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U.S. Department of Agriculture · Agricultural Research Service · January 2013

Agricultural Research

More Than Just a Home Invader!

Story begins on page 18

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

FORUM

Citrus Greening: A Puzzle With Many Pieces

Finding the solution to some crop diseases is like solving a jigsaw puzzle. The solution has many pieces, and fitting them together can be a challenge. Citrus greening, also known as "Huanglongbing" (HLB), is just such a disease. HLB is costing Florida citrus growers millions of dollars each year in lost revenue from unproductive trees. It continues to spread and is the most serious threat to the U.S. citrus industry in history. The disease was recently confirmed in commercial groves in Texas and in a homeowner's yard in California. Both states are important citrus producers, and if HLB spreads in these states the way it has throughout Florida, the economic consequences will be devastating.

The Agricultural Research Service is working aggressively with university and private-industry partners to help mitigate the threat posed by HLB. The goal is to keep the U.S. citrus industry sustainable and profitable and to preserve the nutritious and delicious array of fruits and juices that people have come to treasure.

Solving the HLB puzzle depends on developing a deeper understanding of its complicated nature. HLB is presumed to be caused by nonculturable, Gram-negative bacteria belonging to the genus *Candidatus* Liberibacter. So far, the species of bacteria associated with HLB in the United States is *Candidatus* Liberibacter asiaticus, which is transmitted by the Asian citrus psyllid, an insect.

The puzzle pieces are questions that constitute gaps in our knowledge: Do some types of citrus have a natural resistance that can be bred into commercial varieties? How can psyllid populations be effectively suppressed? Are there molecular tools for tapping into the genomes of the psyllid or bacterium in ways that would "disarm" them? How do we replant young, healthy trees and keep them free of HLB? Is there a cure for infected trees?

In this issue, you can read about some of ARS's efforts, including research in Fort Pierce, Florida, to exploit a fungal pathogen that keeps the psyllid in check and to help juice processors maintain their high standards (see page 4). We also explore investigations in Albany and Davis, California, to determine whether citrus amino acids hold clues to attack strategies used by the Liberibacter bacterium (see page 7).

Other ARS research includes disease modeling and tracking in Fort Pierce, which provides valuable guidance to state and federal regulatory agencies that use the research to make decisions designed to curb the spread of HLB and the psyllid.

The ARS National Clonal Germplasm Repository for Citrus and Dates in Riverside, California, maintains a diverse collection of citrus and its relatives in a disease-free environment. The repository is instrumental in ensuring that citrus trees provided to commercial operations are free of known pathogens. A team of ARS and university scientists there is screening citrus relatives for sources of natural resistance to the disease, the psyllid, or both.

In Fort Collins, Colorado, scientists are working on cryopreservation techniques that show promise for long-term storage of genetic materials and that also have potential for eliminating pathogens from propagative materials.

The psyllid genome has been sequenced and made publicly available by an ARS scientist in Fort Pierce, and researchers there are exploring ways to use RNA interference technology to silence genes critical to the psyllid's survival.

In Albany, scientists completed the genome sequence of Carrizo citrange, an important citrus rootstock, which will help in the search for genes that may be used to genetically improve citrus for HLB resistance.

In Parlier, California, a researcher is using genomic sequences of a wide range of Liberibacter isolates to develop diagnostics based on specific "DNA signatures," so that when HLB is detected in a citrus tree, it can be traced to its source. Such forensic abilities are in demand among scientists trying to track down sources of new infections in California and Texas, where the incidence of HLB is still low.

ARS scientists working in quarantine facilities in Beltsville and Fort Detrick, Maryland, maintain a collection of HLB isolates from around the world for comparative genetic analyses. Those at Fort Detrick have made progress in diagnosing and differentiating world isolates, and in Beltsville, they are using comparative genomics to identify genes and proteins that may provide an understanding of the role of Liberibacter in HLB disease development.

Currently, the only way to control HLB is to remove infected trees and suppress the psyllid vector. This approach presents difficult choices to growers because many are reluctant to destroy infected trees. A wide range of possible treatments and solutions is being explored for successful management in the future. Together, these efforts are moving us toward the ultimate goal of maintaining a profitable and competitive U.S. citrus industry and eliminating this global threat altogether.

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Solving Problems for the Growing World

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DAVID HALL (D2713-2)

Adult Asian citrus psyllid killed by a biocontrol fungus. This beneficial fungus may prove useful in battling the psyllid, which is spreading a devastating citrus disease in the United States. Story begins on page 4.

Cover: The brown marmorated stink bug is easily recognized by many because it's invading our homes. But the pest, shown here feeding on an apple, is also a major economic threat to fruit crops, garden vegetables, and many ornamentals. ARS scientists are fighting back by developing traps, sequencing the bug's genome, and testing parasitic wasps as biocontrols. Story begins on page 18. Photo by Stephen Ausmus. (D2709-1)

Helping Citrus Growers Deal With a Nasty Invader

Growers battling a devastating citrus disease may soon benefit from a one-two punch from Florida researchers. Two Agricultural Research Service scientists at the U.S. Horticultural Research Laboratory in Fort Pierce are using two very different strategies to help citrus growers and processors deal with Huanglongbing (HLB), a disease that poses a major threat to the survival of the citrus industry and is costing it millions of dollars each year.

Elizabeth Baldwin, research leader of the Citrus and Subtropical Products Research Unit, is leading a team developing technology that orange juice processors can use to determine whether their product has the right taste. David Hall, research leader of the Subtropical Insects Research Unit, has found a way to better exploit a fungus that naturally controls HLB.

HLB, or citrus greening, was first detected in Florida in 2005 and is now established in all citrus-growing counties in the state. The disease was confirmed soon afterwards in backyard citrus trees in Georgia, Louisiana, and South Carolina. Citrus greening was recently confirmed in a commercial grove in Texas and in a single residential tree in California in 2012. A 2012 study by the University of Florida Institute of Food and Agricultural Sciences estimates that HLB has cost the state's citrus industry $3.6 billion since 2006. The disease also occurs in tropical and subtropical Asia, India, Africa, and South America.

The presumed cause of HLB is the bacterium *Candidatus* Liberibacter asiaticus, and it is carried from plant to plant by the Asian citrus psyllid *(Diaphorina citri),* a tiny, winged insect that feeds on all citrus relatives. When a tree is infected, the bacterium moves into its phloem tissues, blocking the passage of nutrients through the vascular system until it makes the plant unproductive. Infected trees usually decline in productivity and die within 5 to 10 years of infection. Fruit on infected trees often falls to the ground before harvest, and those that remain on trees may become misshapen, sometimes only partially ripening in color. Juice from symptomatic fruit usually has an acidic, bitter taste.

The best strategy to control HLB is to remove infected trees (the source of the bacterium for the psyllid) and control the psyllid vector. But many Florida growers are reluctant to pull trees if they are still productive. To help citrus growers and juice processors, Baldwin and her team are investigating the effects of HLB on the taste of orange juice produced from diseased trees. Their goal is to maintain the high-quality product that consumers want and expect while scientists search

STEPHEN AUSMUS (D2703-18)

In an orange grove heavily infected with citrus greening disease, entomologists Matt Hentz (left) and David Hall inspect trees for Asian citrus psyllids that have been killed by the beneficial fungus *Hirsutella citriformis*.

STEPHEN AUSMUS (D2705-10)

Horticulturalist Elizabeth Baldwin (left) and chemist Jinhe Bai review data from the “electronic tongue,” a device that can detect chemical differences related to taste to see whether it can distinguish between juice from HLB-infected oranges and from noninfected oranges.

for a permanent solution to the threat posed by HLB.

As the disease continues to spread, the quality of juice from diseased trees is becoming more of a concern to growers, particularly in Florida, where 90 percent of the oranges raised are used primarily for juice. In general, if fruit from a diseased tree is asymptomatic and looks normal, the juice will taste normal, but if it is symptomatic (smaller, greener, and lopsided), the juice flavor may be affected, depending on the number of symptomatic fruit in a batch of juice.

“There is no specific point where fruit becomes so symptomatic that it affects the taste. It’s more of a spectrum or range, so the juice processors need an objective way of analyzing fruit for taste so they can make an informed decision about what they’re buying,” Baldwin says.

