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Agricultural Research

Egg Pathogens Feel the Heat

Stories on pages 4 and 8

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

FORUM

Fighting Diseases That Threaten the Poultry Industry

One approach the U.S. Department of Agriculture uses to ensure food security is to conduct research on animal diseases, both at home and abroad. Poultry diseases, for example, continue to threaten the health and production of chickens, turkeys, and other bird species, and some are even a threat to human health.

At the Agricultural Research Service's Southeast Poultry Research Laboratory (SEPRL) in Athens, Georgia, scientists are pursuing strategies to help prevent and mitigate the impact of important poultry diseases. (See story that begins on page 4 of this issue.) Their discoveries in the areas of detection, prevention, management, control, and eradication help protect the U.S. poultry industry, which generates about $25 billion and contributes $2.5 billion in exports each year.

SEPRL serves as a major international resource for controlling avian influenza and virulent Newcastle disease. Because of their expertise and research skills, scientists at SEPRL are among the first to be notified when an outbreak of avian influenza occurs here in the United States or in other countries. They rapidly sequence the virus and conduct clinical studies to identify and characterize virus strains and determine their virulence and potential to spread among poultry and people. Importantly, they assess whether our diagnostic tests will be able to detect new and emerging strains and, if needed, develop new tests. SEPRL scientists also make significant contributions to the development of vaccines to ensure that we have the best tools to control domestic or exotic diseases (those from other countries) that pose a threat to the United States.

Although avian influenza is not new to domestic poultry, concerns are growing about outbreaks of emerging strains in Asia and the Middle East that are "zoonotic"—meaning they can be transmitted to humans. Virulent (highly pathogenic) forms of avian influenza cause a high rate of death in birds, and mild forms (low pathogenic) result in respiratory and reproductive problems. There is considerable concern, however, that these mild virus forms might change, or mutate, to the severe forms. And as we just experienced in the spring of 2013 with the H7N9 outbreak in China, even low pathogenic strains can emerge in poultry and acquire the ability to cause severe disease in humans.

One research tool scientists have added to their arsenal to fight disease is "reverse genetics." The process involves constructing avian influenza viruses with specific genetic sequences to learn how they cause disease in poultry, adapt to new hosts, and acquire the ability to infect humans. Using this technique, SEPRL scientists genetically altered an H7N2 virus that infected birds in the northeastern United States from 1994 to 2006. They found that the virus needed insertions of amino acids at a key site to become virulent. This research improved our understanding of how viruses become virulent and contributed to our ability to predict the risk of low pathogenic viruses changing to the virulent form.

Newcastle disease, which first appeared in the United States in the 1930s, is another major problem that scientists are tackling. Virus strains native to the United States cause only mild symptoms, similar to a common cold for poultry. But exotic strains can cause devastating losses, and new strains are evolving that can threaten U.S. poultry production.

For example, a Newcastle strain traced to pet birds in California hit the poultry industry in the early 1970s. It took more than 2 years to eradicate the disease, at a cost of $56 million in federal funds. In 1992, thousands of infected turkeys in North Dakota were euthanized after virulent Newcastle disease was detected.

As early as the 1940s, scientists had developed vaccines to prevent this disease and had identified strains that produced symptoms ranging from mild to fatal. Exotic Newcastle disease has been eradicated in U.S. poultry, but identifying the cause for differences in the severity in strains remains a challenge.

Because of the limitations of PCR (polymerase chain reaction) tests used to detect emerging Newcastle disease viruses, it is possible that viral transmission could occur undetected among wild birds and poultry. It is impossible to predict which genotypes represent the most significant threat to the U.S. poultry industry. There is a need for further evaluation of the effectiveness of current U.S. vaccines and diagnostic assays for emerging viruses.

ARS partners with other government agencies and scientists worldwide to help manage animal diseases where they first emerge, controlling them at the source before they have a chance to spread to the United States.

As a leader in animal health research, ARS's main goal is to protect and ensure the safety of the nation's agriculture and food supply. We continue to do this by delivering scientific information and tools to detect, control, and, when feasible, eradicate animal diseases.

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ROB FLYNN (K8253-6)

In a laboratory at Athens, Georgia, ARS veterinarian David Swayne prepares an avian influenza virus inoculum for a chicken vaccine study. Story begins on page 4.

Cover: ARS scientists have determined effective cooking times and temperatures for poultry meat and egg products to inactivate Newcastle disease and avian influenza viruses. Story begins on page 4. Photo by Peggy Greb. (D3121-1)

Reducing the Threat of Exotic Avian Diseases

Before media headlines announced outbreaks of a new type of avian influenza virus in China in 2013, Agricultural Research Service scientists were already working day and night to pinpoint crucial information about the H7N9 virus that was affecting humans as well as poultry.

Inside the high-level, secure Southeast Poultry Research Laboratory (SEPRL) in Athens, Georgia, the scientists studied the virus sample received from the Centers for Disease Control and Prevention (CDC). They labored quickly to determine whether the existing genetic diagnostic assays, the real-time reverse transcriptase polymerase chain reaction (rRT-PCR), could identify the virus or needed to be modified first, and whether new ones needed to be developed. They examined the effectiveness of available avian influenza vaccines and began research to develop new vaccines to protect poultry against the virus. And they investigated the virus's origin to identify the poultry species potentially involved in transmission of the virus in humans and its continuation in China.

This is what the small team, led by David Suarez, research leader of the SEPRL's Exotic and Emerging Avian Viral Diseases Research Unit, does best. The group responds quickly to emergency needs in foreign or exotic animal diseases. The laboratory serves as an international collaborating center for avian influenza and Newcastle disease with global partners, industry, and U.S. governmental agencies, such as USDA's Animal and Plant Health Inspection Service and the CDC. Scientists focus on research to help prevent and control diseases such as avian influenza, sometimes called "bird flu," and Newcastle disease that threaten the poultry industry worldwide.

"Our lab looks at diseases from the poultry angle, but we're also very concerned about public health," Suarez says. "We work closely with CDC and the National Institutes of Health to share data and information for both animal and human health."

Classifying Avian Influenza Viruses

Avian influenza virus strains, which infect poultry and other bird species, are characterized using two main proteins—hemagglutinin (H) and neuraminidase (N)—located on the surface of the virus, says David Swayne, SEPRL director.

Scientists sequence viruses or run serological tests for the 16 different hemagglutinins and 9 neuraminidases to determine the virus subtype. Some of these proteins are found in influenza viruses that grow in birds, and some are found in mammals and other species. Of the different H subtypes, only H5 and H7 have been found to be highly pathogenic for birds, which means they cause severe disease and kill more than 90 percent of infected birds, Swayne says.

"Highly pathogenic avian influenza viruses cause high poultry death losses, spread rapidly, and result in bans on international trade. They must be eradicated immediately. Low pathogenic viruses cause sick chickens and financial losses for farmers, but we can work with them through improved vaccines and other farm-management tools," Swayne says.

However, one exception makes research very essential, he adds.

"We want to know if the low pathogenicity virus can mutate to become highly pathogenic, which has happened multiple times," Swayne says. "That's the big question."

STEPHEN AUSMUS (D240-8)

In an incubator room, veterinary medical officer David Suarez candles, or shines light through, embryonic chicken eggs to look for signs of life. Embryo death may indicate viral infection.

"Ducking" Bird Flu

A major concern is the H5N1 highly pathogenic avian influenza virus that continues to circulate in Asia, the Middle East, and Africa, causing great losses in poultry and disease in humans, says veterinary medical officer Mary Pantin-Jackwood.

Domestic ducks, which are common in Southeast Asia, have been implicated in the spread of the H5N1 virus. There, domestic ducks are raised in backyards and in rice paddies, where they can come into contact with wild ducks and other poultry.

