicing, or shredding salad veggies.

But outbreaks of foodborne illness have, from time to time, been associated with bagged salad greens. The outbreaks have led the fresh-cut produce industry to voluntarily adopt stringent quality-control standards. The standards help ensure the safety of dozens of different kinds of salad staples, from iceberg and romaine lettuces to spinach, radicchio, and many more.

Helping growers and processors keep these fresh-cut veggies safe to eat is a priority of Agricultural Research Service food safety researchers, including scientists in the Produce Safety and Microbiology Research Unit. The team is part of the agency's Western Regional Research Center in Albany, California, in the San Francisco Bay area.

Innovative studies led by ARS microbiologist Maria T. Brandl are providing new information about the impressive array of genes that *Escherichia coli* O157:H7 calls into action when attempting to colonize leaves of fresh-cut lettuce. In such situations, the pathogenic microbe is essentially trying to stay alive while surrounded by natural chemicals leaking from broken lettuce cells.

Cells Get Sliced, Too

Mechanical cutting of lettuce leaves into large pieces or shredding of leaves into narrow strips, like those in taco filling, breaks lettuce cells. Lettuce cells can also be injured if leaves are bruised—during harvest or while at the processing plant, for instance.

For *E. coli,* the good news is that broken lettuce cells exude carbohydrates, which the microbe can use as a source of energy. But the bad news, from the microbe's point of view, is that injured leaf cells can also leak compounds that are problematic for the pathogen.

Oxidants are a good case in point. Wounded lettuce cells may give off a burst of hydrogen peroxide, for example, an oxidant that can, as its name suggests, cause oxidative stress for *E. coli*.

The pathogen's response to oxidative stress is one example of a coping strategy that's of keen interest to Brandl and her colleagues. "Chlorine, the most widely used sanitizer in produce processing, is an oxidant," says Brandl. "Our findings suggest that *E. coli* cells that have already encountered oxidative stress imposed by plant-cell oxidants, and have activated genes to overcome that stress, may be better adapted to withstand chlorine sanitizers during washing and processing than *E. coli* cells that have not been exposed to previous oxidative stress."

This observation and others come from experiments in which Brandl and coinvestigators exposed *E. coli* O157:H7 for either 15 minutes or 30 minutes to a juice made from crushed, liquefied leaves of fresh romaine lettuce to mimic the chemical compounds that are leaked from plant cells when lettuce is injured. An approach known as "microarray-based whole genome transcriptional profiling" enabled the researchers to determine which *E. coli* genes were activated.

"The technology gives us a snapshot or quick overview of all of the genes that were in play at those points in time," says Brandl. "It's an excellent technology for spying on the pathogen and learning about what happens to the pathogen at the molecular and chemical level."

Microbiologists Maria Brandl and Craig Parker study microarray data to identify *E. coli* O157:H7 genes activated after the bacteria encounter liquid leaking from cut lettuce.

Study Is First To Provide Extensive Details

The microarray-based study was the "first to provide extensive information about the biology of *E. coli* O157:H7 in fresh-cut lettuce," according to Brandl. "We showed that *E. coli* adapts well, using its genetic arsenal to protect itself against a multitude of assaults, including oxidative stress, osmotic stress, damage to its DNA, antimicrobial compounds exuded by the plant leaves, and other threats to its ability to survive and multiply. We showed that *E. coli* can adapt quickly. We also showed that *E. coli* exposed to the contents of broken lettuce cells activated genes that are associated with other key traits."

Those traits included virulence, motility (the microbe's ability to propel itself with its flagella), and its ability to attach to surfaces using appendages known as "fimbriae."

"From what we're observing with the microarray analyses," Brandl says, "we hope to help develop new technologies that can overcome *E. coli* defenses. The microarray technology gives us an inside look at the numerous stresses that *E. coli* faces at the cut surface of a lettuce leaf. Each stress is a natural obstacle that *E. coli* has to overcome. We might be able to use these obstacles in a 'hurdle' approach to decontamination. Instead of relying on just one procedure or strategy, hurdle technology combines several strategies, each enhancing the other to weaken and kill the pathogen."

