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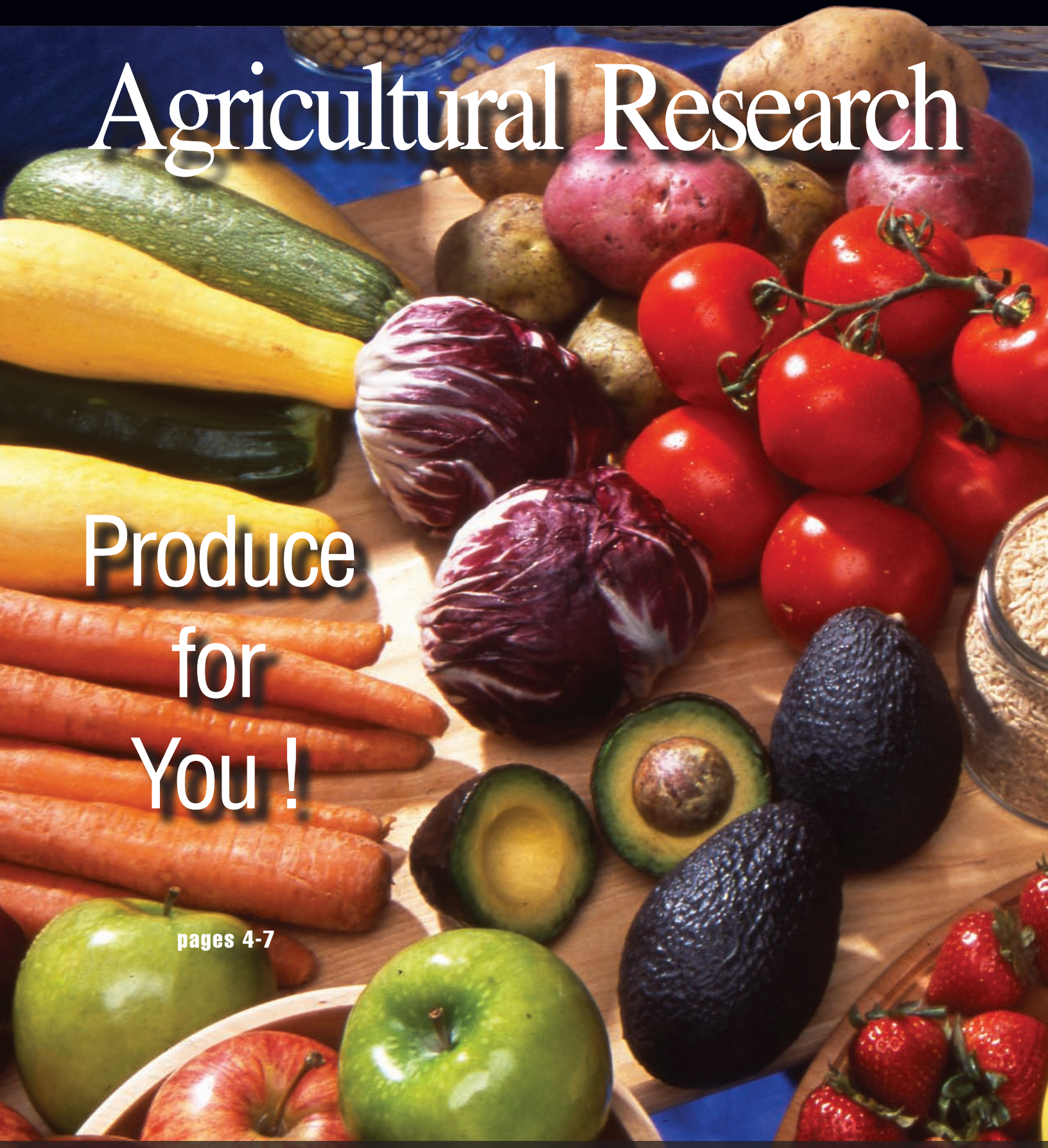
Agricultural Research



Produce for You !

pages 4-7

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

FORUM

Breeding Better Fruits and Veggies

On any given day, the amount and diversity of produce available to U.S. consumers is awe inspiring. To ensure this bounty requires dedicated plant-genetics and breeding research designed to enable farmers and producers to supply high-quality produce for as much of the year as possible.

Look at strawberries, for example. It takes production on two coasts and several varieties to provide these favorites to the nation's consumers for most of the calendar year.

The Agricultural Research Service's Plant Genetic Resources, Genomics, and Genetic Improvement national program (NP #301)—the largest of ARS's 18 national programs—has 177 research projects and a staff of 350 scientists. The program has three components: crop genetic improvement, crop genetic and genomic resources and information management, and crop biological and molecular processes. Its goals are to enhance American agricultural productivity and to ensure a high-quality, safe supply of food, f ber, feed, ornamentals, and industrial products.

To achieve these goals, researchers are breeding improved germplasm and superior crop varieties, developing and applying new genetic and bioinformatic tools, and safeguarding and developing plant genetic resources and associated genetic and genomic databases.

Improving crops often requires developing and accessing new genetic resources. Over the past 60 years, ARS has committed to conserving and managing a broad array of plant and plant-associated microbial genetic resources. In the United States, there are currently 22 genebanks that are used to manage the national plant germplasm collections. These germplasm collections help ARS scientists and other researchers develop breeding stock and new crop varieties to enable crop breeders,

STEPHEN AUSMUS (D103-7)

growers, and producers to meet the needs of a changing world.

Crop production in the United States is challenged by emerging threats from diseases, pests, and environmental extremes; rapidly shifting consumer needs and preferences; expanding needs for bioenergy feedstocks; and the need to ensure that crop products are safe and of high quality.

ARS scientists are conducting genetic research to help plant breeders incorporate genes from ancestral and wild relatives of crop plants. This often includes prebreeding to eliminate undesirable traits that may tag along. The scientists have developed new tools, such as more effective marker-assisted breeding approaches and revolutionary new statistical genetic and genomic methods. They have put these tools and information to good use by developing a host of new varieties and germplasm lines for a full spectrum of U.S. crops, from *Allium* crops, like onions and garlic, to *Zea mays*, or corn.

In Kearneysville, West Virginia, ARS researchers found, in plums, that introducing a poplar tree gene that promotes earlier f owering and fruiting shortened the juvenile stage from 3 to 4 years to less than 1 year. This method, termed "FasTrack," can signif cantly accelerate the breeding of new and improved tree-fruit varieties.

ARS researchers also respond to emerging agricultural threats, such as Ug99 stem rust, a virulent plant disease threatening wheat and barley crops in Africa, the Middle East, and Asia. This disease can cause crop losses of up to 100 percent when cultivars have no resistance. Ug99 may spread broadly throughout these regions and has the potential to reach beyond them.

In Ames, Iowa, ARS researchers and university cooperators have analyzed resistance to the Ug99 stem rust strain in barley using genome-wide expression prof ling, combined with genetic mapping. They identif ed a "master switch" that regulates the expression of hundreds of Ug99-responsive genes and demonstrated that the genetic location of the switch also confers enhanced adult-plant resistance. Further research on the master switch and development of allele-specif c markers can lessen the threat of Ug99.

In Beltsville, Maryland, ARS researchers and their university colleagues bred Peter Wilcox, a fresh-market potato with dark-purple skin, yellow f esh, and a carotenoid content more than 15 percent greater than that of Yukon Gold, the current standard yellow-f esh variety in the country. This new variety provides growers and consumers with a unique combination of skin and f esh colors for niche markets.