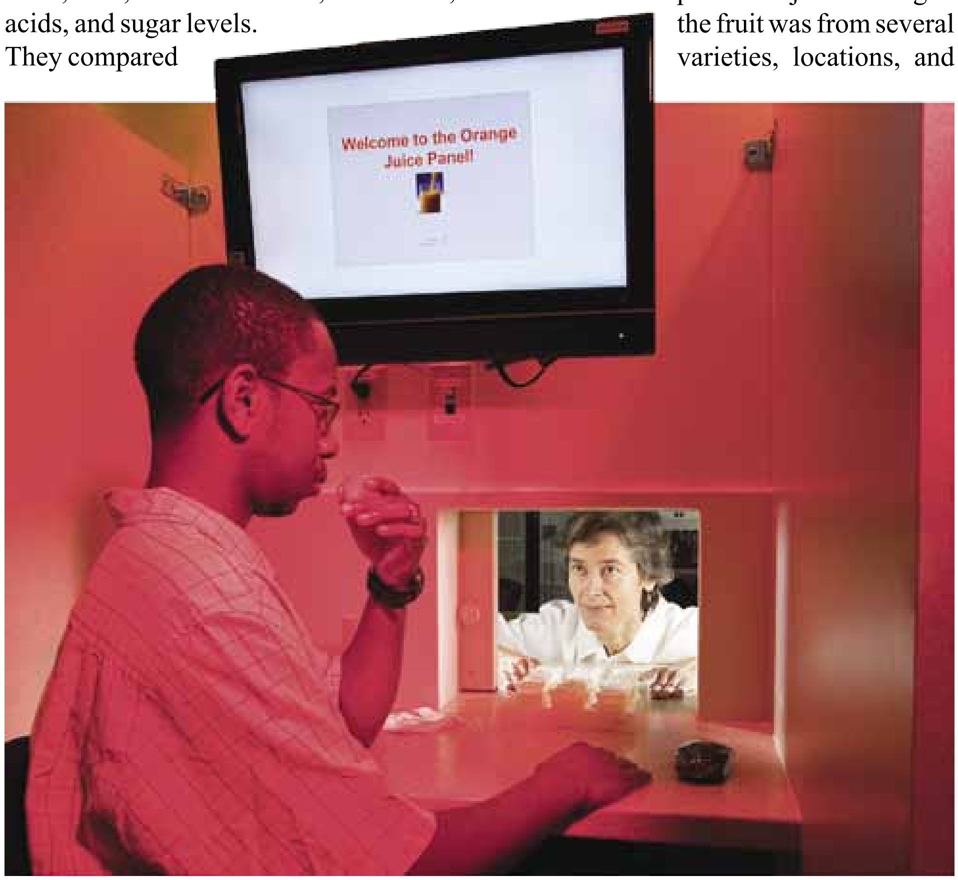
Baldwin and her team are working on two fronts: They are investigating the effects of the disease on the taste and quality of juice and developing new technology that will help processors objectively determine the taste qualities in their juice.

Studying Disease Effects on Taste

The researchers evaluated both asymptomatic and symptomatic fruit produced over two growing seasons for several fruit and juice characteristics, including color, size, aroma volatiles, limonoids, acids, and sugar levels. They compared Midsweet, Hamlin, and Valencia oranges, the three principal varieties harvested for processing, and used gas and liquid chromatography to analyze compounds in fruit that went into the juice.

They found that juice from symptomatic fruits was more often higher in limonin and nomilin, compounds that can give orange juice a bitter taste, but that the compounds were generally detected at amounts below threshold levels in taste panels. Results were published in the *Journal of Agricultural and Food Chemistry* in 2010.

In another study, the researchers investigated how HLB infection affects juice quality with respect to cultivar, maturity, and processing method. They compared the same three varieties of infected and disease-free fruit. The results showed tremendous variability, depending on the harvest date and variety of orange. In general, the researchers found more of a problem with off-flavored juice from diseased Hamlin oranges than with diseased Valencia and Midsweet varieties, and off-flavors were more prevalent earlier in the harvest season. But they concluded that use of some symptomatic fruit would not adversely affect the flavor of commercially processed juice as long as the fruit was from several varieties, locations, and



STEPHEN AUSMUS (D2706-5)

To see whether off-flavors are perceived in juice from HLB-infected oranges, plant physiologist Anne Plotto hands juice samples to taste-tester Keith Williamson. The red lighting in the tasting booth helps him focus only on taste and smell.

DAVID HALL (D2713-1)

Adult Asian citrus psyllid killed by the fungus *Hirsutella citriformis*. A psyllid cadaver, seen here, can remain on a citrus leaf for extended periods and continue to spread the biocontrol fungus to other psyllids.

seasons and the mixture contained juice from healthy fruit.

Results were published in the *Journal of Food Science* in 2010.

Baldwin has been awarded a 2012 cooperative research and development agreement with Southern Gardens Citrus (a subsidiary of the U.S. Sugar Corporation), which grows oranges and processes them into juice, to develop an instrument that processors can use to measure flavor compounds and detect off-flavors in juice. The technology could be designed so that juice is tested in bulk or on an assembly line at the processing plant. From the way the disease is progressing in Florida, Baldwin estimates that the technology will be needed within the next few years to ensure a consistent, delicious, and high-quality product.

Tapping the Effects of a Beneficial Fungus

Many citrus growers apply chemicals to control plant pests (insects and mites) and plant pathogens. Hall has found that some of these inhibit proliferation of a beneficial fungus, *Hirsutella citriformis,* which has the ability to naturally control the Asian citrus psyllid.

Soon after HLB was discovered in Florida in 2005, *H. citriformis* was seen as having a kind of double-barreled impact. It killed Asian citrus psyllids, and the dead psyllids remained on the citrus leaves for extended periods, spreading the fungus to other psyllids.

But field observations in Florida's citrus groves turned up a puzzling pattern, says Hall. "We were seeing fewer cadavers one year than in the next year, and we asked ourselves what could be causing that. Were there environmental factors, such as the humidity levels, affecting these fungal populations? Or were the sprays and chemical treatments applied to citrus groves reducing the numbers?"

Hall and his colleagues methodically counted psyllid cadavers and live adults on mature leaves of 30 trees, randomly selected each week, for 118 weeks from January 2006 to April 2008 in an orange grove near Vero Beach. Hall's partners included Drion Boucias from the University of Florida and Jason Meyer from Purdue University. As is routine in many commercial operations, the grove was periodically sprayed with petroleum oil and copper hydroxide to control greasy spot, a fungal disease that defoliates citrus. The researchers tagged some leaves where they found psyllid cadavers so they could assess how long they remained on leaves. They also measured rainfall, air temperatures, and relative humidity to see if weather patterns played a role in cadaver and psyllid numbers.

The results showed that the cadavers continued to spread the beneficial fungus on citrus leaves for an average of 68 days and that sometimes there were more cadavers than live adults on leaves. They also found that cadavers were most abundant in the fall and winter months and that cadaver numbers were higher in 2006, a year when no copper hydroxide was sprayed.

The researchers then conducted laboratory experiments to evaluate the effects on *H. citriformis* of six chemical compounds commonly sprayed to control greasy spot and other citrus diseases in Florida citrus groves. In collaboration with insect pathologists at the University of Florida, the team successfully cultured the fungus on media in petri dishes. Five-week-old cultures were sprayed with the compounds. Three of them—copper hydroxide, petroleum oil, and elemental sulfur—reduced the fungus's ability to infect the Asian citrus psyllid. The other three—copper sulfate pentahydrate, aluminum tris, and alpha-keto/humic acid—did not slow it down.

The study, published in March 2012 in *BioControl,* is the first investigation into the field dynamics of a fungal pathogen that attacks the Asian citrus psyllid, Hall says. It opens the door to more widespread use of *H. citriformis* in HLB management programs.

"Knowing that *H. citriformis* is naturally occurring and that it confers this benefit may encourage growers to capitalize on it by using alternatives to some of their current treatments," Hall says.

Because the researchers detailed how they cultured *H. citriformis* in the paper published in *BioControl,* others can expand on the work by developing strategies that use the fungus to control the psyllid in commercial citrus operations. For example, citrus could be sprayed with a water solution containing fungal spores to supplement existing populations. The fungus may be of particular interest to organic producers, who need alternatives to traditional insecticides, and to growers in developing countries less likely to spend money on traditional sprays.

"Now that we know how to culture this beneficial fungus, it becomes a potential weapon that could be more widely deployed in our war with this nasty citrus pest," Hall says.—By **Dennis O'Brien,** ARS.

This research is part of Quality and Utilization of Agricultural Products (#306) and Crop Protection and Quarantine (#304), two ARS national programs described at www.nps.ars.usda.gov.

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Orange Juice Amino Acids May Reveal Secrets to Quelling Citrus Greening Disease

Amino acids in orange juice may hold clues to the mostly secret, highly successful attack strategy of a powerful plant pathogen. Recent studies of these compounds may pave the way to a safe, effective, environmentally friendly way to vanquish this orchard enemy.

Known as *Candidatus* Liberibacter asiaticus, this microbe causes citrus greening disease, also called "Huanglongbing," or "HLB." (See article on page 4).

Agricultural Research Service chemist Andrew P. Breksa III and University of California-Davis professor Carolyn M. Slupsky have compared the amino acid composition of juice from commercially grown oranges. They used nuclear magnetic resonance spectroscopy to study juice from oranges grown on either HLB-positive trees or HLB-negative trees. Their investigation is apparently the first to do so, the scientists say.

The research has yielded distinctive profiles of the kinds and amounts of 11 different amino acids in 3 types of oranges: fruit from healthy trees, symptom-free fruit from HLB-positive trees, and fruit with HLB symptoms from HLB-positive trees.

With further research, the profiles may prove to be "a reliable, rapid, and early indicator of the presence of the HLB pathogen in an orchard," Breksa says. For growers, an early indicator of HLB would be valuable. Here's why: HLB can be a silent killer, living quietly and undetected for years in a grove of oranges, grapefruits, lemons, or other citrus fruit. The amino acid profiles may have another use, as well. They may reveal clues to mechanisms underlying the microbe's mode of attack.