Brandl and Albany colleagues Jennifer L. Kyle, Craig T. Parker, and Danielle Goudeau published their findings in *Applied and Environmental Microbiology* in 2010.—By **Marcia Wood,** ARS.

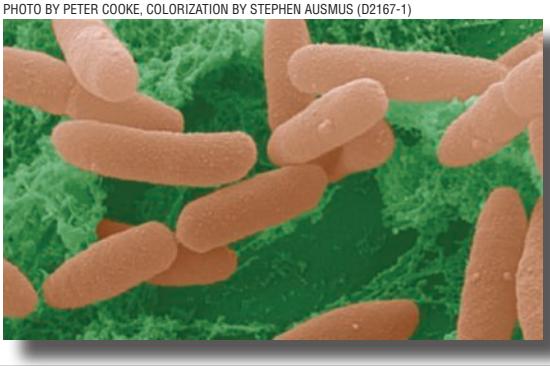
This research supports the USDA priority of ensuring food safety and is part of Food Safety, an ARS national program (#108) described at www.nps.ars.usda.gov.

Maria T. Brandl is in the USDA-ARS Produce Safety and Microbiology Research Unit, Western Regional Research Center, 800 Buchanan St., Albany, CA 94710; (510) 559-5885, maria.brandl@ars.usda.gov.

Left: ARS scientists are working to make leafy greens and other fresh produce safer for consumers. Produce and leafy greens shown (clockwise from top): romaine lettuce, cabbage, cilantro in a bed of broccoli sprouts, spinach and other leafy greens, green onions, tomatoes, and green leaf lettuce.

Colorized SEM (scanning electron micrograph) of pathogenic *E. coli* on a lettuce leaf. Image is shown at about 16,000 times normal size.

PHOTO BY PETER COOKE, COLORIZATION BY STEPHEN AUSMUS (D2167-1)



Oysters, Clams, and Mussels Keeping

You don't have to be a celebrity chef to cook a pot of delicious, freshly harvested mussels. Simmer them

PEGGY GREB (D2168-1)

ARS molecular biologist David Kingsley (foreground) works with Haiqiang Chen (middle) and Dallas Hoover, both with the University of Delaware-Newark, to perform high-pressure processing on virus samples at the university.

for about a half-hour in a simple broth of white wine and garlic, then serve with a ready-to-eat garden salad and some crunchy bread. You'll have a hearty meal for family and friends to enjoy. In fact, mussels—easy to prepare and fun to eat—are one of America's most popular kinds of seafood.

Of course, simmering, or any other means of cooking, helps ensure that you won't pick up a foodborne pathogen when you eat a mollusk. But eating raw or undercooked mollusks—as many seafood fans prefer to do—may pose a safety hazard if the shellfish is harvested from waters polluted with pathogenic microbes.

That's why enhancing the food safety of mouthwatering mollusks is the focus of David H. Kingsley, a molecular biologist; Gary P. Richards, a microbiologist; and technicians Gloria K. Meade, Brad Shoyer, and Michael A. Watson. Based in Dover, Delaware, they are the only ARS group working nearly exclusively on molluscan food safety. The team is in the ARS Eastern Regional Research Center's Food Safety and Intervention Technologies Research Unit.

Microbes that cause human illness can make their way into mollusk tissues when the bivalves open their shells to feed, taking in, filtering, and expelling seawater. As a result of this filter-feeding, pathogens can "bioconcentrate" within shellfish meat.

Microbes of concern include viruses such as norovirus, the number-one cause of foodborne illness in the United States, and hepatitis A virus, which causes a contagious liver disease. Also of concern are bacteria such as *Vibrio vulnificus,* which can cause serious infection, or *V. parahaemolyticus,* a cause of gastroenteritis.