"Because of these contacts, domestic ducks are a source of H5N1 and other

STEPHEN AUSMUS (D241-45)

In a secure containment facility in Athens, Georgia, veterinary pathologist David Swayne and microbiologist Joan Beck (retired) determine the success of a new vaccine technology by taking throat swabs from chickens.

influenza viruses. They're like a mixing vessel," Pantin-Jackwood says. "The influenza virus mutates a lot, but it can also pick up genes from other influenza viruses."

Ducks infected with H5N1 virus show a wide range of responses—from moderate-to-high mortality to no sickness at all—which makes it difficult to recognize and control H5N1 influenza in these birds. This helps explain why the virus remains endemic in countries like China, Vietnam, and Indonesia, where ducks are a large food industry.

In her studies, Pantin-Jackwood showed that young ducklings do not fight off H5N1 infection as well as older ducks.

Successful control of the H5N1 virus in domestic ducks is important for the eradication of the disease in commercial poultry in Southeast Asia, she says. Duck species and husbandry practice should be considered when planning surveillance and control measures in countries with large domestic duck populations.

Pantin-Jackwood examined two commonly farmed domestic duck species, Muscovy and Pekin, and found a big difference in response to infection and vaccination against H5N1. Both species became infected with the virus, but Muscovy ducks developed a more severe disease.

"You need to vaccinate according to species of bird," she says. "In addition, domestic ducklings should be vaccinated before they are 1 month old and released into rice paddies, so they are well protected."

Getting the Most Out of Vaccines

When a highly pathogenic H7N3 virus was reported in Mexico in 2012, microbiologist Darrell Kapczynski examined available vaccines to make sure they would be able to protect U.S. poultry against this virus. He analyzed two USDA-approved H7 isolates and developed inactivated vaccines.

STEPHEN AUSMUS (K10700-1)

A vaccine against Newcastle disease is administered to a baby chick by microbiologist Darrell Kapczynski.

"We demonstrated 100 percent protection in vaccinated birds against a lethal challenge of the virus, showing that the vaccine derived from these isolates could protect U.S. poultry," Kapczynski says.

Scientists also determined that the new virus was not the same H7N3 virus obtained from ducks in Mexico in 2006. The two viruses were related, but the 2012 virus was not a direct descendant of the 2006 virus, Kapczynski says.

"What is interesting about the 2012 virus is that the genetics that make it highly pathogenic come from the bird itself," he adds. "This virus mutated using the host's own nucleic acid—incorporating part of the chicken genome into the virus genome."

This unusual "genetic recombination" event underscores the need to make sure that poultry are also free of the low pathogenic forms of the virus, he says.

Kapczynski and his colleagues also demonstrated that the Mexican 2006 low pathogenic virus could be used as a vaccine. All birds vaccinated with the virus strain and challenged with the 2012 virus were protected.

"The economic impact of this newer virus is enormous," he says. "The affected region produces around 55 percent of the table eggs in Mexico. More than $720 million in losses were reported by the industry since the outbreak. Fortunately, the virus hasn't moved into the United States."

Keeping an Eye on Egg Production

The 2009 pandemic H1N1 influenza virus—known as "swine flu"—does not kill birds; it infects the reproductive tract of poultry, causing decreased egg production, Kapczynski says. First identified in Mexico, the virus spread quickly around

KEITH WELLER (K8098-2)

STEPHEN AUSMUS (K10003-17)

A new vaccine for turkeys (left), developed by ARS scientists, is effective against the H1N1 influenza virus. Diseases of chickens (right) and other poultry are the focus of ARS's Southeast Poultry Research Laboratory in Athens, Georgia.

the world. It was found in swine in Canada and in breeder turkeys in Chile, Canada, and the United States.

"This was a 'reassortant' virus, meaning it contains two or more pieces of nucleic acid from different parent viruses," Kapczynski says. "Typically, influenza viruses segregate based on species they can infect." This virus had gene segments of avian, human, and swine influenza viruses and was capable of infecting mammals as well as poultry.

The poultry industry wanted to know whether the vaccines in stock would protect turkeys against the 2009 H1N1 influenza virus. Kapczynski and his colleagues made a new vaccine from the pandemic H1N1 virus and tested it against commercial inactivated H1N1 vaccines. Birds were vaccinated either with the pandemic H1N1 vaccine or the commercial vaccines and then challenged in the laboratory. Scientists looked at egg production, serology, and shedding. The new vaccine protected against egg-production losses, whereas the commercial vaccines were not as effective.

"The take-home message was that the turkey industry needed to update the isolates in the vaccines to more closely match the field strains in order to protect their flocks against this virus," Kapczynski says.

Taking a "Swab" at Viruses

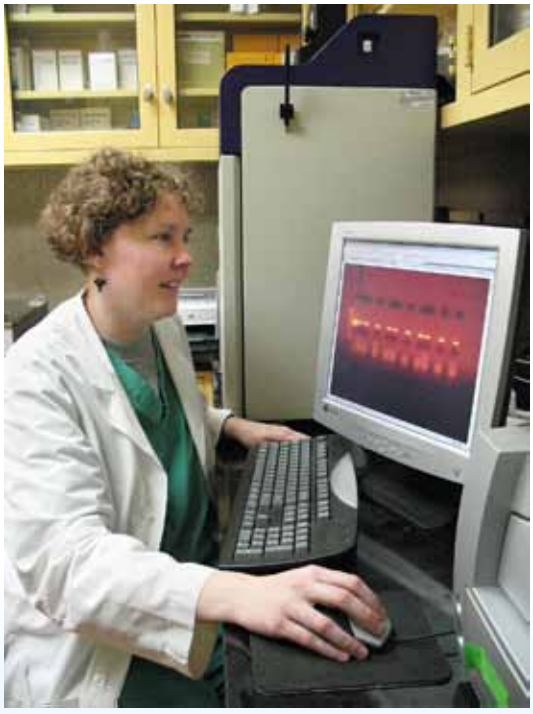
Before poultry is processed in the United States, the birds must be tested for avian influenza. Sample collection plays a key role in this process.

"In poultry, we test nearly 100 percent of all meat chicken and meat turkey flocks for the virus prior to processing. We also do a lot of surveillance in egg-laying chickens," says microbiologist Erica Spackman.

Identifying the best methods to collect avian influenza samples for optimal testing, and making sure the process is economically feasible, is important. Although the current method works well, Spackman found that improvements could be made.

"One of the most important variables is the number of swabs required—the sample size we take from inside the mouth of the chicken or turkey to see if the virus is there," Spackman says. "We need to collect a certain number of swab samples per flock to get a reasonable virus sample."

SUZANNE DEBLOIS (D3119-1)



Microbiologist Erica Spackman reviews results of a reverse transcription polymerase chain reaction test to determine whether there is virus in a sample and to generate material for gene sequencing.

Swab samples are collected from the same flock and put into tubes for testing. Traditionally, each tube contains 1-5 swab samples. The idea was to determine whether more swab samples could be pooled together into a single tube without inhibiting or affecting the sensitivity of the test.

Spackman found that putting 1, 5, or 11 swab samples in the same tube did not affect testing. A similar experiment with Newcastle virus samples had the same results.

Industry groups are already using the new process, which saves money without comprising test performance, Spackman says.

Investigating Newcastle Disease

Exotic Newcastle disease, an extremely virulent form of the virus, is not found in the United States, but it is widespread in Asia, Africa, South America, and Mexico. This contagious disease is costly, often fatal, and affects chickens and other bird species.

Like avian influenza, Newcastle disease threatens food security, particularly in countries like Africa, where poultry is the main source of meat protein, Suarez says. Most outbreaks are severe, killing about 80 to 90 percent of infected birds that have not been vaccinated or previously exposed to a less virulent form of the virus.