"No one understands precisely how the pathogen overcomes the defense system a citrus tree can ordinarily mobilize when it's under siege," Breksa says.

Trees need amino acids for growth, development, and defense. But what if the HLB pathogen were causing havoc with the trees' ability to create, use, and recycle these amino acids?

That information could be used as a starting point for a tightly focused counterattack strategy, Breksa points out.

Building up and tearing down amino acids is part of the everyday life of a citrus tree. For instance, a tree can convert the amino acid phenylalanine into cinnamic acid, a precursor to compounds thought to be important to the tree's defense system. But juice from oranges of HLB-positive trees "had significantly higher concentrations of phenylalanine," Breksa notes. "This means that the HLB pathogen may have interfered with the orderly conversion of phenylalanine to cinnamic acid."

Another example: Juice from oranges grown on HLB-positive trees contained significantly less of the amino acid proline. Says Breksa, "When a tree 'knows' something is wrong, it synthesizes proline. In the case of HLB-infected trees, however, the pathogen might be outsmarting the tree by undermining proline synthesis."

Breksa is based at the ARS Western Regional Research Center in Albany, California. He and Slupsky collaborated in the work along with Thomas G. (Greg) McCollum of the ARS Horticultural Research Laboratory in Fort Pierce, Florida. An article by Breksa, Slupsky, McCollum, and Anne M. Slisz and Darya O. Mishchuk of Slupsky's lab documents the findings and was published in the *Journal of Proteome Research*.

ARS, the Citrus Research Board, and the State of California Department of Food and Agriculture helped fund the research.—By **Marcia Wood,** ARS.

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

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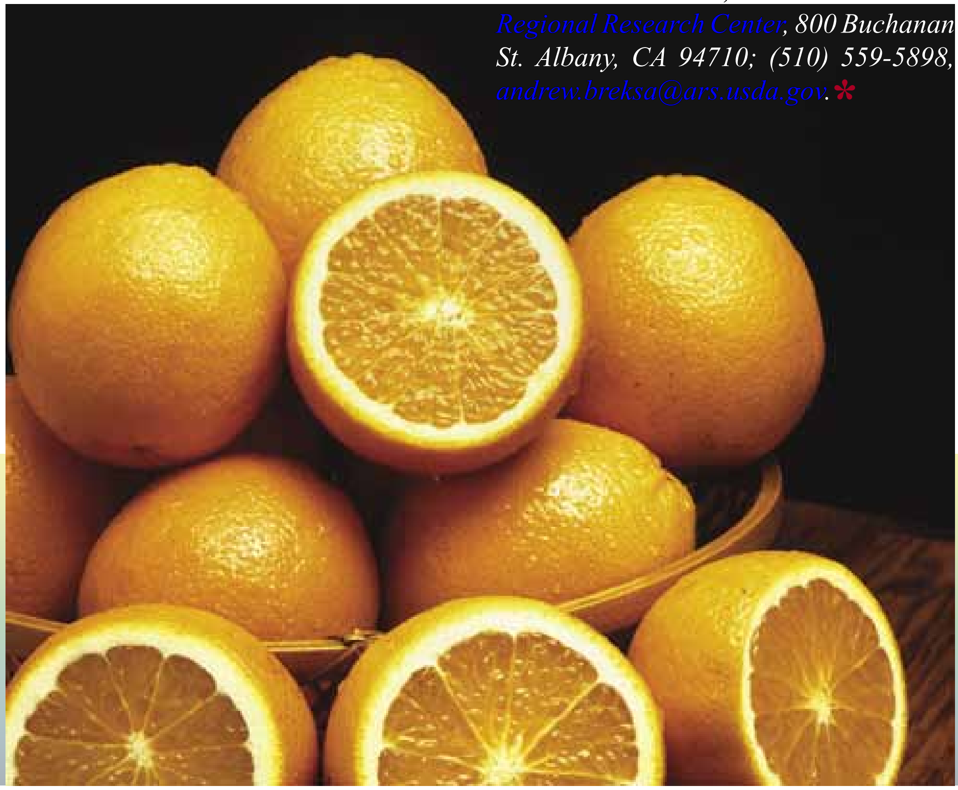

ARS and university researchers have found, in oranges, distinctive amino acid profiles that may be early indicators of the presence of citrus greening disease in orchards.

(K3644-12)

Drainage Ditch Research Reveals Opportunities for Cleaning Up Runoff

"There is no typical drainage ditch," says Agricultural Research Service ecologist Matt Moore. "And until recently, farmers just thought of them as something they need to drain water off their fields. But we can use these ditches to minimize pesticide and nutrient losses in runoff—and it can be done without taking any cropland out of production."

Moore, who works at the ARS National Sedimentation Laboratory in Oxford, Mississippi, has been wading through edge-of-field drainage ditches since he was a boy planting rice on his family's farm in Arkansas. The ditches—as common in agricultural landscapes as the fields they drain—range from shallow gullies that sometimes run dry to much larger channels that hold either standing or flowing water throughout the year.

Many farmers control ditch vegetation with trimming or dredging to eliminate barriers that could impede the flow of runoff. But Moore has conducted a number of studies over the past 10 years showing that drainage ditches where plants are allowed to flourish are strikingly effective at keeping agricultural pollutants in field runoff from reaching surrounding surface waters.

Blocking a Path for Pesticides

In one of Moore's first studies, he spent 28 days evaluating the transport and capture of atrazine and lambda-cyhalothrin in a 160-foot section of a vegetated agricultural drainage ditch in Mississippi. One hour after he started a simulated runoff event, 61 percent of the atrazine and 87 percent of the lambda-cyhalothrin had transferred from the water to the ditch vegetation, and at the end of the ditch, runoff pesticide concentrations had decreased to levels that were generally nontoxic to downstream aquatic fauna.

"I was surprised by the short distance the runoff needed to travel in the drainage ditch to lose its pesticide load," says Arkansas State University researcher Jerry Farris, who was one of the coauthors on the study. "This suggested that we could use these ditches as one tool in managing agriculture from an ecological perspective."

PEGGY GREB (D2723-1)

In tests to see how well vegetated drainage ditches like this one reduce agricultural pesticide and nutrient runoff, ecologist Matt Moore (left) and soil scientist Martin Locke assess the biomass of plants growing in the ditch.

Encouraged by initial results, Moore conducted a followup study to measure the capture of lambda-cyhalothrin and bifenthrin, both pyrethroid pesticides that can be extremely toxic to aquatic fauna even in low concentrations. Just 3 hours after the start of a simulated runoff event, he found that 96 percent of the lambda-cyhalothrin and 99 percent of the bifenthrin were already captured by the ditch vegetation.

Just 7 days after the simulated runoff event, lambda-cyhalothrin and bifenthrin concentrations in some ditch water samples collected downstream had declined so significantly that they were well within acceptable toxicological threshold ranges. This meant that the pesticide concentrations now posed a much lower risk to aquatic ditch fauna. Thirty days after the simulated event, water samples from all the collection sites contained pesticide concentrations within acceptable toxicological threshold ranges.

Sample analyses also suggested that in a worst-case event, a ditch would need to be at least 395 feet long to reduce both lambda-cyhalothrin and bifenthrin to concentrations below 1 percent. A ditch would need to be at least 920 feet long to reduce both pesticides to concentrations below 0.1 percent.

Moore and colleagues conducted a similar study on the pyrethroid esfenvalerate with similar results—3 hours after the runoff simulation began, 99 percent of the pesticide was associated with the ditch vegetation. Using data from the study, the researchers constructed a model that suggested esfenvalerate concentrations in runoff that traveled the length of a 1,675-foot vegetated ditch could be reduced to 0.1 percent of the initial concentration.

Finds on Fertilizers

Robert Kröger, a South African Ph.D candidate who was completing his studies at the ARS laboratory in Oxford, decided to investigate whether drainage ditches could help mitigate nutrient loads in field runoff.

"You can manage nutrients that contribute to the development of oxygen-deficient 'dead zones' in downstream ecosystems in three ways," says Kröger, who is now a research professor with Mississippi State University. "You can just not use them; you can use edge-of-field systems like buffer strips to capture them; or you can use drainage ditches—and every single agricultural landscape has a drainage system."

For 2 years, Kröger and Moore collected runoff samples from two Mississippi drainage ditches adjacent to experimental no-till cotton fields. They collected monthly samples and also obtained samples of runoff generated by storms.

Sampling analysis indicated that the ditches alternated throughout the year between being a sink and source for dissolved inorganic phosphorus and particulate phosphorus. Around 5.5 percent of the fertilizer applied annually to the fields was transported into the ditches, where around 44 percent of inorganic phosphorus in the runoff was removed by attaching to ditch sediments or vegetation before the runoff was discharged.

The ditches reduced runoff concentrations of dissolved inorganic phosphorus during the growing season by 61 percent. When the fields were fallow, average loads were decreased 47 percent. But it wasn't possible to determine whether some of that phosphorus load was from the accumulation of "legacy" nutrients—those that linger in the soil years after they are applied.

The team used the same experimental fields to determine whether the ditches also helped reduce inorganic nitrogen from field runoff. Runoff samples collected during the 2-year study contained 2.2 percent of the initial fertilizer application, but only 1.1 percent of the inorganic nitrogen remained in the runoff when it was discharged from the ditch. This means that the ditch was responsible for reducing runoff levels of inorganic nitrogen by 57 percent over 2 years.