Enlisting Science To Detect, Identify, and Deactivate Microbes

In current investigations, the researchers are developing new, high-tech assays that regulatory agencies, public health officials, and seafood processors could use to detect and identify these or other pathogens in shellfish. The scientists are exploring new ways to decontaminate mollusks while protecting the seafood's flavor, texture, and color.

Kingsley, for example, is investigating the use of a specialized procedure known as "high-pressure processing," or HPP, to inactivate viruses, specifically norovirus and hepatitis A virus.

HPP isn't new. For example, it is used commercially for deli meats and to pasteurize some juices. Some shellfish processors use it to deactivate *Vibrio* bacteria. But Kingsley and co-workers are the first to show that HPP can inactivate some foodborne viruses.

How HPP Works

The HPP equipment compresses water in a tank to create intense pressures—as high as 90,000 pounds per square inch (psi). That's in contrast to normal atmospheric pressure, which is about 15 psi at sea level.

In early studies to determine whether norovirus is susceptible to HPP treatment, the researchers worked with a mouse norovirus as a substitute, or surrogate, for the norovirus that causes illness in humans. Human norovirus can't be grown in the laboratory, but the mouse norovirus can.

"The mouse norovirus is closely related to human norovirus, so it is a relevant surrogate," says Kingsley. Results indicated that 99.99 percent of the mouse norovirus, bioconcentrated by oysters grown in laboratory tanks, was inactivated by treating the oysters with HPP for 5 minutes at 60,000 psi.

As a further test of the HPP treatment, the scientists blended oyster meat and mouse norovirus together and fed the mixture to laboratory mice known to be highly susceptible to the virus. None of the mice tested positive for the virus.

The impact of the HPP treatment on human norovirus is still being explored, Kingsley notes.

HPP Inactivates Hepatitis A Virus, Too

In tests targeting the hepatitis A virus, Kingsley and coinvestigators determined that the same pressure treatment of 60,000 psi for 5 minutes inactivated 99.9 percent of the virus that had been bioconcentrated by oysters in laboratory tanks. The results were the same whether the treatment targeted in-shell oyster meat or oyster meat that had been removed from the shells.

At ARS's Eastern Regional Research Center in Wyndmoor, Pennsylvania, technician Brad Shoyer (foreground) and David Kingsley load oysters into a high-pressure processing machine.

PEGGY GREB (D2169-1)

Popular Mollusks Safe To Eat

PEGGY GREB (D2172-1)

At the University of Delaware Marine Laboratory, in Lewes, Delaware, David Kingsley inspects oysters grown for research.

The hepatitis A studies led to collaboration with researchers in Italy, where raw or lightly cooked Mediterranean mussels, popular in European markets, are sometimes a vector for the virus. The Dover scientists and colleagues from the University of Bari in Italy found that the 5-minute, 60,000-psi treatment inactivated 99.9 percent of the virus in North American blue mussels and in Mediterranean mussels. The Bari researchers are looking into possible commercial use of the process in Europe.

HPP works by damaging a virus's outer layer, known as a "capsid," or a bacterium's membrane. Without protection of a capsid or membrane, the microorganisms can't survive.

With all of its promise, HPP is not yet perfect. The equipment is expensive. The pressures needed to inactivate norovirus and hepatitis A are higher than what is commercially used currently for *Vibrio* inactivation and may alter the meat's taste and texture somewhat. "We have some ideas that might resolve this," says Kingsley.

In addition to his ARS colleagues at Dover, Kingsley's coinvestigators include Kevin R. Calci with the U.S. Food and Drug Administration, Dauphin Island, Alabama; Haiqiang Chen and Dallas G. Hoover at the University of Delaware-Newark; George J. Flick and Daniel R. Holliman (deceased), Virginia Polytechnic Institute and State University-Blacksburg; Robert M. Gogal and Richard Kerr, University of Georgia-Athens; Juan S. Leon and Christine L. Moe of Emory University, Atlanta, Georgia; and Valentina Terio at the University of Bari.