At SEPRL, microbiologist Claudio Afonso and veterinary medical officer Patti Miller study viruses from countries where the disease is endemic. They characterize the viruses, make sure existing tests and vaccines are effective against them, and develop strategies for

better vaccines to control them. Recently, they proposed a new classification system for Newcastle disease isolates.

Newcastle disease virus comprises a diverse group of viruses. Historically, two systems have been used to classify isolates. The lineage system grouped isolates into six lineages and a host of sub-lineages. The genotype system grouped isolates into class I or class II.

Both systems were being used simultaneously, which generated confusion and sometimes the assignment of viruses to multiple genetic groups.

To produce reliable and consistent results, Afonso and his colleagues developed a single system to group viruses. They evaluated gene sequences of more than 700 Newcastle disease virus strains, comparing genomes to identify and classify specific groups of isolates.

"After our analysis, Newcastle disease virus isolates placed in class I had only a single genotype, while isolates in class II contained 15 genotypes," Afonso says. "Since we have developed guidelines to classify genotypes, three additional genotypes have been identified."

The new system can be used by any laboratory worldwide.

Verifying Newcastle Vaccines

In collaboration with the poultry industry, the SEPRL team evaluates the capacity of current vaccines to protect against emerging isolates and tests improved vaccines.

"The genetics of Newcastle disease field strains differ from those of the vaccine strain," Suarez says. "As with avian influenza, the closer the 'seed'—the virus strain used to make the vaccine—is to the circulating virus, the more effective the vaccine."

Using this approach, scientists modified an existing Newcastle disease virus to include two key proteins in a new vaccine to provide optimal protection against other viruses or the field strain. The new vaccine reduced shedding and was more effective. Plans are being made to commercialize it.

In another study, Miller examined the role a bird's immunity plays in transmission of Newcastle disease virus, protection against it, and relationships among the genotypes. She determined the amount of antibodies produced by vaccinated animals and their capacity to transmit virulent challenge viruses.

ERICA SPACKMAN (D3117-1)

Swabs are taken from inside the mouths of birds, placed in tubes, and then tested for the presence of avian influenza virus.

"While there are multiple factors that affect the transmission of Newcastle disease, our findings suggest that, besides the level of antibodies induced after vaccination, decreasing the time to reach the peak antibody response should be a goal for future vaccines," Miller says.

Delivering Safe Egg Products

Scientists are taking their research a step further in a project that looks at how viruses can be rendered harmless when they are found in poultry products.

In past research, Swayne determined the times and temperatures needed to inactivate Newcastle disease and avian influenza viruses in poultry meat and egg products. "Those data sets are now included in the international regulations and used by the World Organization for Animal Health for cooking meat and eggs to make sure they are free of these disease-causing viruses," he says.

Because liquid egg products are normally pasteurized to eliminate *Salmonella,* Swayne and his colleagues investigated whether those pasteurization times and temperatures would also inactivate Newcastle disease and avian influenza viruses in egg products. They inoculated liquid egg products with both viruses and then heat-treated the eggs at various times and temperatures. The treatments were based on standard USDA pasteurization criteria for each specific product—homogenized whole egg and fortified, sugared, plain, and salted egg yolk.

Findings in one study suggested that one or more standard pasteurization processes killed the viruses in four of the five egg products, and the fifth required an extension of treatment for less than 1 minute.

Staying on Guard

Scientists at SEPRL continue to study existing Newcastle disease and avian influenza viruses and keep a vigilant watch on exotic poultry diseases as they emerge. They work to determine the origin of viruses, the best methods to detect them and to prevent them from spreading, and techniques to control and kill them. This research helps ensure that U.S. poultry is protected against these viruses if they happen to invade our country.—By **Sandra Avant,** ARS.

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

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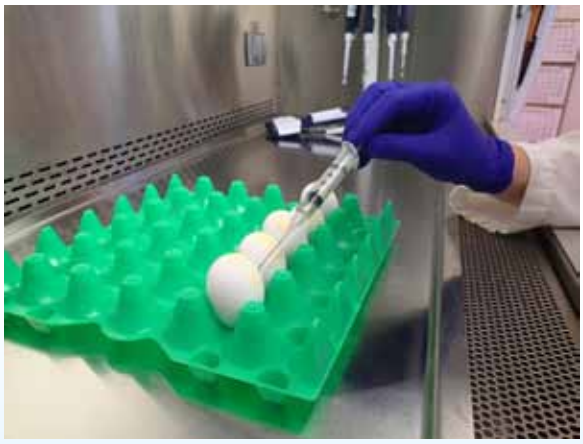
ERICA SPACKMAN (D3118-1)



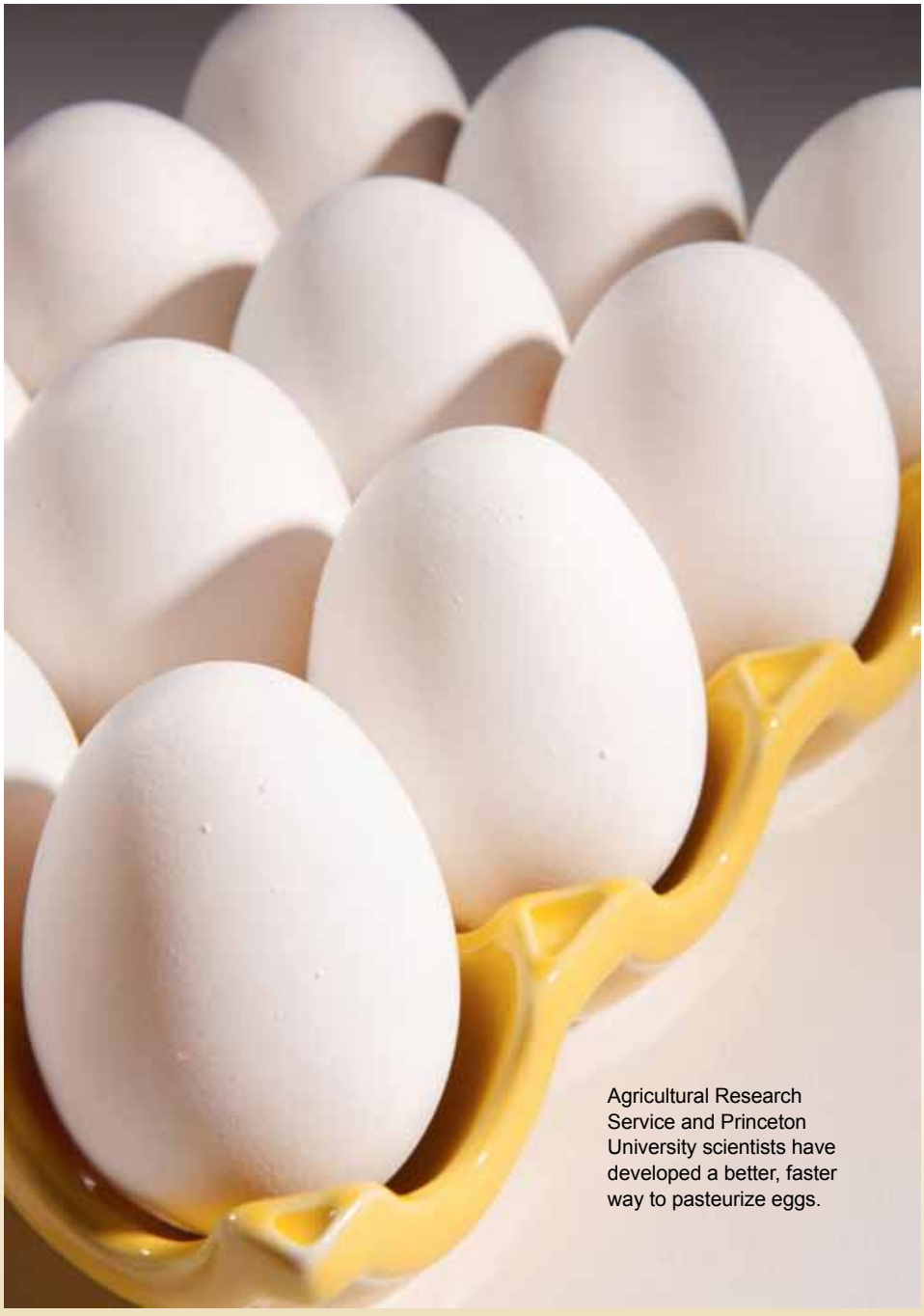
Avian influenza virus is harvested from a chicken egg as part of a diagnostic process.