These are just a few examples of the work ARS has done, and continues to do, to provide improvements in crops for the benef t of U.S. agricultural producers and consumers.

Kay Simmons
ARS Deputy Administrator
Crop Production and Protection
Beltsville, Maryland

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Solving Problems for the Growing World

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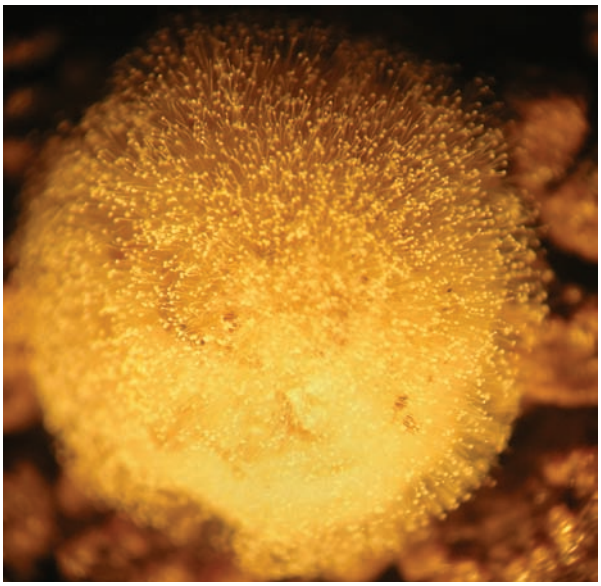
This good-guy fungus strain explodes into action to protect corn from bad-guy strains.

Story begins on page 20.



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Cover: ARS is always working to bring fresh fruits and vegetables to your home's kitchen and garden. Story begins on page 4. Photo by ARS. (K11432-1)

Fruits and Veggies for Now and in the Future

Fruits and vegetables are important parts of a balanced diet, and making sure consumers have an abundant supply of them is part of the Agricultural Research Service's mandate.

Genetic improvements in fruits and vegetables are essential to meet the ever-changing needs of growers, processors, and consumers. Scientists in ARS laboratories around the country strive to meet those needs.

Potatoes—a Kitchen Staple

UNIVERSITY OF FLORIDA (D2646-1)

In Beltsville, Maryland, plant geneticist Kathy Haynes is breeding potatoes with higher levels of carotenoids. Haynes, who is with the Genetic Improvement of Fruits and Vegetables Laboratory (GIFVL), in cooperation with nutritionist Beverly Clevidence, who is with the Food Components and Health Laboratory, is developing yellow potatoes with higher carotenoid levels for fresh consumption. In the July 2011 issue of the *Journal of the American Society for Horticultural Science*, Haynes describes the plant crosses she used to double and triple carotenoid levels normally found in Yukon Gold potatoes, a yellow-f eshed variety familiar to consumers.

Several carotenoids are involved, including neoxanthin, antheraxanthin, violaxanthin, lutein, and zeaxanthin. Of these, lutein and zeaxanthin are of keen interest for eye health; they appear to protect against age-related macular degeneration and, perhaps, cataract formation.

"Zeaxanthin is the one I am most interested in at this point because there are fewer sources of it in the diet than lutein. Both lutein and zeaxanthin are very high in dark-green leafy vegetables like spinach and kale—vegetables that people don't tend to eat a lot of," says Haynes. "My thinking is that if we can elevate their levels in potatoes, which people do eat a lot of, then we could make a signif cant impact on the human diet.

"We have a new potato named Peter Wilcox, which is a purple-skin, yellow-f esh potato introduced in 2007," says Haynes. "It is being grown a lot for use in roadside-market niches. The overall carotenoid levels in that potato are about 15 percent higher than they are in Yukon Gold potatoes."

BENILDO DE LOS REYES (D2655-1)

Top image: Peter Wilcox potatoes. This yellow-fleshed, purple-skinned potato has overall carotenoid levels about 15 percent higher than Yukon Gold potatoes.

Left: ARS plant geneticist Kathy Haynes (left) and University of Maine collaborator Sue Ballou inspect seedling generations arranged by carotenoid content.

More recently, Haynes and her colleagues have, with help from wild potatoes, produced a potato that has even higher carotenoid levels. The potatoes most people can purchase in the grocery store are tetraploids, meaning they have four sets of chromosomes. Most wild potatoes are diploids, possessing two sets of chromosomes, and are often small and lumpy, unlike the potatoes consumers purchase.

"What we found in the diploids is that those with intense yellow f esh have about 13 times more carotenoids than Yukon Gold. So we thought we would be able to cross them with tetraploid potatoes to produce potatoes that have 13 times the carotenoid levels and look like grocery store potatoes. But it didn't happen that way," explains Haynes. "We ended up with tetraploids that have only 2-3 times the carotenoids present in Yukon Gold potatoes—not what we were hoping for. Still, the carotenoid levels are higher than in current varieties on the market."

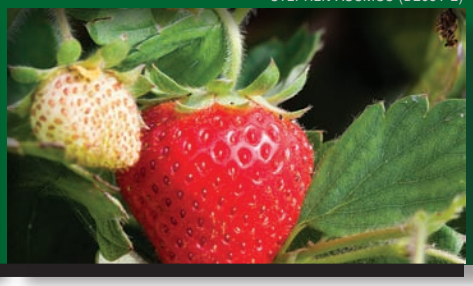
Protecting Spuds From Their Enemies

SCOTT BAUER (K5458-3)

SCOTT BAUER (K7239-8)

ROBERT GRIESBACH (D1267-2)

STEPHEN AUSMUS (D2651-2)



Potato plants can be damaged or killed when they don't recognize an attack by pests and fail to initiate responses to defend themselves. Identifying protective genes and deploying them in modern cultivated potatoes will help ensure a secure future for potato production, benef ting producers and consumers.

Plant pathologists Rick Jones, Ken Deahl, and Leslie Wanner, also at GIFVL, are focusing on resistance to *Phytophthora* late blight and *Streptomyces* scab. Jones and collaborators identif ed key *Phytophthora* enzymes involved in late blight during studies of the *Phytophthora* genome. That work was recently published in *Nature*. Jones continues to study individual gene functions of both *Phytophthora* and potato to determine the best way to protect potatoes from this devastating disease.

"Identifying individual protective genes in potato enables their direct introduction into already popular varieties such as Russet Burbank, avoiding extensive breeding and selection efforts," Jones says.

"In 2011, we isolated and characterized a population of newly occurring strains of *Phytophthora* from tomato and potato hosts growing in heavily blighted f elds in several locations in the United States. This information will be useful to plant breeders who need to test plant material for susceptibility to these new strains," says Deahl.

The group also looked at potato cultivars and characterized them for resistance to common scab. Caused by several species of *Streptomyces*, common scab of potato is a persistent problem for growers. "Identifying plant resistance is diff cult, partly because complex populations of multiple *Streptomyces* strains are usually present in production f elds. Breeders typically screen for resistance in f elds with multiple common scab-causing strains, but strains typical for one part of the country differ from the strains typical for a different geographic region," says Wanner. "In order to strengthen scab-resistance breeding efforts, we successfully characterized three potato families for resistance to common scab in greenhouse assays using individual pathogenic *Streptomyces* strains typical of different parts of the country." This information will be helpful to potato breeders in their efforts to incorporate resistance into new cultivars and is now available online to select potato cultivars for growth in specif c regions (tinyurl.com/potatoscab).