The researchers wanted to see if they could make these good results even better. They were already familiar with the riser pipes producers placed at the edge of drainage ditches to create a dam that temporarily impounds runoff. This reduces runoff volume and velocity, which in turn reduces field erosion. It also helps raise the water table, which improves crop access to soil water.

Working with a water sample collected from a drainage ditch, technician Lisa Brooks extracts a portion of it for pesticide analysis.

The team installed low-grade weirs—small dams—at several points throughout three drainage ditches. They also placed riser pipes at the ends of three other ditches. Then they conducted two simulated nutrient runoff events and tracked nutrient loads in each impoundment pool for the next 7 days.

The scientists observed that levels of several nutrients in the trapped runoff dropped significantly 7 days after the runoff event began. Average median levels of dissolved inorganic phosphate dropped 93 percent, and average median levels of total organic phosphate dropped 87 percent. Average median nitrate levels declined 97 percent, and average median ammonium levels dropped 76 percent. This indicated that using either riser pipes or weirs to temporarily trap field runoff could increase the capacity of drainage ditches to lessen runoff nutrient loads.

Tests in the West

Moore was asked by U.S. Environmental Protection Agency (EPA) environmental scientist Debra Denton to test the effectiveness of vegetated drainage ditches for mitigating pesticide runoff in California crop fields. Moore conducted a preliminary field trial in Yolo County, California, using a U-shaped vegetated ditch, a V-shaped vegetated ditch, and a V-shaped ditch with no vegetation. Each 545-foot ditch was amended for 8 hours with a mixture of diazinon, permethrin, and suspended sediment.

Afterwards, Moore's team analyzed water, sediment, and plant samples for pesticide concentrations. They found that differences in half-distances—the distance that runoff travels in a ditch to reduce initial pesticide concentration by 50 percent—ranged from 69 feet in the V-shaped vegetated ditch to 485 feet in the V-shaped unvegetated ditch.

The researchers followed up with another study that evaluated the ability of existing drainage ditches alongside California tomato and alfalfa fields to mitigate runoff loads of the pesticides chlorpyrifos and permethrin. The scientists planted creeping wildrye and slender sedge—both native to California—in the V-shaped ditches about 5 months before the start of the irrigation season.

Water, sediment, and plant samples collected after the runoff events indicated that chlorpyrifos concentrations in alfalfa field runoff decreased 20 percent by the

PEGGY GREB (D2727-1)

Using a gas chromatograph, technician Renee Russell analyzes the pesticide content of samples collected at various points along a vegetated drainage ditch. The goal is to determine how the ditches affect the fate and distribution of pesticides.

time it left the ditch. Thirty-two percent of the measured chlorpyrifos was associated with ditch plant material. Permethrin concentrations in runoff from tomato fields decreased 67 percent by the time it left the ditch, and suspended sediment concentrations dropped 35 percent.

With these findings in hand, Denton worked with USDA's Natural Resources Conservation Service (NRCS) state office in California to include vegetated agricultural drainage in its Environmental Quality Incentives Program (EQIP). NRCS approved this designation in 2008, which meant that farmers who installed the ditches could be reimbursed for up to 50 percent of the cost.

"One of the best things about this project is that ARS and EPA were working side by side for the same goal—to help the farmers and improve water quality," says Moore.

"Because of this work, other researchers in California are now studying ways to use vegetated drainage ditches to reduce pesticides, nutrients, and sediment loadings into waterways," adds Denton. "But one of the things Moore emphasizes is that using vegetated ditches is just one practice in a suite of practices farmers can use to reduce agricultural pollutants in field runoff."

Moore's research contributed to the decision by NRCS managers in Mississippi to include vegetated agricultural drainage ditches in the state's EQIP. Meanwhile, Moore is continuing his research.

"Our next step is figuring out the best ways to manage vegetation in the ditches," Moore says. "But the farmers we talk with are cautiously optimistic about how the ditches can work for them. They do see that it could be a low-cost option for controlling nutrients and pesticides in runoff."—By **Ann Perry,** ARS.

This research is part of Water Availability and Watershed Management, an ARS national program (#211) described at www.nps.ars.usda.gov.

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Graduate assistant Traci Hudson (left) and ARS soil scientist Martin Locke (center) measure water quality while professor Jerry Farris collects another water sample for analysis. Hudson and Farris are with Arkansas State University.

PEGGY GREB (D2725-1)

Added Incentive To Target Nitrogen

Capturing nitrogen from field runoff is good for water quality. But research is needed to determine how much of the captured nitrogen is transformed and emitted as nitrous oxide, a greenhouse gas that holds 300 times as much heat as carbon dioxide.

Crop-Friendly Bacteria Tapped To Battle Fungal Marauders

Soil-dwelling bacteria that depend on wheat and barley roots for their "room and board" could soon make good on their debt. Researchers are investigating the microbes' potential to biologically control root-rot fungi that cause crop yield losses of 10-30 percent annually in the U.S. Pacific Northwest and other parts of the world.

The bacteria are members of the genus *Pseudomonas* and include 11 strains that stymie the growth of *Pythium* and *Rhizoctonia* fungi, which are responsible for damping-off and root-rot diseases of wheat and barley. The pathogens thrive in cool, moist soils and can reach especially high levels in crop fields where conservation tillage is practiced to save on fuel costs, avoid soil erosion, and attain other ecological and environmental benefits.

"They're most problematic to seedlings of spring crops that are 4 to 6 weeks old," notes Pat Okubara, a geneticist in the Agricultural Research Service's Root Diseases and Biological Control Research Unit in Pullman, Washington. "Fungicides are not very effective, and there are no resistant wheat or barley varieties yet," she adds. Rotating wheat with nonhost crops is difficult too, because of the fungi's extensive plant-host range.

Over the past year, Okubara and university colleagues have evaluated the biocontrol potential of 26 *Pseudomonas* strains. From those, they chose 11 for further study based on 3 important characteristics: rapid colonization of and persistence on roots, high antifungal activity, and reduction of plant disease symptoms.

Prior studies show that *Pseudomonas* bacteria secrete powerful antibiotics to keep their fungal rivals at bay—to the benefit of wheat and other host crops. But other mechanisms are also at work, notes Okubara, whose collaborators are Chris Taylor (Ohio State University, Wooster), Olga Mavrodi (Washington State University, Pullman), and ARS technician Nathalie Walter.

Some strains, for example, help the plants help themselves by triggering a sort of immune-system response called "induced systemic response." Others produce hormone-like substances that spur on root and shoot growth in the plants, enabling them to recover from fungal damage more easily.

PAT OKUBARA (D2715-1)

Digital scan of the roots of a wheat plant treated with a biocontrol bacteria strain. From the scan, root measurements are made, and this information helps ARS researchers evaluate the strain's effectiveness against root pathogens.

Wheat roots being washed to assess disease severity and root growth following a biocontrol treatment.

PAT OKUBARA (D2714-1)

In nature, the bacteria and fungi compete for precious space and nutrients that both need to survive and grow. Unlike the fungi, however, these bacteria generally don't harm the plants and can greatly benefit plants when pathogens are present. It is this phenomenon that the researchers hope to exploit in the form of a biobased-pesticide seed treatment for wheat and barley and for greenhouse-grown ornamental and herb crops, such as lavender and basil.

In greenhouse tests with seedlings from the wheat cultivar Penawawa, use of five of the *Pseudomonas* strains diminished the severity of *R. solani* AG-8 root rot by 30-92 percent and *Pythium ultimum* by 32-56 percent. Two strains also reduced rot caused by *R. oryzae* and *P. irregulare,* which also plague Pacific Northwest wheat and barley crops.

A commercial product isn't likely for another few years. But the arrival of any new antifungal weaponry should be welcome news for wheat growers, especially those who've shied away from direct seeding or other conservation-tillage measures.

"Any means of reducing the pathogen in the field could also benefit crops used in rotation with wheat," adds Okubara.—By **Jan Suszkiw,** ARS.

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

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Advantages of Understanding the Lady Beetle Diet

Understanding the feeding behavior of lady beetles will help agronomists develop cropping systems that best use these important beneficial insects as biological controls of insect pests, such as aphids and Colorado potato beetles.

Agricultural Research Service entomologist Jonathan Lundgren at the North Central Agricultural Research Laboratory in Brookings, South Dakota, and former ARS entomologist Michael Seagraves were part of a team of ARS and university scientists that examined how lady beetle diets alter their feeding patterns and physiology.

Appreciated for their ability to eat insect pests, lady beetles also consume nectar, pollen, and other plant tissue. Indeed, most beneficial predators eat both prey and nonprey foods, and understanding the factors that affect what they eat is important to using them in biological control of crop pests. The foods they consume determine where and when they can be found in a farm field and whether they decide to eat crop pests.

Also, since many field crops are treated with insecticides, an important step in assessing the risk to beneficial species is to know how much insecticide these insects consume when they feed on plants.

For laboratory feeding tests, the team chose a native lady beetle species, *Coleomegilla maculata.* The results of the tests reveal that this lady beetle consumes two to three times more plant tissue after being fed a prey-only diet than after being fed a mixed diet of prey and plant tissue.