Safer Eggs

New Technique Uses Radio Waves to Zap *Salmonella*

If you're a fan of classic Caesar salad or old-fashioned eggnog, you probably know that these foods contain raw eggs. For that matter, so do Béarnaise sauce, hollandaise sauce, conventionally made mayonnaise, some homemade ice cream, and, of course, eggs served sunny-side up or soft-boiled.

Problem is, about one out of every 20,000 chicken eggs produced in the United States has a high risk of being contaminated with *Salmonella* bacteria. Not all kinds of *Salmonella* are harmful to us, but some are, notably *S. enteritidis,* which has been associated with eating raw or undercooked eggs. This and other pathogenic *Salmonella* strains can cause diarrhea, stomach cramps, fever, and—in some instances—death.



Agricultural Research Service and Princeton University scientists have developed a better, faster way to pasteurize eggs.

PEGGY GREB (D3121-3)

Those most vulnerable to salmonellosis, as the disease caused by this microbe is known, are infants, preschoolers, pregnant women, the elderly, and anyone who has a compromised immune system.

Finding a Better Way To Kill the Bacteria

Properly cooking chicken eggs—such as by hard-boiling them—kills *Salmonella.*

So does pasteurizing them. Right now, a hot-water-immersion process is apparently the only technique used commercially in this country to pasteurize fresh "shell" eggs (eggs that are sold in-the-shell, instead of as a liquid product, for example). Many supermarkets offer these eggs as a specialty item in their dairy case.

But the hour-long immersion process may change qualities of these raw eggs, perhaps making them less satisfactory to discerning home cooks and restaurant chefs alike. Studies led by Agricultural Research Service chemical engineer Dave Geveke have resulted in a better, faster way to pasteurize raw shell eggs without ruining their taste, texture, color, or other important characteristics.

Geveke's tests with some 4,000 fresh shell eggs indicate that heating them with the energy from radio waves, or what's known as radiofrequency (RF) heating, followed by a comparatively brief hot-water bath, can kill harmful microbes without lessening the quality of the treated eggs.

Two-Phase Process

Here's how his technique works: Each raw egg is positioned between two electrodes that send radio waves back and forth through it. Meantime, the egg is slowly rotated, and its shell is cooled by spraying it with water—to offset some of the heat created by the radio waves.

Unlike conventional heating, RF heating warms the egg from the inside out. That's critical to the success of the process. It means that the dense, heat-tolerant yolk, at the center of the egg, receives more heat than the delicate, heat-sensitive white (albumen).

The hot-water bath comes next. The warmth of the bath helps the yolk retain heat, to complete the pasteurization. The heat from the water also pasteurizes the white, without overprocessing it.

From start to finish, the treatment takes around 20 minutes, making it about three times faster than the hot-water-immersion technique. And in tests using a research strain of *Salmonella,* Geveke showed that the RF-based process killed 99.999 percent of the *Salmonella* cells.

Inoculating the Eggs

Before the treatment, Geveke's team artificially infected the eggs by poking a small hole in the top of each, injecting the *Salmonella* into the egg via a glass syringe, then sealing the hole with a droplet of quick-setting epoxy glue. In nature, a hen's eggs can become contaminated with *Salmonella* if her ovaries are infected with it.

The idea of using RF heating to kill pathogens in foods isn't new. But using RF heating to kill pathogens in eggs is novel. And Geveke and his colleagues are evidently the first to pair RF heating with a hot-water bath to pasteurize raw shell eggs.

The new process is safe and effective and is expected to be cost-efficient, Geveke notes. Another plus: RF heating is already a familiar technology in the food industry: It's used in cooking, baking, and defrosting, among other chores.

Right now, the research is at the prototype stage. Christopher Brunkhorst of the Princeton Plasma Physics Laboratory in Plainsboro, New Jersey, teamed with ARS chemical engineering technician Andy Bigley and Geveke to build the compact prototype that has been used in their Wyndmoor, Pennsylvania, laboratory for the past 2 years. Geveke, Brunkhorst, and Bigley have applied for a patent for the research. What's more, several companies that process eggs have already expressed an interest in the technology.

A provision of the U.S. Food and Drug Administration's Food Code may contribute to growth of the raw-pasteurized-egg market. Already adopted by some states, the code specifies use of raw pasteurized eggs, or other pasteurized egg product, in place of unpasteurized eggs when foods such as Caesar salad are served to at-risk populations or to people who receive meals through "custodial care-giving environments" such as nursing homes, hospitals, or eldercare centers.

Though the specialty market is an obvious application of the RF-heating process, it could of course be used to pasteurize all of the more than 221 million fresh shell eggs produced in the United States every day. This would undoubtedly add to processors' costs, but might be a convenience for shoppers and would add an extra margin of safety to all fresh shell eggs—not just the specialty product, Geveke points out.

Commercial use of the RF-based method is at least a year or so away. Geveke expects to begin pilot-scale tests this year. After that, regulatory approval would be needed.

Those of us who remember being able to lick leftover cake batter off of the mixing spoon—without having to worry about *Salmonella*—can hardly wait.—By **Marcia Wood,** ARS.

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

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JOSEPH SITES (D3114-1)

Chemical engineering technician Andy Bigley positions an egg as chemical engineer Dave Geveke prepares to inject it with *Salmonella*. Their research has produced a technique for killing *Salmonella* without affecting egg quality.

PEGGY GREB (D3112-1)

What Makes an Avocado Delicious?

Scientists Seek Clues to Fruit's Flavor

As anyone who loves avocados will tell you, the perfect fresh avocado has a smooth, buttery texture and a rich, often nutty flavor.

Aroma is, of course, part of what we perceive as flavor. Scientists already know that Hass avocados, the kind that's most widely sold in the United States, have at least 25 aroma compounds or, technically speaking, aroma volatiles. Though the word "aroma" might imply that they have a role in avocado flavor, the precise contribution of each of these aroma volatiles has, for the most part, not been well studied, according to Agricultural Research Service plant physiologist David Obenland at Parlier, California.

ERIC FOCHT (D3120-1)

Taste-testers help identify key aspects of avocado flavor. In all, they provided more than 4,500 observations about the hundreds of domestic and imported avocados they sampled.

The aroma compound pentanal is a good example. It may be responsible for the nutty flavor of a ripe Hass avocado. Right now, however, there's not enough scientific data to confirm that role, nor to indicate the amount of pentanal needed to impart the nutlike flavor, Obenland says. What's more, no one knows whether other aroma chemicals in avocados contribute to this particular aroma.

But Obenland's collaborative research may help unlock some of the avocado's flavor secrets. He's giving top priority to determining the kinds and concentrations of aroma compounds that are essential to the classic flavor of Hass avocados.

With further work, these key compounds might serve as "markers" that breeders could use in pinpointing the most promising new kinds of avocados. Growers and packers of the future might be able to use the markers to determine the best times to harvest the fruit or to develop new tactics to better protect these compounds—or their precursors—during storage and ripening. Avocados, like pears and some other fruits, "mature" on the tree, but ripening (softening) occurs *after* they are harvested.