Left: Plant pathologist Ken Deahl examines a potato damaged by late blight fungus.

Plant pathologists Rick Jones (left) and Leslie Wanner screen both traditional and genetically enhanced potatoes for disease resistance. The potatoes on the left are uninfected (clean and round), whereas the ones on the right are infected (dark and misshapen).

WARREN GRETZ, NREL/DOE (D2632-1)

At the Vegetable Crops Research Unit in Madison, Wisconsin, plant physiologist Paul Bethke, geneticist Shelley Jansky, and technician Andy Hamernik are working on a phenomenon called "cold-induced sweetening," which causes sugar accumulation during long-term cold storage and results in unwanted dark colors in fried and roasted potatoes. Long-term cold storage is necessary to supply potatoes throughout the year.

Bethke, Jansky, and Hamernik used a recently developed technology to show that decreasing the activity of one key enzyme—invertase—is suff cient to enable cold storage of potatoes without compromising the appearance of potato chips or the growth characteristics of the potato plants.

"We are using molecular tools to improve our understanding of what is controlling the process of cold-induced sweetening. We know potatoes are sensitive to their environment and highly sensitive to low temperatures. Potatoes respond to the cold by producing certain sugars called 'reducing sugars,' primarily glucose and fructose," says Bethke. "When you make chips or fries from those potatoes, they tend to be dark colored and bitter—traits people don't want. In our paper in *Plant Physiology*, we provide a proof-of-concept that the invertase enzyme is critically important in the process."

However, it has never been clear how important, because there are other biochemical steps that might also contribute, Bethke says.

"We found that if you prevent the invertase gene from being expressed and therefore prevent the protein from being made, there is an accumulation of sucrose and a corresponding reduction in the amount of glucose and fructose in tubers stored at very low temperatures."

A Pack of Peppers

STEPHEN AUSMUS (D2657-11)

Tangerine Dream—a sweet, edible, ornamental pepper.

At GIFVL, geneticist and research leader John Stommel is developing peppers to have a dual purpose—culinary and ornamental—for use in a high-value niche market. Stommel and his colleague Robert Griesbach, now with the ARS Off ce of Technology Transfer, are not strangers to the arena of developing novel peppers, having produced Tangerine Dream, Black Pearl, and Lil' Pumpkin, among others.

"One of our f rst releases, Tangerine Dream, which preceded the All America Selections award winner Black Pearl, is a dual-purpose culinary ornamental," says Stommel. "Tangerine Dream is orange, banana shaped, and about 3 inches long."

Tangerine Dream was passed along to Burpee Seed Company through the team's cooperative research and development agreement (CRADA) partner, Pan American Seed Company. "It is a fun-looking plant that grows prostrate, with green foliage and attractive, upright-oriented, orange fruit," says Stommel.

"We are also working on miniature bell peppers for dual culinary and ornamental use. Plants have black or green foliage and cherry-tomato-size bell peppers, which we believe will be very popular for the home garden and commercial specialty markets," he says.

Pepper f avor is also under the research scope for Stommel and his colleagues. Under a CRADA, Stommel has been working with Keygene Inc. (Rockville, Maryland), for the last 4 years. "We are working to identify genes that can impart fruity f avors in pepper. We've made some crosses between sweet pepper and some exotic species of pepper that have very unique fruity aromatics," says Stommel. "We are producing a value-added commodity—a sweet green pepper with the aromatics of ripe red or orange fruit. This exotic species of pepper we identif ed embodies unique f oral aromatics at the green stage, which is very unusual, and we are working to introduce that into cultivated sweet pepper."

The green bell pepper, which is the immature form of the yellow, orange, and red pepper, dominates the market in the United States and South America. Green peppers with new f avors could be a boon to the vegetable industry.

Bountiful Berries

STEPHEN AUSMUS (D2648-20)

Geneticist John Stommel (left) and technician Mikhail Kozlov examine miniature bell peppers being developed for culinary and ornamental use in high-value niche markets.

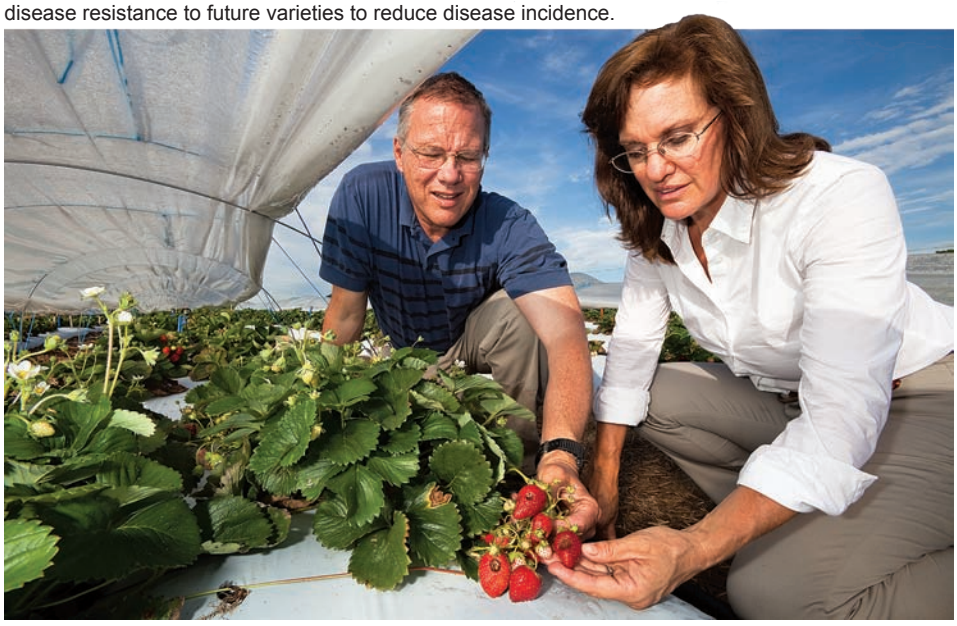
Also at GIFVL, geneticist Kim Lewers is striving to improve strawberries and is conducting f eld trials in several states under materials-transfer agreements with collaborators. While Lewers's focus is on yield, fruit quality, and disease resistance, she is testing some of those strawberry cultivars in a new production system designed to extend the growing season in the northern and eastern United States.

Strawberry is the highest value-per-acre crop that can be grown in this country, but most strawberries are grown in California and Florida. Lewers would like to expand the crop's range to more locations.

"I am trying to make strawberries available locally all along the East Coast, as much of the year as possible, with as little pesticide residue as possible, and we are developing a production system that supports that," says Lewers. "Common strawberry diseases that can affect yield and quality are mainly *Botrytis* and anthracnose. In our research f elds, we use no fumigants and no fungicides, and our program is known for disease resistance. The soils in our Beltsville f elds have a natural population of microbes after a decades-long period with no fumigants and fungicides. Because we don't use anything to protect our strawberries, we know that if they still survive and do well it's because they are resistant or tolerant."

In an effort to extend strawberry production beyond the normal local strawberry season of mid-May to mid-June, Lewers, along with ARS colleagues John Enns,

Reaching inside a low tunnel that extends the fruit's growing season, geneticist Kim Lewers and horticulturalist John Enns observe strawberries with anthracnose fruit rot. The next goal is to transfer disease resistance to future varieties to reduce disease incidence.