The scientists have published their findings in the following journals: *Applied and Environmental Microbiology, Food and Environmental Virology, International Journal of Food Microbiology, Journal of Food Protection,* and *Virus Research.*—By **Marcia Wood,** ARS.

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

David H. Kingsley is in the USDA-ARS Food Safety and Intervention Technologies Research Unit, Eastern Regional Research Center, J.W.W. Baker Center, Delaware State University, Dover DE 19901; (302) 857-6406, david.kingsley@ars.usda.gov.

Steak!

Researchers Give It a Grilling

Steak is an all-American favorite. To help make sure that *E. coli* O157:H7 and some of its Shiga-toxin-producing relatives will not ruin the pleasure of this popular entrée, ARS researchers have tested the effects of grilling on these microorganisms.

In particular, they're learning more about the movement of *E. coli* into "subprimals," the meat from which top sirloin steaks are carved. Their focus is on what happens to the *E. coli* when subprimals are punctured, as part of being tenderized, and the effect of gas-grill cooking on survival of those microbes. The concern is whether tenderizing processes move significant amounts of *E. coli* cells into the deep interior tissues of the meat, says microbiologist John B. Luchansky with ARS's Eastern Regional Research Center in Wyndmoor, Pennsylvania.

In the study, scientists applied various levels of *E. coli* O157:H7 to the lean surface of subprimals, ran the meat (lean side up) through a blade tenderizer, then took core samples from 10 sites on each subprimal, to a depth of about 8 centimeters. In general, only 3 to 4 percent of the *E. coli* O157:H7 cells were transported to the geometric center of the meat, they found. At least 40 percent of the cells remained in the top 1 centimeter. "Knowing where a pathogen is most likely located—in or on steaks—is the first step toward validating proper cooking methods and temperatures for killing it," Luchansky says.

To learn more about the effects of grilling, the group examined other subprimals, applying *E. coli* O157:H7 to the lean surface, running the subprimals once through the blade tenderizer (lean side up), then slicing the meat into steaks either ¾-inch, 1-inch, or 1¼-inch thick. Using a commercial open-flame gas grill they cooked the steaks—on both sides—to an internal temperature of 120°F (very rare), 130°F (rare), or 140°F (medium rare).

"Our findings confirm that if a relatively low level of *E. coli* O157:H7 were to in fact be distributed throughout a blade-tenderized top sirloin steak, proper cooking on a commercial gas grill is effective for eliminating it," Luchansky says.

He did the work with Wyndmoor colleagues Jeffrey E. Call, Bradley Shoyer, and Anna C.S. Porto-Fett; Randall K. Phebus of Kansas State University; and Harshavardhan Thipparredi of the University of Nebraska. Articles published in the *Journal of Food Protection* in 2008 and 2009 document these preliminary findings.

This research was funded by the Beef Checkoff and the USDA Food Safety and Inspection Service as well as ARS.—By **Marcia Wood,** ARS.

Waging War on a Voracious Pest

In Fenton, Michigan, APHIS entomologists Ivich Fraser (left) and Juli Gould release *Spathius agrili,* a parasitic wasp that attacks the emerald ash borer. Logs attached to the tree are used for monitoring purposes.

NICHOLE SMITH (D2150-1)

Efforts To Contain the Emerald Ash Borer

While driving to Michigan to study an infestation of emerald ash borer (EAB) beetles in June 2009, Agricultural Research Service entomologists John Vandenberg and Michael Griggs stopped to check out some defoliated ash trees along a highway in western New York State. What the two scientists discovered, in the town of Randolph, was New York's first infestation of a pest from Asia that has killed tens of millions of ash trees in at least 15 states and two Canadian provinces.

The emerald ash borer is a voracious beetle with stealthy habits. First detected near Detroit, Michigan, in 2002, it is metallic green and about a half-inch long. It will attack a wide variety of ash trees, wiping out huge swaths of wooded tracts and trees that shade many suburban neighborhoods. It poses more than an ecological threat, too. Ash trees are used to make furniture, tool handles, baseball bats, and other wood products.