Ongoing research by Obenland and a team led by Mary Lu Arpaia, of the University of California-Riverside, is providing a start toward developing such markers. In preliminary studies, the scientists tracked changes in the concentrations of individual aroma volatiles as the avocados matured and ripened.

The researchers used two well-established analytical procedures (solid phase microextraction and gas chromatography/mass spectrometry) to extract, identify, and determine the quantities of the compounds. In all, they worked with samples from about 850 domestic and imported avocados and analyzed more than 4,500 observations from 15 to 20 taste-testers.

The studies, described in detail in *Postharvest Biology and Technology* in 2012, are apparently "the first to report the levels of aroma compound sampled during Hass avocado maturation and ripening," Obenland notes.

Among other findings, the researchers confirmed that three chemicals prevalent in the early growth of the fruit [hexanal; (E)-2-hexenal; and 2,4-hexadienal] were probably responsible for a grassy flavor, and that the "likeability" of the fruit—from the taste-testers' point of view—increased as the levels of these compounds decreased in the maturing fruit.

Obenland says the work differs from most prior avocado-flavor studies, which primarily focused on flavor contribution of the fruit's natural oil.

"Our findings," he says, "provide new evidence that aroma compounds actually do influence avocado flavor." ARS, along with the university, the California Avocado Commission, and Pinkerton Avocado Growers Association, funded the research, which was conducted with the help of Mission Produce, Inc., and Del Rey Avocado Company.—By **Marcia Wood,** ARS.

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

*David Obenland is with the USDA-ARS San Joaquin Valley Agricultural Sciences Center, 9611 S. Riverbend Ave., Parlier, CA 93648; (559) 596-2801, david.obenland@ars.usda.gov.**

Opposite page: ARS and university scientists are unlocking flavor secrets of Hass avocados.

Cleaning Honeycombs With Ozone

STEPHEN AUSMUS (D007-1)

Worker bees remove the mummified remains of larvae infected by the chalkbrood fungus, *Ascosphaera apis*. ARS scientists have found that fumigating combs with ozone can destroy spores of this pathogen.

Sometimes, even honey bees need help with "housekeeping"—especially when it comes to tidying up their combs once the honey's been removed. Research by Agricultural Research Service scientists has shown that fumigating combs with ozone gas can eliminate pests and pathogens that threaten honey bee health and productivity. Recent results suggest that ozone fumigation may also help reduce pesticide levels in combs.

The findings stem from a two-part study led by Rosalind James, an entomologist in ARS's Pollinating Insect—Biology, Management, and Systematics Research Unit in Logan, Utah. Results from the first part of her team's study, published in 2011 in the *Journal of Economic Entomology,* demonstrated that fumigating combs with ozone gas at concentrations of 215 to 430 parts per million (ppm) killed all life stages of the greater wax moth, depending on length of exposure. Adult moths and their comb-damaging larvae were most susceptible, and eggs were more resistant, requiring greater exposure levels.

Ozone, a highly reactive state of oxygen, also destroyed spores of the chalkbrood fungus after 24 to 36 hours of exposure using 1,500 ppm. Another honey bee pathogen, however, proved tougher to kill: The American foulbrood bacterium required substantially longer exposure times, along with high humidity levels and an ozone concentration twice as high.

Both pathogens can persist for years on beekeeping equipment and in hives as dormant spores. They germinate when conditions are optimal, and they attack the colony's most vulnerable members, the larvae or "brood." Methyl oxide and gamma irradiation are among treatments that have proven effective for disinfecting comb, but these treatments can be costly and impractical. "Irradiation has to be done in a regulated facility," says James, "whereas an ozone fumigation chamber is something beekeepers can set up on their own."

In January 2013, the team published results from the second part of the study in the journal *Agricultural Science.* That paper details ozone's breakdown of coumaphos, fluvalinate, and several other pesticides that can accumulate in hives. Honey bees are typically exposed to pesticides while visiting flowers that have been sprayed or when the bees are treated for parasitic mites, notes James. The study's coauthors include James Ellis of the University of Florida in Gainesville, and Adrian Duehl, formerly with the ARS Center for Medical, Agricultural, and Veterinary Entomology, also in Gainesville.

The team was particularly interested in coumaphos and fluvalinate because of the chemicals' use against *Varroa* mites. Considered a top threat of honey bees nationwide, the flat-bodied parasites can weaken and eventually kill bees by feeding on their bloodlike hemolymph. Severe *Varroa* infestations can decimate a hive within months if left unchecked.

In experiments with glass vials containing residues of these mite-killing pesticides, ozone exposures of 500 ppm for 10 to 20 hours degraded 93 to 100 percent of coumaphos and 75 to 98 percent of fluvalinate. Higher concentrations and longer exposure times were required to reduce pesticide concentrations in wax and comb samples, which were obtained from a Florida-based commercial apiary to reflect real-world exposure levels. The researchers observed that ozone treatments degraded the pesticides better in new combs (less than 3 years old) than in older ones (more than 10 years old).

"There's something about the wax that can impede this breakdown, especially in a comb that's been reused in hives for many years," says James. "It may be that organic materials build up inside the wax, and these materials adsorb or break down the ozone before it can react with the pesticides."

One approach may be to start with new comb and treat it yearly to prevent pesticide residues from building up; another would be to replace comb more often than is commonly practiced. High-capacity ozone generators may be necessary for ridding comb of especially high pesticide concentrations—albeit at added cost.

During the study, participating beekeepers also reported an off-odor emanating from combs that had been treated for wax moths or small hive beetles and placed back

PEGGY GREB (D1041-1)

Research leader Rosalind James (background) and entomologist Theresa Pitts-Singer evaluate alfalfa leafcutting bees in nesting boards.

into hive boxes. So, in a separate study, the team analyzed breakdown products from hives that had been treated with 2,000 ppm of ozone. They determined the primary source of the odor to be benign substances known as "carboxylic acids" and "straight-chain aldehydes." Fortunately, the odors didn't repel the bees and dissipated after a few months, according to the beekeepers. One also noted that bees accepted comb treated for wax moths better than untreated comb.

Besides coumaphos and fluvalinate, the ozone treatment reduced or eliminated eight other common agricultural pesticides found in Florida comb samples, including esfenvalerate (Conquer or Ortho Bug B Gon insecticides), thymol (a pesticide made from thyme extract), and chlorothalonil (Fung-onil or Daconil fungicides).

James envisions beekeepers fumigating combs after they've been removed from hive boxes and emptied of honey, but just before being placed in storage for the winter. Combs removed from storage aren't necessarily placed in the original hive, but sometimes in a different one.

"This practice potentially transmits disease from one colony to the next, especially if the pathogen produces a spore that can stay dormant for long periods," notes James.

Beekeepers may be reluctant to discard comb—even that which has become discolored from years of use—because of the considerable effort bees put into making comb. "It takes a bee time and energy to make wax, but it's needed for making and storing honey, so creating new comb comes at a cost to honey production," adds James.

Ozone offers an appealing solution for decontaminating combs before reuse because it's a process that beekeepers can carry out using commercially available equipment. Although toxic at the concentrations that are used to kill pests and pathogens and degrade pesticides, ozone rapidly breaks down into water and oxygen, she notes.

Current uses of ozone, which is considered a "generally recognized as safe" substance by the U.S. Food and Drug Administration, include decontaminating pool and drinking water and safeguarding the postharvest quality of fruits and vegetables. Until James's studies, there had been no published reports on using the gas to decontaminate honeycombs.

"In our next field trials, we are going to try using ozone on nesting boards for the alfalfa leaf-cutting bee, which is used for pollinating alfalfa crops intended for seed production," says James. She also plans on collaborating with ARS's Bee Research Laboratory in Beltsville, Maryland, to evaluate ozone's effectiveness at reducing disease transmission and improving colony health.—By **Jan Suszkiw,** ARS.