STEPHEN AUSMUS (D2650-12)

STEPHEN AUSMUS (D2649-8)

Low tunnels can extend the strawberry growing season and allow ARS scientists to develop cultivars that produce fruit over several months.

a horticulturalist, and George Meyers, with Research Support Services, created a production system that uses low tunnels to cover the rows of strawberry plants.

These tunnels provide a long row of shade and rain protection. Rain is bad for strawberries because the two most important diseases, *Botrytis* and anthracnose, thrive in the rain. *Botrytis* occurs in cool, wet conditions, and anthracnose occurs in hot, wet conditions.

"This tunnel system has evolved. We have various sensors that measure environmental factors inside and outside the tunnels, such as temperature and relative humidity," explains Lewers. "High tunnels have been used by others, but they were problematic because the humidity is higher in the tunnel, which causes more *Botrytis* and more powdery mildew, another strawberry disease. But in low tunnels, the humidity is the same as outside the tunnel when the sides of the tunnel are up—a good thing because that doesn't facilitate disease under the tunnel.

"The Maryland peak strawberry season is usually mid-May to mid-June, but our strawberries start earlier and continue through the summer and fall. So we have a whole new season. Monthly yields in the low tunnels can be as high as those from the same cultivars when they are grown in California, where they were developed."

All of the strawberry plant material developed in Lewers's research program has been and still is freely available. The plants are not patented, so they are available without special license to any nursery that wants to grow them.

"These are truly a gift to the taxpayer and the industry. Also, our material is known for having better f avor. Growers call it the 'eastern' f avor, and some California breeders now request our material and expertise about selecting cultivars that would impart different desirable traits," says Lewers.

Another berry—the black raspberry—has been the subject of ARS researchers at the Horticultural Crops Research Unit in Corvallis, Oregon, and at Oregon State University. ARS horticulturist Chad Finn and colleague Michael Dossett, of Agriculture and Agri-Food Canada, are the f rst to f nd and report resistance in black raspberry to the large raspberry aphid. The duo screened seedlings from 132 wild populations of black raspberries for aphid resistance.

"Strong resistance was found in three of these populations—one from Ontario, one from Maine, and one from Michigan," says Finn. "Aphid resistance in the Ontario and Maine populations is dominant and seems to be controlled by different genes. Aphid resistance in the Michigan population is governed by one dominant gene." A trait is dominant when it can be inherited from only one of its parents and still be expressed.

Geneticist Chad Finn evaluates black raspberry plants for resistance to black raspberry necrosis virus.

STEPHEN AUSMUS (D991-36)

STEPHEN AUSMUS (D992-14)

The raspberry aphid, *Amphorophora agathonica,* is a major culprit in spreading black raspberry necrosis virus and raspberry mottle virus.

"Identifying these genes makes it easier for breeders to incorporate aphid resistance into commercial black raspberry cultivars," says Finn.

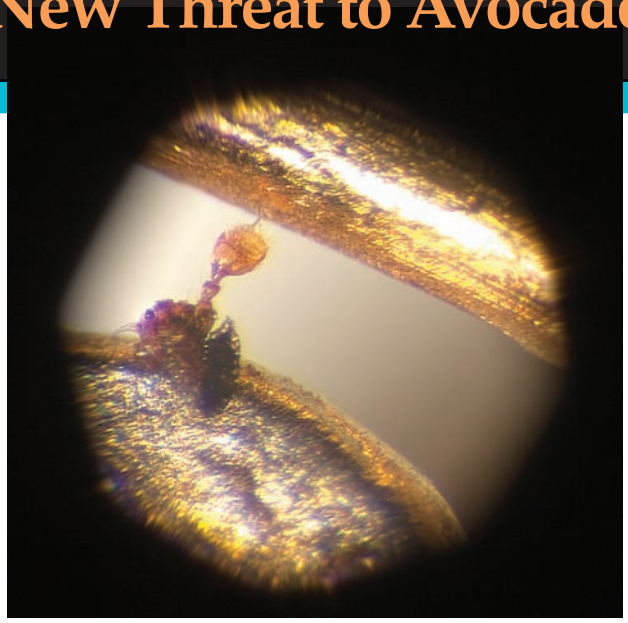
Black raspberry production is severely affected by the black raspberry necrosis virus, which is transmitted by the large raspberry aphid. This and other aphids are important vectors of viruses in raspberry in North America.

"Although breeding for aphid resistance has been recognized as an important tool for protecting red raspberries from viral infection, this is the f rst report of aphid resistance in black raspberry," says Finn.—By **Sharon Durham,** ARS.

This research is part of Plant Genetic Resources, Genomics, and Genetic Improvement (#301), Plant Diseases (#303), and Crop Protection and Quarantine (#304), three ARS national programs described at www.nps.ars.usda.gov.

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

Addressing a New Threat to Avocado: Laurel Wilt



STEPHEN AUSMUS (D2639-5)

Microscopic view of a redbay ambrosia beetle antenna mounted between gold wire electrodes of an electroantennography system. The antennae are exposed to different attractants to determine which ones the insect most readily detects.

First detected 10 years ago near Savannah, Georgia, laurel wilt disease, caused by the fungus *Raffaelea lauricola*, is considered a major threat to the thriving avocado industry. The disease is vectored by the redbay ambrosia beetle, *Xyleborus glabratus*. The tiny Asian import has traveled north to the Carolinas, south to Florida, and west to Mississippi, spreading the wilt fungus along the way. Trees respond to infection by shutting down transport of the fungus through their vascular tissues, which also shuts down transport of water, and that makes the disease particularly lethal. The disease kills 90-95 percent of infected trees and can kill a tree in just 6 weeks.

Laurel wilt was confirmed in Miami-Dade County commercial avocado groves in 2012, and costs to replace Florida's residential and commercial avocado trees are estimated at $429 million. Scientists are concerned that the insect will soon reach Mexico and California, which are major avocado production areas. Its victims also include several other types of laurel trees, including the redbay, swampbay, sassafras, silkbay, camphor tree, spicebush, pondberry, and pondspice. Mountain laurel trees are not in the Lauraceae plant family and are not at risk, but the popular California bay laurel is part of the family and will be at risk if the beetle reaches the west coast. The beetle is also a serious threat to forest ecosystems in the United States. The redbay tree is a popular shade tree in residential areas and one that produces fruit, seeds, and foliage that, in wild areas, sustain songbirds, quail, deer, black bears, wild turkeys, and the palamedes swallowtail butterfly *(Papilio palamedes)*.

"When it arrived in the United States, the beetle had no natural enemies, but it had no trouble finding suitable host trees. Those hosts, never exposed to the foreign fungus, develop an extreme defensive response that has life-threatening consequences. From an ecological standpoint, it was like a perfect storm," says Paul Kendra, an entomologist at the Agricultural Research Service's Subtropical Horticulture Research Station (SHRS) in Miami, Florida.

Plant pathogens that are vectored (transmitted by one organism to another) are often best managed by controlling the vector. Kendra and his colleagues are working on several fronts to minimize the threat posed by the beetle. They are searching for chemical attractants for potential use in traps, treating trees in the ARS avocado collection with fungicides to slow the spread of the disease, and shipping trees from the Miami avocado germplasm collection to disease-free sites for safe storage. ARS scientists Tomás Ayala-Silva, Osman Gutiérrez, Nancy Epsky, David Kuhn, and Jerome Niogret, who are all based at SHRS, are working with Kendra on the project.

Previous research has shown that, like other bark beetles, the redbay ambrosia beetle essentially "sniffs out" volatile compounds released by host trees that it needs to survive. Kendra and his colleagues found that certain lychee varieties are also highly attractive to the beetle, even though lychee trees are not hosts.