"This suggests that plant material is providing some key nutrients lacking in prey-only diets," says Lundgren. "It is important to recognize that nonprey foods contain different nutrients from insect prey, and predators fed mixed diets are often more fit than those fed only prey."

In a follow-up study, Lundgren and his colleagues looked at sugar consumption by lady beetles in the field. Sugar, whether in a sugar-syrup spray provided by the farmer or in nectar from nearby flowering plants, is an important nutrient, allowing female lady beetles to survive and produce more eggs than those denied this sweet treat. This feeding behavior is known to exist, but its effect on lady beetle physiology is less understood.

"Foods like sugar and pollen are important components of their diets, and it is thought that lady beetles rely heavily on sugar resources in the field, although no one has ever quantified their feeding," says Lundgren. "In this study, we applied sugar sprays to soybeans and quantified the frequency of sugar feeding using gut content analysis of common agronomic lady beetles in South Dakota, Maryland, and Kentucky."

Says Seagraves, "We found that all the lady beetles we tested regularly consumed sugar—like nectar—in soybean fields, even when it wasn't applied as a supplement. However, the sugar-sprayed plots had more lady beetles than the untreated plots, although soybean aphid populations were similar in the two treatments. This research makes the case that sugar-feeding is very important for lady beetle populations in cropland and suggests one way to maintain these beneficial species in agroecosystems."

The research team's findings were reported in the journals *BioControl* and *Biocontrol Science and Technology*.—By Sharon Durham, ARS.

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

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JONATHAN LUNDGREN (D2717-1)

Coccinella septempunctata is a predatory lady beetle introduced in North America to control wheat pests, but its diet also includes pollen and nectar. ARS scientists are studying the lady beetle's feeding behavior to learn how to better use it for insect pest control.

New Uses for Sugar Beet Pulp

More than 1 million tons of sugar beet pulp are generated annually by U.S. beet sugar industries. Finding profitable uses for the biodegradable pulp, which is the leftover residue from sugar extraction, is critical for the long-term economic viability of U.S. agribusiness.

Agricultural Research Service researchers and colleagues have long been studying the potential of sugar beet pulp utilization. Now, chemist LinShu Liu and plant physiologist Arland Hotchkiss, both with the Dairy and Functional Foods Unit at ARS's Eastern Regional Research Center in Wyndmoor, Pennsylvania, and colleagues have found new uses for sugar beet pulp.

In collaboration with professor Jinwen Zhang of Washington State University (WSU) in Pullman, Liu and ARS-WSU colleagues developed a biodegradable thermoplastic (meaning plastic that becomes soft when heated) that could be used in disposable food containers.

The bioplastic is manufactured from both sugar beet pulp and a biodegradable polymer called polylactic acid, or PLA, using a twin screw extruder. PLA is a commercially available polymer derived from the sugars in corn, sugar beet, sugarcane, switchgrass and other plants—renewable feedstocks. Extrusion is a cost-effective manufacturing process that is popularly used in large-scale production of food, plastics, and composite materials. Many biopolymers and their composite materials with petroleum-based polymers also can be extruded.

The scientists showed that up to 50 percent sugar beet pulp can be incorporated with PLA, and the resulting thermoplastic composites retain mechanical properties similar to those of polystyrene and polypropylene—the compounds used to make white, spongy food packages. The new thermoplastic is cost competitive with commonly used petrochemical plastics. The study was published in *Industrial & Engineering Chemistry Research* in 2008.

ARS-WSU researchers also developed other sugar beet pulp materials. Under extrusion compounding, sugar beet pulp was turned into a thermoplastic-like material with the assistance of water and/or glycerol. This material can be subsequently processed by extrusion or injection molding to produce neat (meaning pure) sugar beet pulp products.

The resulting thermoplastic sugar beet pulp possesses mechanical properties that are similar to those of low-density polyethylene—the commonly produced materials used for opaque plastic containers, bags, and film coverings. It can also be blended with PLA and other biodegradable polymers for enhanced water resistance. The composite could function as a lightweight-bearing material comprising up to 98 percent sugar beet pulp.

This continued development of the sugar beet pulp plastic—for example as yogurt cups, cottage cheese tubs, or other thin, opaque plastic containers—could benefit sugar beet growers and beet sugar processors. More findings were reported in the *Journal of Polymers and the Environment* and *Industrial & Engineering Chemistry Research* in 2011.

The new composite plastics containing sugar beet pulp are cost competitive when compared to materials that are made solely of PLA, according to Liu and Hotchkiss. "The technology is promising and provides a 'green' material for food packaging," says Hotchkiss.—By **Rosalie Marion Bliss,** ARS.

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

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PEGGY GREB (D2729-1)

Plant physiologist Arland Hotchkiss (right) adds sugar beet pulp and polylactic acid to an extruder to make the bioplastic strips that chemist LinShu Liu is inspecting.

PEGGY GREB (D2719-1)

Going Beyond the Barnyard To Stop Stable Flies

On the research farm at the University of Nebraska, ARS entomologist Jerry Zhu checks stable fly captures from a trap baited with his newly designed attractant, or lure, in a cattle feedlot.

Livestock producers may not be able to see the difference between stable flies and other flies at a distance, but they can definitely see the stable flies' effect on their cattle as the animals stop grazing and bunch together to minimize the number of bites they're getting.

Stable flies are among the most important arthropod pests of cattle in the United States. Their painful bites can reduce milk production in dairy cows, decrease weight gain in beef cattle, and reduce feed efficiency.

Generally, insecticide sprays are used to help keep stable flies off animals, especially their legs, where the flies mainly bite. But as cattle walk through wet grass or wade through water, the spray washes off—making the treatment ineffective. Management of this pest is further complicated by the fact that larval development sites exist for only a short time, are difficult to find, and can produce huge numbers of the aggravating flies.

Scientists at the Agricultural Research Service's Agroecosystem Management Research Unit (AMRU) in Lincoln, Nebraska, are looking at better methods to locate stable fly habitats, finding easier and more efficient ways to control them, and assessing the damage they cause.

A Heavy Cost for Cattle Producers

What's the cost of stable fly damage? It's something livestock owners need to know.

"If you tell a producer that a site is the source of lots of flies and needs cleaning up, that producer wants to know if it is worth the time and expense," says AMRU entomologist David Taylor. "We wanted to provide a cost-benefit analysis."

Taylor and his colleagues developed a model to assess the economic impact of stable flies using four classes of production: dairy, cow/calf, pastured and range stocker, and animals on feed. They found that each year, stable flies cost the U.S. cattle industry more than $2.4 billion, making them the most damaging arthropod pest of U.S. cattle.

As their name implies, stable flies have historically been associated with stables and barnyards. But over the last 30 years, they have become a significant pest in pastures too. Research indicates that the problem is partly due to the large bales of hay placed in fields as supplemental feed for cattle during the winter.

"The accumulation of wasted hay, manure, and urine at these feeding sites creates an ideal habitat in the pasture for stable fly larval development," Taylor says. "We identified hay-feeding sites as producing a lot of flies, but we wanted to know how the timing of the flies coming off the sites correlates with adult population levels."

In Nebraska, stable fly populations peak twice a year—in mid-June to July and again in September or October. Scientists determined that the hay-feeding sites are the primary sources of flies in the June-July peak.

Stopping Stable Flies Before They Mature

Cleaning up infested sites has been the main stable fly control method for about 100 years, Taylor says. The problem is that hay-feeding sites are often in remote locations.

As for insecticide use, says Taylor, “This kind of habitat has an active microbial community that can break down most traditional insecticides very quickly. You might get a couple of days of control before the effectiveness wears off.”

The team found that using an insect growth regulator to interrupt the development of stable flies can be effective. In one study, Taylor used cyromazine to control immature stable flies. Cyromazine, a commercial product, has been used to control other species of flies, mainly in poultry production. It interferes with molting and inhibits proper development of the insect’s external skeleton.

“We wanted to develop a method where the producer could apply a single treatment and be done,” Taylor says.

Scientists found that a single application of granular cyromazine sprinkled on a hay-feeding site reduced the number of adult stable flies emerging by 97 percent. Treatments took about 10 minutes, cost $10 per site, and remained effective for 10 to 20 weeks.

“It’s something producers can put in a pickup truck and don’t have to mix or spray,” Taylor says. “They can quickly treat sites while doing other chores or checking on cattle.”

A “Push and Pull” Strategy

Identifying the attractants or substances that lure females to a particular site to lay their eggs may help scientists find ways to reduce their populations.

“When gravid females—flies with eggs—reach an egg-laying site, we believe they use the olfactory sensors on their antennae to gather information related to nutrition,” says AMRU entomologist Jerry Zhu. “They then make a decision as to whether it is the right area to lay their eggs.”

Zhu is using what he calls a “push and pull” strategy to control stable flies. The “push” involves driving stable flies or other filth flies, like house flies and horn flies, away from livestock with a repellent. Plant-based chemicals that are low in toxicity, such as those found in catnip, are being used as experimental treatments.