This research is part of Crop Production, an ARS national program (#305) described at www.nps.ars.usda.gov.

*Rosalind James is in the USDA-ARS Pollinating Insects—Biology, Management, and Systematics Research Unit, Utah State University, Logan, UT 84322-5310; (435) 797-0530, rosalind.james@ars.usda.gov.**

Interior view of the ozone generator that supplies ozone to the fumigation chambers used in the studies.

ROSALIND JAMES (D3108-1)

Dietary Products

Some are Underresearched, Overmarketed

While surfing the Web, you may have seen ads promising "one tip to a flat belly." After clicking on the ad, you jump to a website where official-looking major-news-outlet logos appear. After reading more details about the advertised product, if you clicked "purchase," you took a step closer to achieving the product promoter's goals.

There are several products, such as African mango supplements, that appear at the end of these "one tip" ads. But the real tip is accurate consumer information about the products.

The popular African mango (AM) supplements are based on extracts from the seeds of *Irvingia gabonensis*. At the Beltsville [Maryland] Human Nutrition Research Center, Agricultural Research Service chemist Pei Chen and postdoctoral associate Jianghao Sun studied AM supplements and found that none of the labels on the ones they tested provided accurate information for consumers.

During the past decade, other researchers have conducted human clinical trials and published results indicating that AM seed extracts *may* have some effect on lowering body weight.

"All of the labels of African mango dietary supplement products sold in the United States list African mango seed extract as the major ingredient," Chen says. Chen and Sun conducted studies to find out whether the supplements contain what they say they contain.

Noting a lack of past chemical analyses of AM seeds, seed extracts, and dietary supplements in the scientific literature, Chen and Sun used a method called "ultra high-performance liquid chromatography-mass spectrometry" to perform such an analysis.

For the study, the team procured AM seeds that had been imported directly from Africa. The samples came with a voucher verifying their authenticity and were further authenticated by a U.S. Pharmacopeia scientist. The team also procured three AM seed extracts and five different AM dietary supplements to analyze. The three seed extracts were imported from China, and the supplements were purchased online in the United States.

During testing, Chen and Sun identified a group of major components in the verified AM seeds: ellagic acid; mono-, di-, and tri-*O*-methyl-ellagic acids; and their related glycosides. "These components can be used as authentication markers when testing the contents of AM extracts and related AM dietary supplements for quality control," says Chen.

Among the five AM dietary supplements tested, only one contained trace amounts of AM seed. The other four supplements and the three AM seed extract samples did not contain any detectable amount of authentic AM seed.

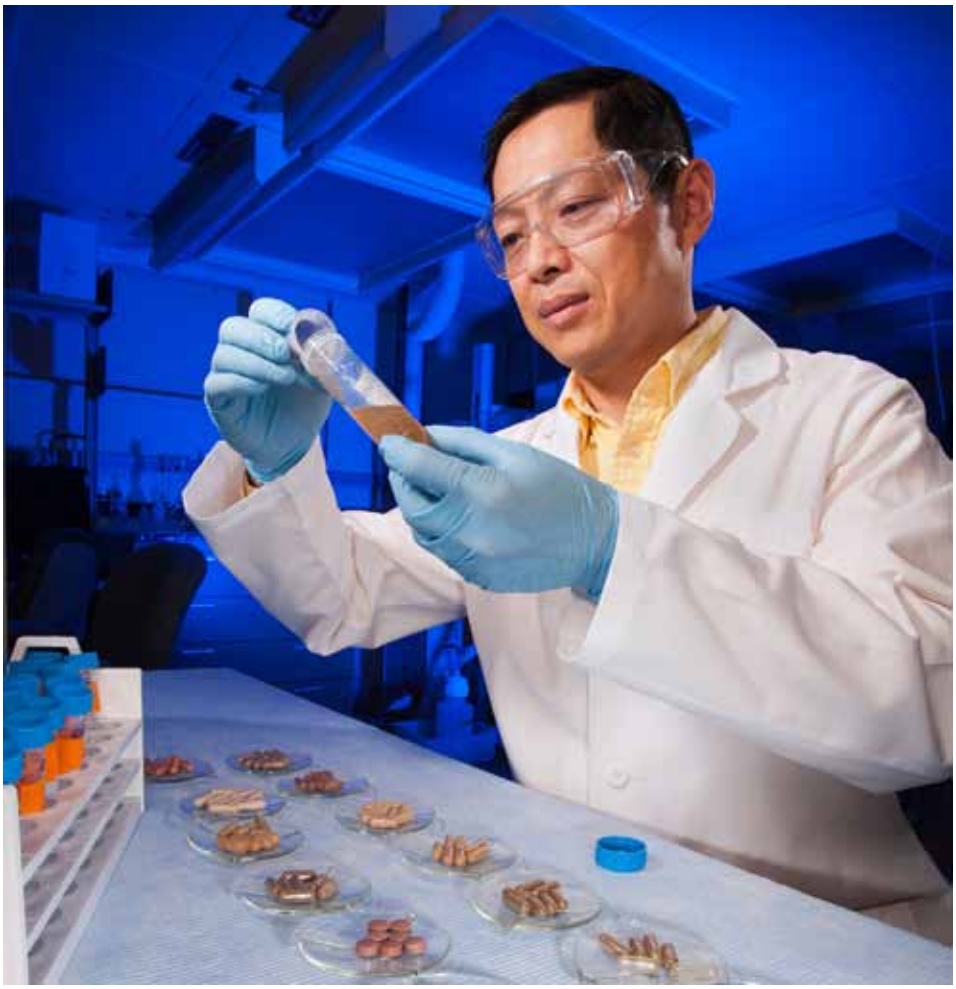
MARSHALL G. MILLER (D3110-1)

In the Neuroscience and Aging Laboratory at the Jean Mayer USDA Human Nutrition Research Center on Aging at Tufts University in Boston, research psychologist Barbara Shukitt-Hale (left) and molecular biologist Shibu Poulose prepare açaí fruit extract samples for cell studies.

When Foods Are Hyped in the Media

"Super foods," is a non-scientific term sometimes used by both media and advertisers to promote certain whole foods that are high in nutrients or phytochemicals. These compounds are often extracted and put into dietary supplements or added to other foods by the food and supplement industries. The aim is to appeal to the consumer's hope for health benefits from consumption.

PEGGY GREB (D2474-1)



Chemist Pei Chen prepares extracts from dietary supplements to study differences in the content of phytochemical compounds.

Significantly, there is scientific evidence that eating a wide variety of foods is important to the body's ability to absorb nutrients, and the *Dietary Guidelines for Americans* promotes a diet that consists of "co-consumption" of a variety of recommended foods to achieve a balanced diet and sufficient nutrient intake.

ARS scientists in Boston, Massachusetts, have authored a book chapter focusing on one widely touted food—açaí (pronounced ah-sah-EE). Açaí is a reddish-purple berry, which grows on large palm trees that are in the *Euterpe* genus and are native to Central and South America. Açaí has received attention among food scientists, and now a variety of açaí products—juices, tablets, capsules, and dissolvable powders (freeze dried or spray dried)—are being marketed to consumers.

The book chapter was authored by molecular biologist Shibu Poulose and research psychologist Barbara Shukitt-Hale, who are with the Neuroscience and Aging Laboratory, which is part of the Jean Mayer USDA Human Nutrition Research Center on Aging at Tufts University in Boston. Studies in humans are needed to assess potential health benefits of any single food, and the authors report that there have been a limited number of studies exploring the health effects of açaí berry in humans.

Focusing on Facts

In 2013, ARS nutritionists Seema Bhagwat, David Haytowitz, and Joanne Holden (retired) prepared and launched the USDA-ARS Database for the Flavonoid Content of Selected Foods: Release 3.1. The amount of flavonoids in foods is of interest because of their purported beneficial health effects.