Two tree oils are now used in traps to monitor the beetle's movement in Florida—manuka oil and phoebe oil. But both oils are nonspecific lures, and supplies of phoebe oil have dried up, so the ARS researchers hope to find other natural compounds that attract the beetle and can be used in traps for better detection and pest control.

Seeking the Irresistible

To find natural attractants, Kendra and his colleagues began by exploring several questions, including whether the

Left: Female redbay ambrosia beetle, *Xyleborus glabratus* (about 2 mm long). Center: An indicator of ambrosia beetle infestation is "sawdust" around the beetle's entry hole (shown here is another female redbay ambrosia beetle). Right: A cross section of a swampbay tree trunk shows the redbay ambrosia beetle's galleries (white lines) that are filled with the fungus *Raffaelea lauricola,* the cause of laurel wilt disease.

STEPHEN AUSMUS (D2634-2)

STEPHEN AUSMUS (D2634-10)

STEPHEN AUSMUS (D2640-2)

beetle prefers any one of the three races of cultivated avocado tree (West Indian, Guatemalan, and Mexican), whether avocado or lychee trees attract more beetles, and which of the two lures now used in monitoring efforts is more effective.

In f eld experiments at the Lochloosa Wildlife Conservation Area, in north central Florida, the researchers compared the number of beetles attracted to manuka oil lures, phoebe oil lures, and bolts of wood cut from lychee and from the three races of avocado. The conservation area was an acceptable setting because the beetle has infested trees there since 2007. In laboratory tests, the researchers also conducted "choice" experiments by placing lychee and avocado wood on opposite ends of a plastic bin and placing the beetles in the middle to see which wood they preferred. Compounds released by the two types of wood were analyzed using gas chromatography-mass spectroscopy (GC-MS). Results, published in the *Journal of Chemical Ecology,* showed that the beetles had no strong preference among the three avocado races and that lychee was the most attractive wood. Of the 29 compounds detected, 3 were found to attract the beetle, and the lychee had large amounts of all three.

Subsequent research, published in the *Journal of Economic Entomology,* showed that phoebe oil lures were effective for 10 to 12 weeks, but the manuka lures lasted only about 2 to 3 weeks in Florida. The fact that phoebe lures were effective for longer periods is unfortunate, because phoebe oil comes from an endangered Brazilian walnut tree that only grows in the Amazon. Knowing how long the manuka lures work should prove useful to Florida agriculture off cials, who use them in monitoring efforts. Off cials and growers will also benef t from the researchers' discovery that the beetles prefer freshly cut wood surfaces, which indicates that trees are vulnerable to attack during pruning.

In another study, Kendra and his team evaluated compounds released by seven tree species known to attract the insect: a variety of lychee tree, the redbay, the avocado, and four other species of laurel trees. Volatile chemicals released by the trees were collected using methods previously shown to be effective—manually rasping bark and outer tissues from freshly cut tree samples and placing the samples in glass chambers containing purif ed air and special f lters that collect emitted gases. The compounds in the f lters were then analyzed using GC-MS. The results, published in *Florida Entomologist,* showed that the laurel trees collectively released a "generalized bouquet" of 11 compounds and that of those, 4 are also released by lychee. The compounds were the same as those identif ed in the *Journal of Chemical Ecology* study, and efforts to f nd attractants are now focused on those compounds.

The researchers are also beginning electrophysiology studies, exposing the beetle's antennae to different attractants to determine which ones the insect most readily detects. Results of those studies are pending.

Safeguarding an Important Collection

The Subtropical Horticulture Research Station in Miami is also home to the part of the ARS National Germplasm Repository that serves as the collection site for avocado trees and many other species of tropical and subtropical fruits and ornamentals. To prevent infection among those avocado trees, Ayala-Silva injected 260 mature trees with propiconazole, which is known to control laurel wilt in redbay trees as well as fungal diseases in oak and elm trees. Tree roots were injected in the summer of 2009 and again between February and March of 2011 with a commercial formulation of the fungicide known as "Alamo."

Injecting individual tree roots with the treatment is too expensive for most growers, and its use in commercial production would require regulatory approval, but the researchers wanted to see if the treatment would protect the trees in the research collection.

Follow-up evaluations showed the fungicide was still present in the tree branches 14 months after injection, a result that shows the treatment can deter infection for lengthy periods. Results were published in *Acta Horticulturae*.

The researchers are continuing to monitor the collection for signs of the disease and have shipped young avocado trees to ARS facilities in Hilo, Hawaii, for safekeeping. In addition, plans are under way to begin a screening program for avocado varieties with resistance to laurel wilt.—By **Dennis O'Brien,** ARS.

This research is part of Methyl Bromide Alternatives, an ARS national program (#308) described at www.nps.ars.usda.gov.

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

STEPHEN AUSMUS (D2637-5)

During field trapping studies at Lake June State Park in central Florida, entomologist Paul Kendra sets up a sticky panel trap baited with an essential oil lure for evaluations of attractants for redbay ambrosia beetle.

ARS Scientists Tee Up To Tackle Golf Course Pesticide Runoff

At the University of Minnesota Turf Research, Outreach, and Education Center in St. Paul, Minnesota, a rainfall simulator is used to generate runoff. Plots were equipped with rain gauges and automated runoff samplers to measure precipitation, runoff, and flow rates, and to collect periodic runoff samples.

At last count, around 27 million golfers in the United States have been teeing up for rounds on approximately 16,000 golf courses. Each golf course has either 9 or 18 holes, so well over 100,000 fairways—which typically make up a third of a golf course—are carefully tended and pampered.

Golf courses are often close to ponds, streams, and lakes, and the chemicals used to maintain the grounds have been found in surface waters of urban watersheds. Now, studies by Agricultural Research Service chemist Pamela Rice on pesticide and nutrient losses from fairways have given landscape crews some environmentally friendly ideas for maintaining the popular green playgrounds.

"Our research indicates that there are management practices you can use as a preventative measure to protect water resources around golf courses," says Rice, who works at the ARS Soil and Water Management Research Unit in St. Paul, Minnesota.

Cultivation Method Affects Pesticide Fate

Rice worked with University of Minnesota professor Brian Horgan to design a series of studies at the University of Minnesota Turf Research, Outreach, and Education Center in St. Paul. One project simply measured the quantity of pesticides in runoff from creeping bentgrass *(Agrostis palustris)* turf managed as a golf course fairway.

The scientists applied the pesticides chlorpyrifos; flutolanil; mecoprop-p; 2,4-D; and dicamba to the experimental fairways and then measured the amount of the pesticides in runoff from simulated rain events that occurred within 33 hours of the applications. Samples taken from edge-of-plot runoff contained less than 1 to 23 percent of the total amount of pesticides applied. With the exception of chlorpyrifos, all the other chemicals were detected in the initial runoff samples and in samples taken throughout the runoff events.

Rice and Horgan also evaluated the effects of different types of core cultivation on pesticide concentrations in runoff. In hollow-tine cultivation, soil cores are removed from the turf, air dried, and then brushed back into the open holes. Solid-tine core cultivation uses less labor and is less disruptive to the turf surface, but can cause soil compaction. Core cultivation on golf fairways controls thatch, alleviates surface compaction, improves water inf ltration, and stimulates root and shoot growth.