“Catnip oil and its active compounds—nepetalactones—are powerful repellents against stable flies,” Zhu says. “Catnip is probably the best repellent identified, so far, for flies that bite. Catnip oil is also a good larvicide,” meaning it can be used for reducing stable fly larval development, he adds.

Zhu and his colleagues developed several sprayable catnip oil formulations for reducing stable fly field populations. Through a cooperative research and development agreement, Zhu partnered with Microtek Laboratories, Inc., to test a novel granular catnip product that can deter egg-laying.

The “pull” part of Zhu’s strategy involves developing attractants to lure stable flies into a trapping system that can be combined with a low-toxicity insecticide or a sticky substance.

Zhu and Taylor are also working with AMRU entomologist Kristina Friesen, who is studying microbial communities associated with stable fly larval development sites.

“In my mind, the long-term solution to stable fly control is a cultural solution,” Taylor says. “Even though we’re developing strategies such as chemical control, our long-term objective is to provide producers with methods to raise cattle without providing larval developmental sites for flies. That’s the real goal.”—By **Sandra Avant,** ARS.

This research is part of Veterinary, Medical, and Urban Entomology, an ARS national program (#104) described at www.nps.ars.usda.gov.

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Entomologist David Taylor adjusts the settings on a sprayer used to automatically treat cattle with a stable fly repellent.

PEGGY GREB (D2721-1)

David Taylor checks a stable fly emergence trap near a hay-feeding site. The trap is used to evaluate the effects of chemical and physical treatments on stable flies.

PEGGY GREB (D2722-1)

The Real Dirt on Biosolids as Soil Amendments

The U.S. Environmental Protection Agency (EPA) estimates that more than 60 percent of dry biosolids—treated wastewater solids that can be recycled or stored—are applied to land, composted, or used to cover landfills. The biosolids have been processed to kill pathogens, and EPA strictly regulates biosolid use to ensure the materials don't harm the environment, human health, or animal health.

Farmers who follow pre- and postapplication management regulations can obtain permits to use biosolids for amending fields where food and feed crops are grown. Now, research by an Agricultural Research Service scientist is helping to clarify the long-term effects that biosolid amendments can have on some soil types—and how the amendments could affect crop production.

"Processed biosolids contain nitrogen and phosphorus that can be used for fertilizer," says agronomist Eton Codling, who works at the ARS Environmental Management and Byproduct Utilization Laboratory in Beltsville, Maryland. "They also contain copper, manganese, and zinc, which are plant micronutrients. But biosolids also have lead and cadmium, which can contaminate the soil."

Tracking a Timeline

Codling decided to investigate how long plant-available phosphorus and other minerals remain in soils amended with biosolids and how much phosphorus, copper, cadmium, lead, manganese, and zinc was taken up by wheat grown on those soils. He measured mineral levels in three different soils that had previously received a single amendment of a biosolid processed via one of the following methods: high heat, additions of lime, anaerobic digestion, or air drying.

The amendments had taken place 16 to 24 years earlier during a series of studies on biosolid amendments, and they had been applied to the soils at several different rates. As part of the earlier work, the fields had been cropped after the biosolids had been added, so the biosolid nutrients in the experimental fields had been available for crop uptake for at least 16 years before Codling began his research.

Still, the scientist observed that phosphorus levels were generally higher in the biosolid-amended soils than in control soils, which strongly indicated that soluble phosphorus levels in biosolid-amended soils could exceed typical plant requirements for years after the addition of the soil amendments. This meant that the excess phosphorus could wash out of the biosolid-amended soils into adjacent water channels and contribute to the development of oxygen-deficient "dead zones."

Codling also noted that phosphorus solubility varied with the biosolid type and application level. For instance, a soil amended with heat-treated biosolids contained higher levels of water-extractable phosphorus than the same soil type amended with lime-treated biosolids. This occurred even though the soil with the lime-treated biosolids had received amendments at levels that were three times that of the heat-treated biosolid amendment. The lime-treated biosolids had most likely sequestered phosphorus in low-solubility

STEPHEN AUSMUS (D2710-1)

Agronomist Eton Codling inspects wheat plants grown in biosolid-amended soils. Yields from some biosolid amendments were higher, but yields from lime-treated biosolids were severely reduced. The unhealthy plant on the left is growing in soil amended with lime-treated biosolids.

Safe Food, Safe Water

Since there are health risks associated with cadmium ingestion, the EPA has established maximum contaminant levels for cadmium in drinking water. The U.S. Food and Drug Administration has established limits for cadmium in bottled water and for several food products. The agency monitors the metal in the food and feed supply and would take appropriate regulatory action if the cadmium levels were found to be injurious to health.

calcium phosphate compounds. Most of the biosolid-amended soils also had higher levels of plant-available cadmium, copper, and zinc than the nonamended soils, and soil mineral levels generally increased as amendment levels increased.

Crop Response

Codling then conducted a study in which wheat was planted in pots filled with each type of amended soil. The researcher observed that yields from wheat grown in three of the five biosolid-amended soils were higher than from wheat grown in control soils. The highest yields were recorded for wheat grown in soils amended with biosolids created via anaerobic digestion, and yields in these experimental soils increased as amendment levels increased. But yields from wheat grown in lime-treated biosolids were severely reduced, probably as a result of manganese deficiency.

Codling also measured mineral levels that had accumulated in the above-ground biomass of the experimental crops. He observed that wheat grown in any of the biosolid-amended soils had higher phosphorus concentrations than wheat grown in the control soils. This coincided with the soil's elevated levels of plant-available phosphorus and provided additional indications that phosphorus was readily available for crop uptake 16 years after test soils were amended with biosolids.

Overall wheat tissue levels of lead were low, because most plants typically do not bioaccumulate lead to any significant degree. But tissue cadmium levels ranged from 1.2 parts per million (ppm) to more than 20 ppm in wheat cropped in the biosolid-amended soils. (Cadmium levels in the control soils averaged around 1.4 ppm.)

In addition, all the soil mineral levels were reduced after one cropping of wheat. Since Codling had collected leachate from each pot after watering and returned it to the pots, he surmised that the lower levels of extractable metals and phosphorus in the soils most likely resulted from plant uptake.

Taken together, these results, which are scheduled for publication in the *Journal of Plant Nutrition,* confirmed to Codling that minerals in biosolids can linger in soils long after the soils are amended. In addition, the way biosolids are processed before they are applied to soils may affect soil mineral levels to some degree.

"Even though I was evaluating mineral levels in vegetative tissue, not grain, the results still show that food and feed crops can take up minerals left over from biosolids years after the soils have been amended," Codling says. "Since sewage treatment facilities have different processes for treating biosolids, this information could help us manage biosolid amendments more effectively."—By **Ann Perry,** ARS.

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

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With Hot Air Treatment, Bacteria Fly the Coop

ANNA BATES (K9516-1)

While being transported in hauling coops on trucks, poultry that have been colonized with bacteria such as *Campylobacter* can contaminate, through fecal shedding, pathogen-free poultry. Those pathogens can also be passed on to the next group of birds during the next trip, and so forth, unless the cycle is broken.

That's where Agricultural Research Service microbiologists Mark Berrang and Richard Meinersmann and colleague Charles Hofacre at the University of Georgia in Athens come in. The team has reported a treatment that reduces poultry cross-contamination from transport-cage flooring.

Campylobacter are foodborne pathogens that can be present in raw or undercooked poultry. Since the bacteria are commonly found in the digestive tracts of poultry, they're readily deposited, through fecal shedding, onto coops and trucks when contaminated animals are transported to processing plants.

Berrang and Meinersmann are in ARS's Bacterial Epidemiology and Antimicrobial Resistance Research Unit in Athens.

Earlier work has shown that drying soiled or washed cages for 24 to 48 hours could lower or eliminate detectable *Campylobacter* on cage flooring. But extended drying times are impractical, so the researchers tested the use of hot flowing air to speed the process.

To determine whether the effect was due to heat alone or flowing air alone, hot flowing air was compared with unheated flowing air and static hot air as well as with a control. The numbers of *Campylobacter, Escherichia coli,* and coliforms on small squares of washed or unwashed fecally soiled transport cage flooring were measured after drying treatments.

When applied after a water-spray wash treatment, flowing hot air for 15 minutes lowered the numbers of *Campylobacter* to an undetectable level. The authors reported that the treatment could provide significant savings in drying time if used by industry, suggesting a potential commercial application. Static heat at similar temperatures was not nearly as effective, and unheated flowing air was moderately effective, but less so than hot flowing air.

The authors concluded that processors may be able to use a forced-hot-air treatment to dry cages between transporting flocks, lessening the number of *Campylobacter* on cage flooring, thereby decreasing the potential for cross-contamination during live haul.

More findings are reported in the *Journal of Applied Poultry Research,* December 2011.—By **Rosalie Marion Bliss,** ARS.

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ARS Works Toward Control of Brown Marmorated Stink Bug

The brown marmorated stink bug (BMSB) is wreaking havoc in U.S. homes, gardens, and agricultural operations, causing personal and economic woe. Agricultural Research Service scientists are exploring various aspects of monitoring and control of this increasingly important insect pest, which is an invasive Asian species known as a sporadic pest of many tree fruit crops in China, Korea, and Japan. Along with being a household nuisance, they are a major economic threat to producers of orchard fruits such as apple, peach, and pear; garden vegetables and row crops; and many ornamental species.