The beetle spends much of its early life feeding under the bark of the ash tree, so that by the time it is detected, it is usually too late to save an infested tree. Ash borer beetle larvae feed on the phloem tissue of the tree, which weakens the tree and eventually kills it. One sign of infestation is damage from woodpeckers that feed on borer larvae.

"The ash borer is a really hard pest to detect in its early stages and really hard to study because of how it spends most of its life cycle," says Vandenberg.

A Variety of Control Strategies

Vandenberg and Griggs, both with ARS's Robert W. Holley Center for Agriculture and Health in Ithaca, New York, and Jian Duan, an entomologist at the ARS Beneficial Insects Introduction Research Unit in Newark, Delaware, are working with Leah Bauer, an entomologist with the

STEPHEN AUSMUS (D2138-6)

Emerald ash borer, *Agrilus planipennis*.

U.S. Department of Agriculture's Forest Service, and other federal and state partners on long-term efforts to control the spread of the EAB. Strategies include evaluating use of a fungal pathogen and three species of nonstinging parasitic wasps imported as biocontrol agents from the beetle's native lands, northeast Asia. Other ARS researchers are exploring whether pheromones can be used to keep the pest in check and developing cryopreservation techniques as a way of ensuring a future supply of ash trees. Partners along with the Forest Service include scientists from USDA's Animal and Plant Health Inspection Service (APHIS); Cornell University; the State University of New York's College of Environmental Science and Forestry; and foresters and scientists in New York, Maryland, Michigan, and Massachusetts and a number of other states.

APHIS plays an essential role in the effort. The agency joined with the Forest Service to conduct extensive studies that now make it possible to release nonstinging wasps in infested areas. APHIS also operates a facility in Brighton, Michigan, where large numbers of wasps are reared for field releases. "Before these natural enemies could be released, we conducted host-specificity testing to see if they would attack other wood-boring insects. We found that they preferred the emerald ash borer," says Juli Gould, an APHIS entomologist.

The beetle can be spread when people transport infested firewood and nursery stock, and infested trees are often found along highways. After the discovery by Vandenberg and Griggs in New York, a follow-up survey turned up a pattern of infestation that prompted state and federal quarantines restricting the movement of firewood, lumber, and logs from ash trees growing in the area. Many of the nearby properties are wooded, with up to 80 percent of their acreage covered by ash trees. "If you have an ash tree that is really important to you, you can inject insecticide into it once every year or two and that may save it. But that's really expensive, and it's not practical to do that for a forest of infested trees," Vandenberg says.

Crews in New York have been "girdling" ash trees by removing a 6-inch-wide rim of bark from around the tree to expose the wood. The girdled trees become attractive to the beetles, so that they leave other trees alone. The girdled trees are removed the following winter and spring, which prevents a new crop of EAB adults from emerging and dispersing, according to Bauer, the Forest Service entomologist.

Vandenberg is helping with control efforts in New York, girdling trees in Randolph and setting up sticky traps near them to study the extent of the infestation.

STEPHEN AUSMUS (D2137-14)

Entomologist Jian Duan (left) and technician Jeff Wildonger dissect EAB-infested ash logs to confirm that the borers have been parasitized by *Tetrastichus planipennisi,* a wasp from China.

STEPHEN AUSMUS (D2141-2)

A brood of *Tetrastichus planipennisi* pupae that developed on an EAB larva inside an ash log.

Scientists and technicians have girdled 17 clusters of ash trees in and around Randolph and 120 single girdled trees, known as "sentinel trees," within about 5 miles of the epicenter. The trees will be cut down and carved up into sections to assess the level of infestation, Vandenberg says. But newly discovered infestations in other parts of New York and other states pose a challenge for regulators and researchers alike.