Studying the same group of pesticides 63 days after the plots were cultivated and within 39 hours of chemical application, the scientists measured a 10-percent reduction in runoff volume and a 15- to 24-percent reduction in pesticide transport in runoff from plots receiving hollow-tine core cultivation compared to those receiving solid-tine core cultivation. Samples taken 2 days after the plots were cultivated a second time and within 39 hours of a subsequent chemical application showed a 55-percent reduction in runoff volume and a 35- to 57-percent reduction in pesticide transport.

Rice and Horgan calculated the environmental concentrations of these pesticides in surface water receiving runoff from turf managed with solid-tine core cultivation and found that they would exceed levels that are harmful to nine sensitive aquatic organisms. But hollow-tine core cultivation reduced surface-water concentrations of the pesticides to levels below these for most of these aquatic fauna.

Models: Room For Improvement

Along with ARS agricultural engineer Kevin King, who works at the ARS Soil Drainage Research Unit in Columbus, Ohio, the researchers used the data they collected to evaluate a turfgrass runoff model called "TurfPQ." This model estimates pesticide levels in runoff associated with moderate rainfall, and they wanted to see how accurately TurfPQ predicted pesticide transport in runoff associated with more intense rainfall. They compared runoff data from 13 artif cial

Pesticides being applied to experimental turf plots maintained at a golf course fairway. Following a simulated rain event, runoff was collected from the plots and analyzed for five different pesticides.

rainfall events to estimates provided by TurfPQ for the same conditions.

The scientists found that the model's estimates were lower than the actual measurements for transport of dicamba; 2,4-D; f utolanil; and chlorpyrifos. The model predicted that runoff would begin later than it actually did, which in turn increased error estimates for the amount of pesticides available for offsite transport via runoff. As a result of these f ndings, they concluded that with some tweaking, TurfPQ could provide better pesticide-loss estimates during intense storm events.

What About Fertilizers?

Rice and Horgan also used their experimental plots to study differences between how hollow-tine cultivation and solid-tine cultivation affected nitrogen and phosphorus retention on fertilized fairways. In surface waters, these two nutrients feed the growth of algae, and when the algae die, their decomposition depletes oxygen levels in the water. These conditions contribute to deterioration of local waterways and downstream aquatic environments. The U.S. Environmental Protection Agency (EPA) has established phosphorus limits for lakes and streams and nitrate nitrogen limits for our drinking water.

Using the same experimental turfgrass fairways that were used for the pesticide studies, the researchers measured runoff volume and amounts of soluble phosphorus, ammonium nitrogen, and nitrate nitrogen that were lost via runoff.

As with their pesticide studies, Rice and Horgan found lower nutrient concentrations in runoff from fairway plots that received hollow-core cultivation compared to solid-core cultivation—up to 77 percent less 2 days after the plots were cultivated and up to 27 percent lower 63 days after cultivation.

They also estimated the environmental concentrations of nitrogen and phosphorus in surface water receiving runoff from the experimental plots. They found that with one exception, phosphorus concentrations usually remained above EPA water-quality criteria established to limit eutrophication, which can occur when water bodies receive excess nutrients that stimulate excessive plant growth. (The exception was observed in phosphorus concentrations found in runoff 2 days after hollow-tine core cultivation.) However, all estimated environmental concentrations of nitrogen were below levels associated with increased algal growth, and nitrate levels in runoff from plots receiving either type of core cultivation were not high enough to threaten human health.

"We've seen that the total amount of applied chemicals lost from golf courses is more a function of the volume of runoff than the concentrations of chemicals in the runoff," Rice concludes. "Our studies also show that, even though it is more labor intensive, hollow-tine core cultivation can help reduce the loss of applied nutrients and pesticides from fairways, which helps protect nearby surface waters."—By **Ann Perry,** ARS.

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

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Core cultivation of plots. Some plots were cultivated with solid tines, others with hollow tines. Runoff was collected from plots cultivated by each method and analyzed for pesticide and fertilizer (nitrogen and phosphorus) content.

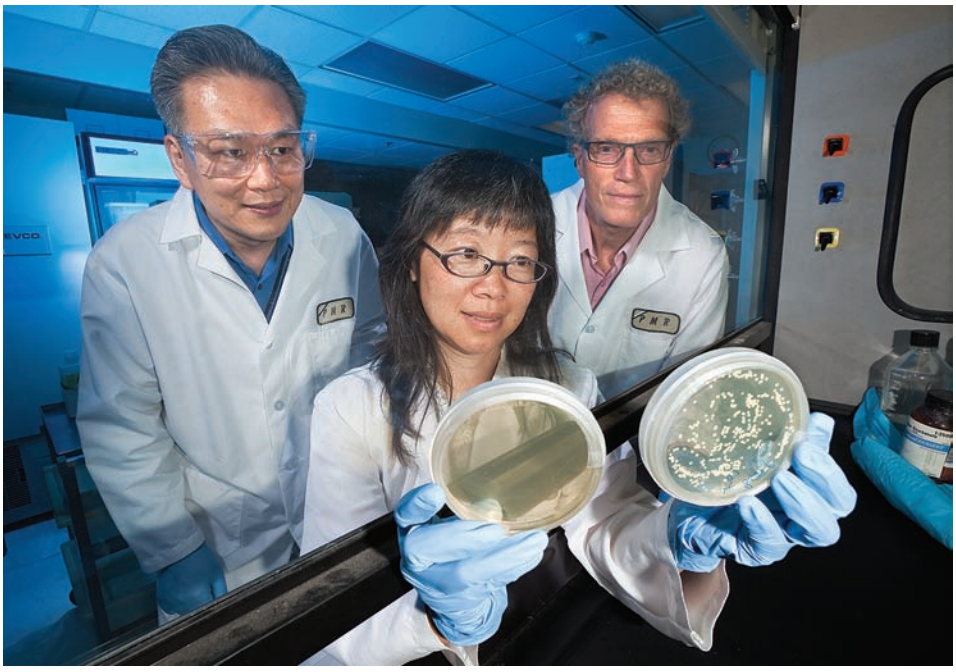
JENNIFER RITTENHOUSE (D2562-1)

STEPHEN AUSMUS (D2654-4)

Human Medical Advances May Result From Tree Nut Research

Prescription drugs that today help patients f ght severe fungal infections might tomorrow be even more effective. Laboratory studies by former Agricultural Research Service research leader Bruce C. Campbell, ARS molecular biologist Jong H. Kim, and their colleagues suggest that pairing conventional antifungal medications with natural, edible plant compounds such as thymol—extracted from the popular herb thyme—can work synergistically with some of these drugs to boost their healing effects.

The studies are an unexpected "spillover" from the scientists' ag-based, food-safety-focused research, which they conduct at the ARS Western Regional Research Center in Albany, California.



STEPHEN AUSMUS (D2641-3)

Each year, millions of Americans are diagnosed with diseases caused by pathogenic fungi. The most serious of these, invasive fungal infections, are more than skin deep and can be deadly. What's more, some of the fungi that cause these infections are developing resistance to well-established antifungal drugs.

For their work, Campbell and Kim have targeted several key groups of fungi: *Aspergillus* molds, which can severely damage the lungs or other organs; *Candida* yeasts, which sometimes cause life-threatening conditions if they enter the bloodstream; and a *Cryptococcus* yeast that can lead to fungal meningitis, a dangerous swelling of tissue around the brain and spinal cord.

"With the appropriate medication, administered early, most healthy people can

Above: The popular herb thyme. ARS scientists in Albany, California, are determining whether the natural compound thymol, extracted from thyme, can work synergistically with conventional antifungal meds to boost their effects.