Though there are thousands of individual flavonoids, the new release contains composition values for 26 commonly occurring dietary flavonoid compounds in 506 food items. For example, for each 100 grams of açaí, the flavonoid database lists the amount of cyanidin in fresh berries at 53.64 milligrams (mg), frozen berries at 61.94 mg, and powdered açaí fruit/pulp/skin at 200.96 mg.

"The freeze-dried açaí powders that are commonly used for nutrient-composition testing and analysis have had almost all of the fruit's moisture removed," says Haytowitz.

In their book chapter, Poulose and Shukitt-Hale report that published scientific tests and analyses have shown that açaí fruit and pulp contain other phytochemicals, including different flavonoids, phenolic acids, and stilbenes; and nutrients, including vitamin A, beta-carotene, copper, iron, retinol, calcium, protein, and fiber. Açaí also is rich in polyunsaturated fatty acids, such as omega-3 and omega-6, and monounsaturated fatty acids. For example, its oleic acid content reportedly rivals that of olive oil, according to the authors.

Gauging how well the body breaks down phytonutrients in foods and whether such compounds are available for absorption in the body requires separate testing and analysis. At this time, there is a dearth of scientific evidence proving health effects in humans from consuming açaí. And there is a need to test foods associated with health claims by nonscientific sources.

The book chapter appears in "Tropical and Subtropical Fruits: Flavors, Color, and Health Benefits," published in 2013 by the American Chemical Society.—By **Rosalie Marion Bliss,** ARS.

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

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KATHY EYSTAD (D3116-1)

Coats Protect Seeds Planted in Cold, Wet Soils

Farmers in the northern Corn Belt have a narrow springtime window to plant their crops—an interlude when the snow is gone, the soil has thawed, and the fields are not too wet and not too dry. But farmers live in an imperfect world, and planting delays can seriously reduce yields. So Agricultural Research Service plant physiologist Russ Gesch and his colleagues have conducted several studies to see whether adding a protective coating to corn and soybean seeds could help optimize planting schedules.

"In Minnesota, farmers want to get into their fields as soon as possible, because some of them are farming thousands of acres. But early planting in cold, wet soils can damage seeds," says Gesch, who is in the ARS Soil Management Research Unit in Morris, Minnesota. "Seeds that sit in wet soil for too long can rot. Or maybe the seeds germinate, but emergence is uneven—a few plants emerge one day, a few more plants emerge a few days later—there's a mix of developmental stages across the field. And even when farmers follow a normal planting schedule, a cold snap later on can interfere with seed response and result in poor emergence and poor yields."

Time To Cover Up

Gesch's research began with a 3-year study he conducted with agricultural economist David Archer, who works at the ARS Northern Great Plains Research Laboratory in Mandan, North Dakota. The two researchers compared the performance of coated and uncoated corn seeds planted in early spring—when soil temperatures were still below the optimum 50°F needed for germination—and coated and uncoated seeds planted 4 to 6 weeks later. The coated seeds were covered with a temperature-activated polymer that prevented water from reaching the seeds until soils were warm enough for germination and emergence.

The scientists observed that coated seeds planted in early spring had significantly greater levels of emergence and establishment than uncoated seeds planted at the same time. And the rate of emergence—how long it took 50 percent of the seeds to emerge and be established—was faster for coated seeds than for uncoated seeds.

Opposite page: Uncoated soybean seeds (left) and polymer-coated seeds (right). The ultra-thin coating protects seeds from damage caused by the cold, wet soils of early spring.

Below: Plant physiologist Russ Gesch works with young crop plants in the lab. Gesch's research on protective seed coatings is helping North Central Plains farmers optimize planting schedules and improve crop production.

Coated seeds planted in early spring generally had similar or greater rates of emergence than uncoated seeds planted in late spring. However, coated seeds planted in late spring generally had slower emergence rates than uncoated seeds planted at the same time. To the scientists, these findings strongly indicate that farmers could use coated seeds to get a jump-start on their spring planting, because the seeds would be protected from cold, wet soils until conditions favored germination and emergence.

KATHY EYSTAD (D3115-1)

But the soil conditions that help or hinder planting schedules aren't just linked to seasonal changes. Farmers use no-till management to help protect soil moisture, mitigate soil erosion, and preserve soil structure. However, in late winter and early spring, no-till soils are also generally wetter and colder than conventionally tilled soils.

"Early planting is more challenging with no-till, even though no-till systems have environmental and economic benefits," says Gesch. "Some farmers prefer to use conventional tillage because it helps to warm and dry the soil more quickly and gives them a head start in planting."

So in his next study, Gesch worked with ARS soil scientist and research leader Brenton Sharratt to evaluate the effects of seed coats in conventional-till and no-till fields. Sharratt works at the ARS Land Management and Water Conservation Research Unit in Pullman, Washington. The scientists compared the performance of coated and uncoated seeds of corn and soybean, planted in no-till and conventional-till fields in early spring and in late spring. Unlike corn seeds, soybean seeds do not germinate well until soil temperatures reach about 55°F.

The scientists didn't find any consistent differences in germination and emergence between coated seeds planted in conventional-till systems and those planted in no-till systems. As in the previous study, these results suggested that farmers could accelerate their production schedules by planting coated seeds in no-till fields in the early spring. But Gesch and Sharratt also found that coated soybean seeds planted in late spring were less successful because they were exposed to soil temperatures that were too high for successful germination and establishment.

When the Time Is Right

In a followup 2-year study of no-till production, Gesch, Archer, and soil scientist Kurt Spokas monitored the responses of coated and uncoated soybean seeds in no-till fields planted in early spring and late spring. Spokas works at the ARS Soil and Water Management Unit in St. Paul, Minnesota.

As in the other studies, coated seeds emerged later than uncoated seeds. The coated seeds planted in early spring took longer to reach 90 percent emergence than seeds that were planted in late spring. But in both years of the study, planting coated seeds 4 to 5 weeks earlier than usual did not have any negative effects on eventual emergence or plant population establishment.

However, planting coated seeds in late spring—after soil temperatures had risen and moisture levels had declined—did result in slower emergence and slower stand establishment.

"Seed coats can be detrimental with late planting because we don't usually want to delay emergence at that point," says Gesch. "The seed coat doesn't break down just because soil temperatures warm up; there also needs to be enough moisture. And if the seed coat doesn't break down completely before germination and emergence, the coat can actually strangle the seed."

These studies were published in *Agronomy Journal, Journal of Agricultural and Applied Economics,* and *Field Crops Research.*

"Not many farmers are using seed coats as a management tool yet, because the coating technology is not widely available," says Gesch. "But the farmers we've talked with are interested in our findings, because they realize that using seed coats could help them optimize their planting schedules."—By **Ann Perry,** ARS.

This research is part of Crop Protection and Quarantine (#304) and Crop Production (#305), two ARS national programs described at www.nps.ars.usda.gov.

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SCOTT BAUER (K9441-1)

Probiotics for Pigs

Less Manure, Better Health

Pigs that consumed a fiber-rich diet and a bacterial probiotic supplement reduced their manure output by 20 percent, gained more weight, and had better blood cholesterol and glucose levels.

Pig producers would like to keep their costs down by supplementing livestock feed with dried distiller's grains with solubles (DDGS) and other agricultural coproducts generated from biofuel production. But adding hard-to-digest fiber to livestock diets also increases the production of manure—never a good thing, especially when it threatens to exceed on-farm storage capacities. So Agricultural Research Service microbiologist Cherie Ziemer and others conducted the first published investigation of using bacteria as a probiotic to increase fiber fermentation rates and reduce manure output in pigs consuming high-fiber diets.