Emerging from the trunk of an ash tree, an emerald ash borer is infected with *Beauveria bassiana,* an insect-pathogenic fungus that may prove to be a valuable biocontrol for this pest.

HOUPING LIU (D2146-1)

STEPHEN AUSMUS (D2140-19)

The parasitic wasp *Tetrastichus planipennisi* is native to China and is showing promise as a biocontrol in the United States for emerald ash borer.

Wasp Watch

Duan is working with Bauer and Gould to try to determine how well the three wasp species that are natural EAB enemies, *Oobius agrili*, *Tetrastichus planipennisi,* and *Spathius agrili,* will survive the winter in different northeastern areas and whether any one of them is more effective than the others. "This is a new habitat for them, and we don't know how late in the year they remain active," Duan says.

The researchers attached cages containing the wasps to green ash trees infested with EAB larvae between August and October in areas of Michigan and Maryland to assess the wasps' abilities to parasitize the ash borer and survive in those areas. The results are promising, says Duan. "We found that they successfully overwintered and survived in Michigan, and if they can survive the winter in Michigan, they most likely would successfully overwinter in New York and Pennsylvania," he says.

Duan is also assessing the potential use of several species of wasps native to North America. In collaboration with other researchers, he identified optimal rearing techniques for one of the parasitic wasps *(T. planipennisi)* to help ensure a sufficient supply. The rearing-techniques research was published in the *Journal of Economic Entomology.*

Wasps have been released in Michigan, Illinois, Indiana, Ohio, West Virginia, and Maryland, and releases are planned in several other states. Generally, scientists and technicians will release 1,200 individuals of each species at each release site, 600 at a time. In many states, there have been multiple release sites. "We also have long-term monitoring plots to look at the impact in all these states," Gould says.

Recently, Duan published a preliminary assessment of the establishment and impact of those newly released parasitoids on EAB populations in three natural forest stands in Michigan. Findings, published in the journal *Environmental Entomology,* showed that at least one of the wasps *(T. planipennisi)* had become established in three release sites in Michigan and that it was the most abundant species of the parasitoid wasps attacking EAB larvae a year after release.

Vandenberg is also testing use of an insect-pathogenic fungus, *Beauveria bassiana,* as a biocontrol agent along with the wasps. The fungus is the active ingredient in BotaniGard, a commercially available insecticide labeled for use against a variety of insects. The researchers think the fungus could be applied to infested trees as a first step before the wasps are released. Preliminary results show that it kills the beetles but leaves the wasps unharmed, says Vandenberg, but those studies are ongoing.

Using Chemical Attractants

Since 2007, ARS entomologist Allard Cossé has worked with a multidisciplinary team of scientists from ARS, APHIS, and the Forest Service to identify naturally occurring chemicals that the ash borer and its parasitoids simply cannot resist. Early success came with the identification by APHIS and Forest Service colleagues of several compounds emitted from the bark and leaves of girdled ash trees. These compounds, which are sensed by the antennae of adult ash borers, led to the development of traps baited with manuka oil—a less expensive proxy. These traps are now used to detect infestations of ash borer and support the establishment of new quarantine areas to contain the pest.

Cossé and colleagues have also discovered components of the ash borer's chemical attractant, or pheromone, and synthesized it for use in traps—either alone or combined with attractants derived from ash trees. Their target, macrocyclic lactone, is a compound that adult female ash borers emit while feeding. This compound's role as a sex attractant for adult male borers has recently been determined in large-scale field tests in Canada and the United States, adds Cossé, who is with ARS's National Center for Agricultural Utilization Research (NCAUR) in Peoria, Illinois.

His collaborators include, among others, Gould, Damon Crook, Victor Mastro, Jonathan Lelito, and Ivich Fraser—all with APHIS's Plant Protection and Quarantine program; Bruce Zilkowski and Richard Petroski, with ARS-NCAUR; Peter Silk and Krista Ryall, with the Canadian Forest Service; Ashot Khrimian, with ARS's Invasive Insect Biocontrol and Behavior Laboratory in Beltsville, Maryland; along with Bauer and Therese Poland, who are both with the Forest Service's Northern Research Station.