Left: Molecular biologist Jong H. Kim (left), technician Kathleen L. Chan, and entomologist Bruce C. Campbell inspect petri dishes containing pathogenic fungi. The dish on the left contains thymol and antifungal medications that are inhibiting fungal growth.

fend off infections caused by these fungi," says Campbell. "But current treatments may take many months, can be expensive, may lead to serious negative side effects, and can contribute to the increasing presence of resistant strains."

People with a compromised immune system, such as cancer patients undergoing bone marrow or organ transplants, and individuals with AIDS (acquired immunodef ciency syndrome), are among the most susceptible to invasive fungal infections.

In their ongoing research, Campbell and Kim have shown that drug and natural agent duos are signif cantly more effective in debilitating target fungi than if either the drug or agent had been used alone.

Using plant-derived compounds to treat fungal infections is not a new idea, nor is that of pairing them with antifungal medications. The Albany team's studies have, however, explored some apparently unique pairs and provided some of the newest, most detailed information on the mechanisms likely responsible for the impact of powerful drug-natural agent combinations.

Food Safety Studies Targeting *Aspergillus* Attract Medical Researchers' Interest

It was Campbell and Kim's research on *Aspergillus* species that initially attracted the attention of medical and public health researchers in the United States and abroad. This mold is found worldwide in air and soil. Growers of pistachios, almonds, corn, cotton, and some other crops know that it can infect their harvests and produce af atoxin, a natural carcinogen.

"Af atoxin-infected crops must be identif ed and removed from the processing stream," says Campbell. "At times, this can result in huge economic losses." That's why there's an ongoing demand for research that will yield new, robust, affordable ways to control the fungus.

To meet that need, Campbell, Kim, and coinvestigators have—since 2004—carefully built a portfolio of potent, plant-based compounds that either kill *A. f avus* or thwart its ability to produce af atoxin. Further research and testing might enable tomorrow's growers to team the best of these natural agents with agricultural fungicides that today are economically infeasible to use.

The *A. f avus* of orchards and f elds is also among the *Aspergillus* species that can cause lung damage in immunocompromised people. If exposed to the fungus in a moldy home, for instance, they may inhale more of its spores than their bodies can handle. Though an *A. f avus* relative, *A. fumigatus*, is the main pathogen associated with this invasive infection, *A. f avus* and a third causative species, *A. terreus*, are also of concern.

Thymol Performs Well in Many Tests

In a study published in 2010 in *Fungal Biology*, the team zeroed in on these three *Aspergillus* species. The study showed that thymol, when used with two systemic antifungal drugs—f uconazole or ketoconazole—"inhibited growth of these fungi at much lower than normal doses of the drugs," says Kim.

The study also showed that some combinations don't work. For example, thymol improved the effectiveness of the drug amphotericin B against *A. f avus* and *A. fumigatus*, but it undermined the drug's effectiveness against two of three strains of *A. terreus* tested, according to Kim.

In an investigation reported last year in *Applied Microbiology*, thymol was one of a half-dozen promising natural compounds that were teamed with drugs and tested against *Candida* and *Cryptococcus* species.

Other natural compounds selected for this battery of tests included cinnamic acid from cinnamon tree bark; salicylic acid, like that found in willow; and 2,5-DBA (dihydroxybenzaldehyde), found in chard.

Each natural agent was paired with each of three different antifungal medications—amphotericin B, f uconazole, and itraconazole.

Importantly, none of the natural compounds interfered with the effectiveness of any of the drugs. Also notable: 2,5-DBA "worked synergistically with almost all of the drugs used against the *Candida* and *Cryptococcus* species," Kim says.

Thymol added to the impact of all three drugs in the *Candida* tests and one of the three in the *Cryptococcus* experiments.

What Fungal Genes Do Natural Compounds Target?

Additional studies by this team are providing new evidence to support earlier f ndings at Albany and elsewhere, which suggest that the natural agents sabotage the target fungi's ability to recover from oxygen-related problems referred to as "oxidative stress."

"Antifungal drugs can trigger oxidative stress," Kim explains. "Fungi may respond by quickly activating a complex defense system, which can include producing protective enzymes that act as antioxidants."

In this research, the team used both natural and laboratory-built versions, called "analogs," of certain compounds, including modified forms of thymol, cinnamic acid, and vanillin, like that from vanilla bean.

The studies suggest that these modif ed agents interfere with fungal genes. Normally, those genes enable the fungi to produce the necessary antioxidant enzymes. Some examples are *sod1* and *sod2*, which cue production of superoxide dismutases, and *glr1*, which contains the blueprint for another powerful antioxidant enzyme, glutathione reductase. An article published in 2011 in *Annals of Clinical Microbiology and Antimicrobials* tells more.

It's Called "Chemosensitization"

The scientists refer to the synergistic interaction of med and natural agent as "chemosensitization"—a term borrowed from chemotherapy treatments for cancer patients. "The natural agent 'sensitizes' the target fungi, making it more vulnerable to the effects of the drug," Campbell explains.

"Our petri-dish tests," says Campbell, "are a starting point. Many years of medical research are needed to provide the data necessary for federal approvals of new medical uses of natural agents. But the fact that the chemosensitizing agents are natural may help simplify the approval process."—By **Marcia Wood,** ARS.

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

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PEGGY GREB (D2665-1)

Reducing Sorghum's Major Limitations

Cold Tolerance and Diseases

Plant pathologist Louis K. Prom examines sorghum seeds infected by *Colletotrichum sublineolum,* the cause of sorghum anthracnose.

In the world of cereal crops, sorghum is as versatile as they come. Extremely drought tolerant, it grows in marginal areas and produces so much biomass that some researchers are exploring whether it can be used as a biofuel. It is an important part of the human diet in India, Africa, and parts of Japan, and while it is used in the United States primarily in animal feed, it is a major U.S. export and is sold domestically to make gluten-free f our.

But sorghum has its limitations. It is highly susceptible to diseases and is not cold tolerant. Agricultural Research Service scientists in College Station, Texas, are developing tools to help combat the diseases that can wipe out entire f elds of sorghum. Colleagues in Lubbock, Texas, are hunting for genes that they can use to develop cold-tolerant lines and expand the range of what was originally a tropical plant.

Some Like It Cold

Commercially, sorghum will not grow well in areas where soil temperatures fall below 59°F during early-season planting. But a team of ARS scientist and technicians in Lubbock is out to change that. Plant physiologist Gloria Burow and colleagues John J. Burke and Zhanguo Xin, along with technicians Halee Hughes and Charlie Woodf n, have joined with Cleve Franks, a scientist at Pioneer Hi-Bred International, to search for genes in cultivars from China that can be used to develop cold-tolerant lines. If they can increase sorghum's cold tolerance, it could be planted earlier, and its range in the Midwest could be extended beyond the Sorghum Belt that now stretches from Texas to Kansas and parts of Nebraska. The researchers want to extend it into the Dakotas and west to Colorado, Burow says.

Burow, Xin, and Burke are in the Plant Stress and Germplasm Development Research Unit at the ARS Cropping Systems Research Laboratory in Lubbock.

Burow and her colleagues spent 3 years evaluating 200 sorghum accessions from China, where the plant is known for its cold tolerance. They took one of the most promising cold-tolerant lines, accession PI610727, crossed it with a cold-sensitive sorghum variety, and produced 171 inbred lines. They raised those lines at sites in Texas and recorded soil and air temperatures to assess the cold tolerance of each plant. They also extracted DNA from the leaves and used simple sequence repeat markers to genotype them, essentially matching up markers found in individual plants with their cold hardiness, measured as the ability to germinate early and to produce robust seedlings under cold conditions.