Adult and late-instar nymph stink bugs, *Halyomorpha halys,* feed on a Honey Crisp apple, a popular cultivar among consumers.

STEPHEN AUSMUS (D2709-6)

Since its detection in the northeastern United States a decade ago, the BMSB has been detected in 38 states and has earned the distinction of being classified as the top invasive insect of interest by the U.S Department of Agriculture. With economic losses to the apple industry estimated at $37 million in 2010, the bug's threat to apple growers prompted a Member of Congress to organize a public hearing in western Maryland. There is also concern about the potential damage it could cause to vineyards in California and other states.

Tracy Leskey, with the Appalachian Fruit Research Station in Kearneysville, West Virginia, is the principal investigator of the research group, which includes several scientists in ARS's Invasive Insect Biocontrol and Behavior Laboratory in Beltsville, Maryland; Jana Lee, an ARS entomologist in Corvallis, Oregon; and Kim Hoelmer, director of the USDA-ARS European Biological Control Laboratory in Montpellier, France.

A major project led by Leskey, funded through the USDA-National Institute of Food and Agriculture's Specialty Crop Research Initiative Program, is called "Biology, Ecology, and Management of Brown Marmorated Stink Bug in Orchard Crops, Small Fruit, Grapes, Vegetables, and Ornamentals." While this is a mouthful, it goes to the heart of the damage that can be caused by this pest. The project is funded for 3 years with $5.7 million in federal funds and $7.3 million in matching funds. The group includes ARS, Pennsylvania State University, Washington State University, North Carolina State University, Virginia Polytechnic Institute and State University, Rutgers University, Northeastern IPM Center, Oregon State University, University of Maryland, University of Delaware, and Cornell University. The project will take advantage of research that ARS scientists have conducted on BMSB since it was detected in the United States in 2001. The project's progress can be followed on their website, stopbmsb.org.

Support scientist Starker Wright (left) and entomologist Tracy Leskey inspect traps baited with experimental pheromone lures. The lures are being tested for brown marmorated stink bug attraction.

STEPHEN AUSMUS (D2707-4)

Setting the Trap

Growers need as much in-the-field information as possible to find ways to manage BMSBs. "Monitoring tools are used to assess the presence, abundance, and seasonal activity of pests and natural enemies to determine the need for and timing of insecticide applications," says Leskey. "Specifically, our group evaluated responses of brown marmorated stink bugs using different visual stimuli, compared the effectiveness of commercially available traps from Asia with a black pyramid prototype trap, compared relative attraction to different doses of odor attractants, and conducted a field cage experiment designed to establish how often the brown marmorated stink bugs reproduce."

Leskey has focused on visual stimuli that can, in addition to odor stimuli, attract the BMSBs to traps that will help farmers monitor the level of infestation in fields.

"We used pyramid-shaped traps of different colors—black, green, yellow, clear, white. In field trials in 2009 and 2010, we found significantly more stink bug adults and nymphs captured in the baited black pyramid traps than in the other traps," says Leskey. "Further, more adults and nymphs were captured in a trap placed on the ground than in a commercially available baited trap from Japan that we hung from a tree limb."

"We also found that in 2010 and 2011, brown marmorated stink bugs produced two generations in 1 year in Kearneysville, based on presence of eggs and newly molted adults in field cage experiments," says Leskey. "Although it has been reported that these bugs produce only one brood in eastern Pennsylvania, it appears that in more southerly locations within the Mid-Atlantic, they can produce two generations."

Secrets of Attraction

Researchers at the ARS Invasive Insect Biocontrol and Behavior Laboratory (IIBBL) in Beltsville, Maryland, are leading the pivotal pheromone research efforts and genomics studies and partnering with Leskey on field tests of potential attractants for use in commercial traps.

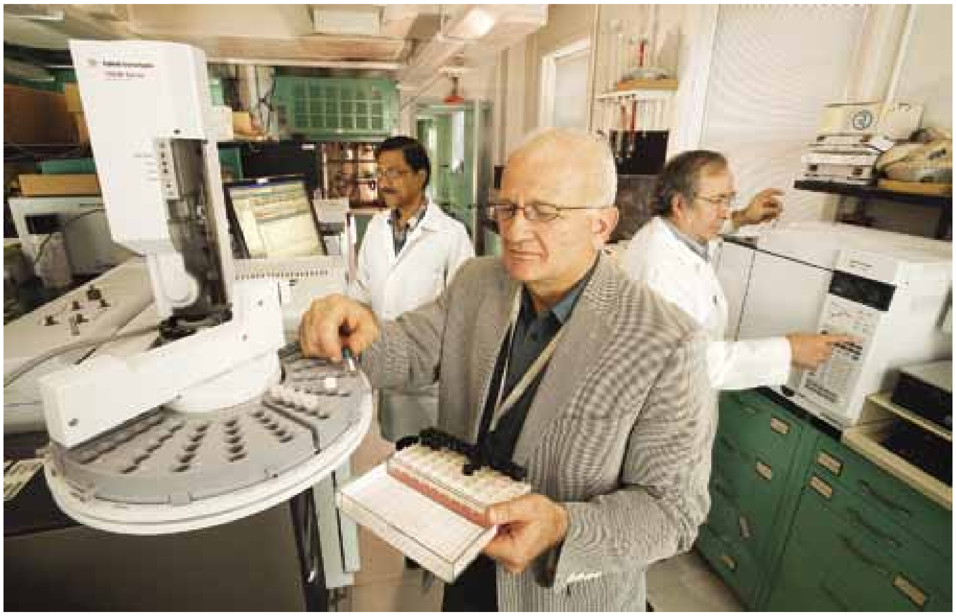
STEPHEN AUSMUS (D2708-6)

Technician Brent Short counts the number of adult males and females and other life stages of brown marmorated stink bugs captured in traps baited with experimental lures.

Scientists at IIBBL were working on the BMSB long before it became such a huge problem in the United States. Aijun Zhang, an analytical chemist, started looking for the BMSB pheromone in 2003, along with Ashot Khrimian, a synthetic chemist, and Jeff Aldrich, an entomologist who retired in 2011. Khrimian and Aldrich published results in the *Journal of Agricultural and Food Chemistry* and in *Tetrahedron,* showing that a compound identified as a pheromone of another stink bug was also a late-season attractant for the BMSB. When the BMSB emerged as a major pest in the United States, Aldrich and Khrimian began helping U.S. manufacturers develop traps with the attractant.

"Our work has already led to successful commercial products now on the market. But what we now have is only a late-season attractant, and because that doesn't help growers as much as we would like, we still have work to do," Khrimian says.

In 2010, the team of scientists at IIBBL found an "aggregation pheromone" that shows promise as the main pheromone attractant for BMSB. This pheromone is released by males when they feed, and it attracts both males and females. The scientists are trying to determine the chemicals that make up the pheromone. They are working on identifying the specific isomers (structurally related chemicals) that the stink bugs may



STEPHEN AUSMUS (D2712-2)

ARS scientists at Beltsville, Maryland, analyze reaction products to evaluate purities of synthetic lures for brown marmorated stink bug. Left to right: chemists Shyam Shirali, Ashot Khrimian, and Filadelfo Guzman.

be releasing to attract other stink bugs to feeding sites. They are trying to identify the various combinations or ratios of attractant isomers that will produce an affordable and efficient lure, Khrimian says.

The mixture and components were also evaluated in field trials this summer in Beltsville, Kearneysville, and elsewhere. Don Weber, who is overseeing the Beltsville field studies, set up traps with the different candidate formulas and twice each week counted the numbers of male, female, and nymphal (immature) stink bugs they attracted. These pyramid traps, based on those designed by Leskey, are similar to those developed for weevils and pests of woody fruit. They have a screen funnel that allows the stink bugs entry, but inhibits exit. Lures with the experimental formulas hang alongside kill strips inside clear plastic containers.

A provisional patent application was filed, and the researchers hope to include results from the summer field trials in supplemental data that will be filed as part of the completed patent application.

STEVE VALLEY, OREGON DEPT. OF AGRICULTURE (D2730-1)

A female parasitoid wasp, *Trissolcus mitsukurii,* from Asia. This species is one of several parasitoids being evaluated as potential biocontrols of brown marmorated stink bug.

Help From Genes and Natural Enemies

Dawn Gundersen-Rindal, research leader of the ARS Beltsville group, has been working with scientists at Baylor College of Medicine to sequence the stink bug's genome. The sequencing is part of an international effort, known as the "i5K Project," to sequence the genomes of 5,000 insects. Because it is such a nuisance to homeowners, a threat to agriculture, and rapidly spreading in the United States, the BMSB is one of the group's top priorities, she said.

"Sequencing the genome will tell us about the genes that give this insect its defense mechanisms and its ability to respond to threats, such as pathogens that we might want to use against it. It might give us clues, for instance, how it may develop resistance to insecticides," she says.

Separate from the sequencing project, Gundersen-Rindal is looking for genes that might be unique to the stink bug or make it vulnerable to specific treatments. "We hope we can find critical genes and use them against the stink bug by developing molecular biopesticides that address some weakness unique to its genetic makeup," she says.