A key tool has been the electro-antennogram, a device that records the strengths of electrical signals generated by the EAB's antennae when connected to electrodes and exposed to different odors the pest encounters in nature. The device, coupled with gas chromatography analysis and wind tunnel experiments, has also proved invaluable in finding and developing attractants to help monitor ash borers.

Now these tools have been harnessed to identify attractants for the three parasitic wasps being released to control the pest. So far, the researchers have developed an experimental pheromone formulation for one of the three wasp species, namely *S. agrili.* Cossé reports the formulation is a blend of five compounds produced by male *S. agrili,* and it attracts other males as well as females. Efforts are now under way to develop pheromones for the other two species and then to blend them with ash tree attractants for added effect.

The researchers have made rapid progress, but their efforts are a race against the clock, given the rate that the pest is spreading. "If we can slow down the spread

LOUELA CASTRILLO (D2145-1)

ARS entomologist John Vandenberg sprays a formulation of spores of the fungus *Beauveria bassiana* on an ash tree at a test site in Michigan.

of the emerald ash borer and establish populations of natural enemies, it's possible we can create a kind of equilibrium whereby fewer trees are lost to the pest," says Cossé.

Ensuring Ash's Future

As added insurance, a team of ARS researchers in Ames, Iowa, and Fort Collins, Colorado, has devised a procedure for putting ash tree budwood material into a "deep freeze" for future use.

Using cryopreservation techniques that have been very effective for apple and sour cherry, horticulturist Mark Widrlechner and plant physiologist Gayle Volk showed that dormant budwood can be safely stored in liquid nitrogen vapor for prolonged periods and later thawed for use in propagating elite clones or cultivars.

Seed-storage methods can safeguard much of the diversity in North America's ash populations. "But there are selected ash cultivars with superior form and stress tolerance that never produce seeds—or that may have special characteristics useful in fighting EAB," says Widrlechner, with ARS's North Central Regional Plant Introduction Station in Ames. "For these trees, having a reliable method to preserve and propagate them in the future would be extremely valuable."—By **Dennis O'Brien** and **Jan Suszkiw,** 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.

Native to China, the parasitic wasp *Spathius agrili* is being used as a biocontrol for emerald ash borer.

STEPHEN AUSMUS (D2139-4)

PCR Fine-Tuned for Better Plant Disease Detection

Plant diseases not only cause significant crop losses, but can also severely damage export markets. The key to controlling any plant disease is a rapid, sensitive, and accurate diagnosis. Polymerase chain reaction (PCR)-based tests are prized tools for diagnosing plant diseases. But PCR's ability to obtain a genetic fingerprint that conclusively identifies the pathogen hinges on there being a minimum number of target cells. Otherwise, its genetic material can't be probed and multiplied in amounts necessary for detection. This diagnostic shortcoming can be especially costly when asymptomatic seed or plants intended for commercial sale are certified as pathogen free when, in fact, they're not.

Now, a solution to the problem is at hand, thanks to a patented procedure devised by Agricultural Research Service plant pathologist Norm Schaad and colleagues. Their technique increases numbers of the target organism in a sample by using growth-promoting agar or liquid media before PCR. In 4 to 72 hours, depending on the pathogen, "the target cells make many thousands of copies, enabling detection by direct PCR," explains Schaad, now retired from the ARS Foreign Disease-Weed Science Research Unit at Fort Detrick, Maryland.

BOB NICHOLS (K7721-7)

A more sensitive test should prove useful for early detection of many bacteria, including the one that causes Pierce's disease on grapes. Shown is an ARS-developed seedless variety, Autumn Royal.

"What makes the procedure so easy to design is that we only need to grow pinpoint-size colonies for PCR," he says. These small colonies are washed from the agar-media plates and used directly for PCR. This eliminates the need for chemicals such as phenol, which is used to extract the DNA needed for conventional PCR.