A high-fiber diet can also serve as a source of energy if the fiber can be broken down during digestion. Other researchers have investigated using carbohydrase enzyme supplements to break down dietary fiber and release sugars that can then be absorbed in the small intestine. Bacterial fermentation of these same fibers, on the other hand, takes place in the large intestine and results in the production of small-chain fatty acids that pigs use to meet metabolic energy demands.

Ziemer and animal scientist Brian Kerr, who works with Ziemer in the ARS Agroecosystem Management Research Unit in Ames, Iowa, fed the pigs in their study either a typical diet or a high-fiber diet. The high-fiber diets contained 10 percent soybean hulls and 20 percent corn DDGS. The pigs were also given one of three bacterial supplements the scientists developed from different strains of *Bacteroides ovatus,* which had been obtained from human fecal samples and cultured in fiber-rich media. The three bacterial supplements were designated Bacterium B, C, and D.

The pigs that consumed the fiber-rich diets and the three different probiotics had strikingly different levels of manure production. The pigs given Bacterium D produced about the same amount of manure daily as the pigs that did not receive a probiotic. The group given Bacterium C produced 4 percent *more* manure than the probiotic-free group.

But pigs that received the bacterial supplement designated as Bacterium B reduced their manure output by 20 percent. These pigs also gained more weight and had better blood cholesterol and glucose levels—both indications of an improved energy status—than pigs not given probiotics.

Ziemer believes the probiotic could improve pig performance and reduce manure volumes, which in turn would increase producer profits and reduce the environmental footprint of pork production. She thinks the bacterium could be fed in a liquid supplement or possibly freeze-dried and mixed with feed.

This work was supported by a grant from the Defense Advanced Research Projects Agency as part of the Intestinal Fortitude Program, which investigates how to help people obtain more energy from fiber. Results were published in the *Journal of Animal Science* in 2012, and ARS has submitted a patent application for Bacterium B.—By **Ann Perry,** ARS.

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

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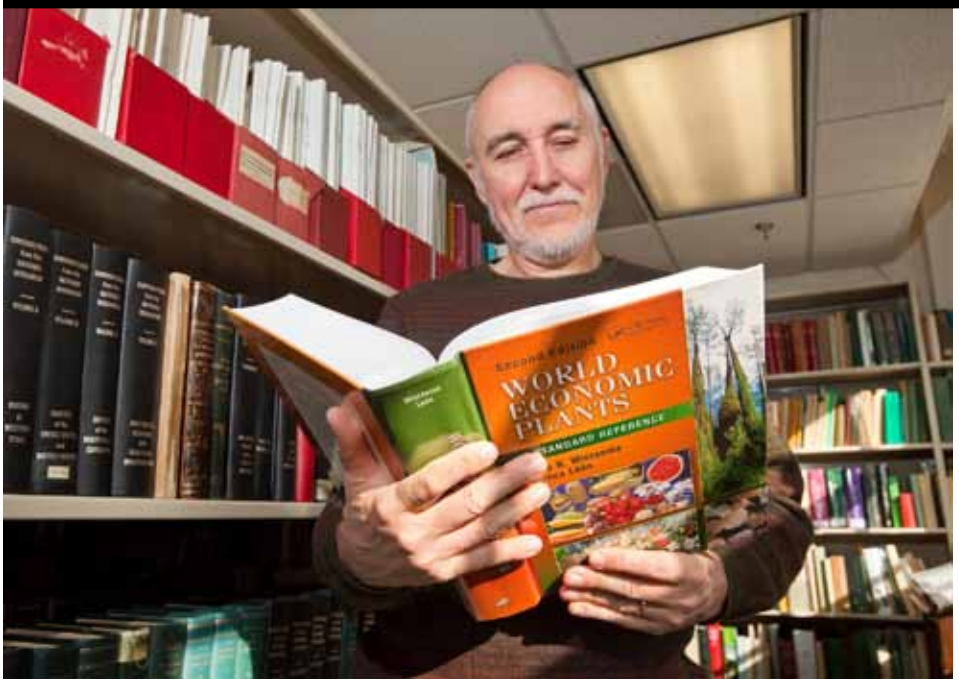
STEPHEN AUSMUS (D3107-7)



New Reference Provides Uses and Origins of Economically Important Plants

At the ARS Germplasm Resources Information Network (GRIN) taxonomy botany library in Beltsville, Maryland, botanist John Wiersema reviews the new edition of "World Economic Plants: A Standard Reference," which he coauthored.

At 1,336 pages, "World Economic Plants: A Standard Reference" is more for professionals and scientists than the casual reader. The book, compiled by an Agricultural Research Service botanist and a University of Texas taxonomist, could also be considered a testament to the diversity of our plant life.

Authors John Wiersema and Blanca León link the list of scientific names with the geographic origins, uses, and relationships of 12,235 plants. They also provide over 50,000 common names for those plants in 27 languages, among them Arabic, Chinese, and Russian. Plants often have different names and uses in different countries, says Wiersema, who is with the ARS National Germplasm Resources Laboratory in Beltsville, Maryland.

The book, published by CRC Press, focuses on plants that are "directly or indirectly important to international [or interstate] commerce ... or have recognized potential for widespread economic usage," according to the text. Plants used for food, fiber, timber, medicines, ornamental purposes, crop breeding, and many other uses are included, along with those having negative impacts, such as invasive weeds and poisonous plants.

The book is an update of an edition the researchers published in 1999 that inventoried 9,500 plants. DNA studies have revolutionized what scientists know about plants and their classifications in recent years. The new edition incorporates that recent molecular data, as well as information gleaned from other types of plant studies.

Along with including 25 percent more plants, the 2013 version indicates more "use classes," such as whether a plant is, for instance, a food source or has medicinal value. Some of the most common categories are ornamentals (5,361), medicines (2,997), food and food additives (2,212), and weeds (2,136).

Readers can look up a plant under its common name or its scientific name, and using the latter, they can learn about the plant's geographic distribution, what it is called in other languages, and how it is used. To supplement data on a plant's native range, the authors have added information on where a plant has been introduced or cultivated.

Information in the book and its sources are fully elaborated on in the ARS Germplasm Resources Information Network, which is part of the ARS National Plant Germplasm System and is publicly available online.

This updated edition, requested by the publisher, offers the advantage of serving as a condensed summary of that information in an easily retrievable format. It will be useful to researchers, plant breeders, librarians, companies involved in regional or global marketing of plants or plant products, regulatory officials, or anyone who needs basic, accurate information about economic plants.

The publication took over 2 years to complete, and the material was reviewed for accuracy by more than 150 experts.

The book costs $149.95 and can be ordered online at www.crcpress.com.—By **Dennis O'Brien,** ARS.

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

*John Wiersema is with the USDA-ARS National Germplasm Resources Laboratory, 10300 Baltimore Ave., Beltsville, MD 20705-2350; (301) 504-9181, john.wiersema@ars.usda.gov.**

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

Locations Featured in This Magazine Issue

Locations listed west to east.

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

San Joaquin Valley Agricultural Sciences Center, Parlier, California
3 research units ■ 112 employees

Pullman, Washington
6 research units ■ 110 employees

Logan, Utah
3 research units ■ 77 employees

Northern Great Plains Research Laboratory, Mandan, North Dakota
1 research unit ■ 32 employees

North Central Soil Conservation Research Laboratory, Morris, Minnesota
1 research unit ■ 31 employees

Ames, Iowa
8 research units ■ 398 employees

St. Paul, Minnesota
3 research units ■ 48 employees

Athens, Georgia
9 research units ■ 167 employees

Center for Medical, Agricultural, and Veterinary Entomology, Gainesville, Florida
4 research units ■ 150 employees

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

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

USDA Jean Mayer Human Nutrition Research Center on Aging, Boston, Massachusetts
1 research unit ■ 8 employees