Another approach to reducing the population of BMSBs is classical biological control—using its natural enemies to help keep its populations in check. Hoelmer continues work he began at the Beneficial Insects Introduction Research Unit in Newark, Delaware, to find parasitoid insects that may lend a hand. Surveys conducted in the United States found that native stink bug parasitoids are not capable of controlling BMSBs, so it is important that more effective biological control agents from Asia be identified, tested, and eventually imported to the United States. Hoelmer has collected some of these parasitoids during foreign exploration in collaboration with the USDA-ARS-SINO-American Biological Control Laboratory, in Beijing, China, and is now testing them in quarantine culture in Newark to determine their specificity for the BMSB.

Each of these research disciplines is needed to control BMSB populations in the United States, which will help farmers and homeowners alike. The project is an example of how USDA and ARS have the organization, infrastructure, and expertise to move quickly toward solving an emergent problem for agriculture.—By **Sharon Durham** and **Dennis O'Brien,** ARS.

This research is part of Crop Protection and Quarantine (#304) and Methyl Bromide Alternatives (#308), two ARS national programs 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. ✻

An adult parasitoid insect emerging from an egg of a stink bug. After a parasitoid female wasp lays an egg into a stink bug egg, the parasitoid offspring (one per egg) develops inside the egg, eating it from the inside out.

BRIAN T. CUTTING (D2731-1)

New Technology for Harvesting the Power of Beneficial Fungi

Biopesticides containing beneficial fungi are often grown on grains or other solids, but Agricultural Research Service scientists have found that a liquid diet might be cheaper and better.

The approach, known as "liquid culture fermentation," offers several advantages, including lower material costs and increased yields of certain forms of pest-killing fungi like *Isaria* or *Metarhizium* that can be sprayed directly onto crop plants or applied to soil as a biological alternative to using synthetic pesticides.

For decades, biopesticide makers have cultured fungi like these on moistened grains or other solid substrates to prompt them into churning out billions of specialized cells called "conidia," or spores, which latch onto and then penetrate the cuticles of silverleaf whiteflies, aphids, and other soft-bodied insect pests, killing them within a few days.

Over the past several years, however, ARS microbiologist Mark Jackson and colleagues have sought to improve on the approach using liquid-culture fermentation methods in special tanks called "bioreactors."

"We've made good strides," reports Jackson, who is in ARS's Crop Bioprotection Research Unit in Peoria, Illinois. "Optimizing fermentation conditions has increased the yield of spores, and we've identified low-cost nutrients that reduced production costs by 80-90 percent."

One of the greatest reductions has been in costs associated with nitrogen as a primary fungal nutrient. One source, hydrolyzed forms of protein, is typically derived from agricultural commodities like milk casein, which can sell for more than $6 a pound. Jackson used less-expensive nitrogen sources, including soybean flour or cottonseed meal, which cost 30-50 cents a pound.

Conidia have long been the spores of choice for biopesticide uses, but other fungal cells can be just as effective, including yeastlike structures called "blastospores" and clumps of pigmented fibers known as "microsclerotia." The latter can be easily and cheaply formulated as granules of almost any size for most application needs.

In the case of *M. brunneum* (formerly *M. anisopliae*), for example, Jackson, together with Stefan Jaronski, an entomologist in ARS's Pest Management Research Unit in Sidney, Montana, demonstrated that the soil-dwelling fungus performed best when cultured and applied as microsclerotia. Fungi in this intermediate survival stage only produce conidia in soil when conditions are optimal. In laboratory tests, conidia resulting from microsclerotia treatments killed 100 percent of sugarbeet root maggots in 1 week versus 25 percent killed in 3 weeks using a conidia-only, corn-granule formulation. (See "Multiplying *Metarhizium*," September 2008, pages 4-5.)

Jackson has also used the system to formulate microsclerotia of the fungus *Mycoleptodiscus terrestris* to biologically control hydrilla, a noxious aquatic weed that's infiltrated lakes, ponds, canals, and other water systems in the southern and western United States. In aquarium- and pond-scale trials conducted by collaborator Judy Shearer at the U.S. Army Corps of Engineer's Engineer Research and Development Center, in Vicksburg, Mississippi, hydrilla plants showed significant reductions in growth after being dusted with granules containing the fungus's microsclerotia.

Jackson says an advantage of the liquid-culture fermentation technology is that it isn't limited to mass-producing one particular fungal species or even one particular form of fungus. Blastospores can be cultured for use in sprays to control leaf-feeding pests like aphids as easily as microsclerotia for use in granular formulations to control soilborne insects, like root maggots.

"This flexibility opens all kinds of doors in terms of where and when you can apply the fungi," says Jackson. "Regardless of the fungal species or the requirement for sprayable blastospores or microsclerotia granules, the production platform we've developed for these biopesticides is the same."—By **Jan Suszkiw,** ARS.

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

Mark Jackson is in the USDA-ARS Crop Bioprotection Research Unit, National Center for Agricultural Utilization Research, 1815 N. University St., Peoria, IL 61604; (309) 681-6283, mark.jackson@ars.usda.gov. ✻

Microbiologist Mark Jackson (foreground) inspects pure cultures of an insect-killing fungus growing in petri dishes as lab technician Angela Payne inoculates a 100-liter fermenter with a liquid culture of the fungus.

STEPHEN AUSMUS (D2711-7)

Corn Cobs On Deck for Cellulosic Feedstock

When energy officials proposed using crop residues to produce cellulosic ethanol, concerned soil scientists took to the fields to learn more about how these residues protect soil from erosion and enhance soil quality. Agricultural Research Service soil scientist Brian Wienhold focused on a single component of residue—the corncob.

"We didn't have data on how postharvest cob residues might protect soil quality," says Wienhold, who works in the ARS Agroecosystem Management Research Unit in Lincoln, Nebraska. "But corncobs make up 20 percent of residue by weight, which means that the average U.S. production of corn could provide 40 to 50 million tons of cobs for feedstock every year."

Wienhold led colleagues in studies that compared runoff from no-till corn fields where postharvest crop residues were either removed or retained. The scientists also removed the cobs from half of the test plots that were protected by the residues. Then they generated two simulated rainfall events; the first occurred when the fields were dry, and the next occurred 24 hours later when the soils were almost completely saturated.

During the first event, on plots where residue was removed, runoff began around 200 seconds after the "rain" began, whereas runoff in the residue-protected plots didn't start until around 240 seconds after it started to "rain." Runoff from the residue-free plots contained 30 percent more sediment than runoff from all the residue-protected plots. The presence or absence of cobs on the residue-protected plots did not affect sediment loss rates.

Wienhold's team concluded that even though cob residues did slightly delay the start of runoff, they did not affect rates of sediment loss. The results showed that the cobs could be removed from other residue and used for feedstock without significantly interfering with the role of crop residues in protecting soils.

In related studies, Wienhold examined how the removal of cob residues affected soil nutrient levels. He placed litter bags containing cob pieces on the surface of no-till fields or buried them 0 to 4 inches deep in the soil. Every 2 months, he tested cob samples from the bags for levels of carbon, nitrogen, phosphorus, potassium, sulfur, calcium, manganese, iron, magnesium, copper, and zinc. Over the course of a year, his sampling indicated that cobs were a source of soil potassium, but that they weren't a significant source of any other plant nutrients.

Wienhold believes these findings demonstrate that harvesting cobs for biofuel production would not result in any notable loss of soil quality. This means that Nebraska's York County—where average corn production results in around 0.2 million tons of cobs—could potentially provide enough cob feedstock every year to keep two 10.5-million-gallon ethanol plants in business.

Results from this work have been published in *Agronomy Journal*.—By **Ann Perry,** ARS.

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

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PEGGY GREB (D2732-1)

Soil scientists Brian Wienhold (left) and Gary Varvel compare corncob residue in various stages of decomposition in no-till corn in Lincoln, Nebraska.

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

Locations Featured in This Magazine Issue

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

Plant Gene Expression Center, Albany, California
1 research unit ■ 12 employees

Corvallis, Oregon
3 research units ■ 133 employees

Davis, California
3 research units ■ 114 employees

San Joaquin Valley Agricultural Sciences Center, Parlier, California
3 research units ■ 125 employees

U.S. Salinity Laboratory, Riverside, California
2 research units ■ 36 employees

Pullman, Washington
6 research units 136 employees

Fort Collins, Colorado
7 research units ■ 141 employees

Northern Plains Agricultural Research Laboratory, Sidney, Montana
2 research units ■ 72 employees

North Central Agricultural Research Laboratory, Brookings, South Dakota
1 research unit ■ 39 employees

Lincoln, Nebraska
2 research units ■ 81 employees

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

Oxford, Mississippi
3 research units ■ 102 employees

Athens, Georgia
9 research units ■ 195 employees

Appalachian Fruit Research Station, Kearneysville, West Virginia
1 research unit ■ 65 employees

Fort Detrick, Maryland
1 research unit ■ 46 employees

U. S. Horticultural Research Laboratory, Fort Pierce, Florida
4 research units ■ 148 employees

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

Eastern Regional Research Center, Wyndmoor, Pennsylvania
6 research units ■ 190 employees

Newark, Delaware
1 research unit ■ 18 employees

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