Besides increasing sensitivity by 100- to 1,000-fold over conventional PCR methods, the enrichment technique, dubbed "Bio-PCR," stops substances called "inhibitors" from interfering with the action of a key enzyme, Taq polymerase. These inhibitors can come from plant extracts and even bacterial cells. During the actual PCR procedure, polymerase mass-produces, or "amplifies," specific fragments of the targeted bacterium's DNA so that it can be detected.

Schaad codeveloped Bio-PCR with Nikolas J. Panopoulos and Efstathios Hatziloukas, both formerly with the University of California-Berkeley.

Bio-PCR works best with such fast-growing bacteria as *Ralstonia solanacearum* (bacterial wilt of potato and tomato) and *Acidovorax avenae* (bacterial fruit blotch of watermelon), where only 48 hours are needed for enrichment.

Even detection of the extremely slow-growing *Xylella fastidiosa* (Pierce's disease of grapes and leaf scorch of shade trees) is improved by Bio-PCR. Indeed, in studies with *X. fastidiosa,* Bio-PCR detected the bacterium in 90 percent of infected grape samples, whereas conventional PCR detected just 13 percent.

"Conventional PCR does not work well with *Xylella* because of inhibitors," says Schaad. "That's a big advantage of Bio-PCR: By plating (growing bacteria) on agar media, the inhibitors are absorbed, and you can get a positive result in 4 to 5 hours."

Other researchers have shown Bio-PCR's inhibitor-eliminating enrichment step works in ferreting out hard-to-detect cells of human pathogens such as *Escherichia coli* O157:H7, offering food-safety applications as well. In the case of *E. coli,* only 2-4 hours of enrichment are needed.—By **Jan Suszkiw,** ARS.

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

To reach scientists mentioned in this article, contact Jan Suszkiw, USDA-ARS Information Staff, 5601 Sunnyside Ave., Beltsville, MD 20705-5129; (301) 504-1630, jan.suszkiw@ars.usda.gov.

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

Locations Featured in This Magazine Issue

Western Regional Research Center, Albany, California
8 research units ■ 250 employees

Fort Collins, Colorado
5 research units ■ 143 employees

Red River Valley Agricultural Research Center, Fargo, North Dakota
6 research units ■ 150 employees

Roman L. Hruska U.S. Meat Animal Research Center, Clay Center, Nebraska
6 research units ■ 120 employees

Ames, Iowa
8 research units ■ 501 employees

National Center for Agricultural Utilization Research, Peoria, Illinois
7 research units ■ 226 employees

Poultry Production and Product Safety Research Unit, Fayetteville, Arkansas
1 research unit ■ 13 employees

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

Eastern Regional Research Center, Wyndmoor, Pennsylvania
6 research units ■ 205 employees

Beneficial Insects Introduction Research Unit, Newark, Delaware
1 research unit ■ 21 employees

Foreign Disease-Weed Science Research Unit, Fort Detrick, Maryland
1 research unit ■ 50 employees

Henry A. Wallace Beltsville Agricultural Research Center, Beltsville, Maryland
30 research units ■ 953 employees

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

U.S. Department of Agriculture
Agricultural Research Magazine
5601 Sunnyside Ave.
Beltsville, MD 20705-5130

Official Business

Please return the mailing label
from this magazine.

To stop mailing _____ ☐
To change your address _____ ☐

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

STEPHEN AUSMUS (D2138-10)

Subscribe

for $60.00 per year
($84.00 foreign
addresses)
Visa and Mastercard
accepted.
Prices subject to change.
Order By:
Fax: (202) 512-2104
Phone: (202) 512-1800
Mail:
New Orders
Superintendent of
Documents
P.O. Box 371954
Pittsburgh,
PA 15250-7954

bookstore.gpo.gov

PEGGY GREB (D2185-2)