They also evaluated each line in petri dishes at cold temperatures and at optimal

A grain mold-resistant sorghum line with bright red seeds.

PEGGY GREB (D2666-1)

temperatures for seed germination. The results, published in the September 2011 issue of *Molecular Breeding*, include a genetic map of 141 genetic markers that will make it easier for breeders to identify cold tolerance in sorghum plants. The researchers also found several lines with superior cold tolerance that could be useful for breeding commercially viable cold-tolerant cultivars.

They have released the 171 inbred lines to breeders and research groups through the ARS Germplasm Resources Information Network, and at least two research groups have requested additional information on that population so far. The work is continuing, with germplasm being evaluated at four locations in Texas, Kansas, and South Dakota. Support for that work is being provided by the U.S. United Sorghum Checkoff Program, a grower-funded effort to improve sorghum.

"We have to be able to show breeders and growers that what we can offer them will grow in different locations and under different soil conditions," Burow says.

The effort could lead to higher yields and plants that tap more moisture from the soil. "If you have cold-tolerant sorghum, you can plant your crop earlier, and if you can plant earlier, you can take advantage of moisture in the soil in the springtime, so that indirectly you have a mechanism for drought tolerance. You could also have two growing cycles in a year," says Burow.

Fighting Sorghum Diseases

At the ARS Crop Germplasm Research Unit in College Station, Texas, plant pathologist Louis K. Prom is developing tools to help combat diseases that can wipe out entire f elds. He is focused on three of the pathogens that are evolving threats to sorghum worldwide: head smut, anthracnose, and grain mold.

Head smut is a soilborne fungal pathogen that infects sorghum plants at the seedling stage, but symptoms appear at f owering, and infected plants are unable to produce grain. Head smut can't be controlled with rotation or chemicals, and it is found all over the world. Once the fungus is in the soil, it is diff cult to eradicate, and the only way to prevent head smut is by growing resistant cultivars. But breeders and growers can only screen for head smut by growing plants in f elds where there are high levels of it and then observing the results. Only after several years can they tell whether plants are resistant. Even then, if soil temperatures are too high or too low, it can reduce infection rates and throw off the results.

Head smut is also an increasing problem in coastal Texas. "Two years ago, we toured an area in South Texas, and the growers and extension agents showed us infected areas where they had never seen head smut before," says Prom.

To speed up monitoring efforts, Prom and his colleagues evaluated 4 techniques for screening sorghum for head smut resistance using 32 isolates of the pathogen collected from different parts of Texas. Inoculated plants were grown in greenhouses to ensure uniform results. The results, published in May 2011 in *Plant Disease*, show that the most reliable method involves injecting 18- to 20-day-old plants with a solution that includes head smut fungal spores in suspension.

Anthracnose is the most important foliar disease of sorghum worldwide, and grain mold is the crop's most signif cant overall threat. Grain mold costs U.S. growers $130 million a year. Host plant resistance remains the most effective control mechanism for these threats, too.

To help growers combat the diseases, Prom screened 39 sorghum accessions over 2 years for resistance to anthracnose and several of the most common types of grain mold fungi. He found one accession from Uganda, labeled "PI534117," that is both highly resistant to anthracnose and shows very little evidence of grain mold infection. The results, published in May 2011 in *Crop Protection*, also show that the cultivar has a high germination rate, good seed qualities, and a short stature, which altogether make it a good candidate for Texas growers.—By **Dennis O'Brien,** ARS.

The research is part of Plant Diseases (#303) and Plant Genetic Resources, Genomics, and Genetic Improvement (#301), two ARS national programs described at www.nps.ars.usda.gov.

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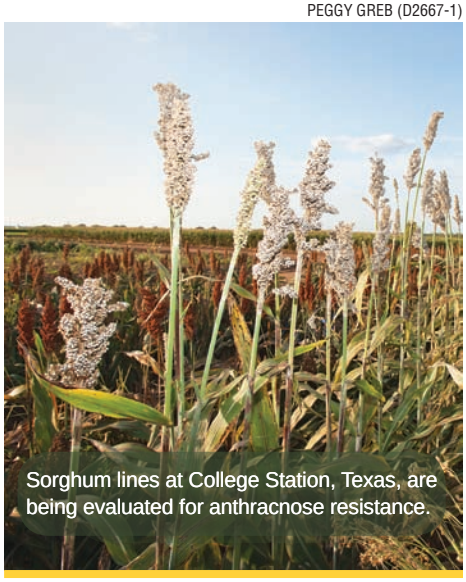
PEGGY GREB (D2667-1)



Sorghum lines at College Station, Texas, are being evaluated for anthracnose resistance.

A recombinant inbred line of sorghum.

JOHN BURKE (D2676-1)

Using Manure To Mend Mine-Damaged Soils

From 1850 to 1950, the Tri-State Mining District of southwestern Missouri, southeastern Kansas, and northeastern Oklahoma produced 50 percent of the zinc and 10 percent of the lead in the United States. The last active mine closed in 1970, but mining's ecological legacy remains throughout the region—lead-contaminated acidic soils, toxic smelter sites, large quantities of mine tailings called "chat," and thousands of acres of land with little or no vegetation.

Paul White, a soil scientist in the ARS Sugarcane Research Unit in Houma, Louisiana, was part of a team that studied whether adding beef cattle manure compost to postmining sites would help jump-start revegetation. "Soil microbes recycle nutrients from soil organic matter, and this nutrient cycling is important for vegetation growth. But there is limited soil organic carbon at these sites," White says. "So we added carbon to the soil via compost to see if that would get these systems going."

Kansas State University agronomy graduate student Luke Baker and professor Gary Pierzynski also partnered in this project.

The scientists also wanted to see whether compost could reduce levels of lead and zinc that could contaminate runoff during heavy rain. High levels of zinc can harm aquatic fauna in surface waters, and lead is linked to a number of serious health conditions in humans. Heavy metals in soils also disrupt the activity of soil microbes by damaging proteins or disrupting cell membranes.

The researchers amended soils in 3- by 6-foot test plots with either 20 or 120 tons of beef cattle manure compost per acre. No manure was put on control plots. Then they applied switchgrass seed on all of the plots and took soil samples from the plots f ve times during the 2-year study.

Two years after they amended the plots with the compost, White and his colleagues found that soils in the high-compost plots had signif cant increases in pH, plant-available phosphorus, total nitrogen, carbon, and available water. High-compost amendments also increased microbial biomass, enzyme activity, and nitrif cation potential, all of which create and support favorable conditions for plant establishment and growth.

"Nitrif cation potential is a sensitive indicator of stress because nitrifying bacteria are especially sensitive to toxic conditions," White explains. "Soil microbes also produce the enzymes that convert organic phosphorus into an inorganic form that can be used by plants."

In this study, the researchers also found that high rates of compost lowered lead and zinc availability by about 90 percent, which may reduce the amount of lead and zinc that could run off and pollute nearby waterways. This reduction occurred because heavy metals generally bind tightly to the organic matter in composted material, which limits their solubility and potential bioavailability in soil. Since high levels of bioavailable zinc inhibit plant growth, this binding action also helps to promote the establishment of a vegetative cover, which in turn can minimize runoff and soil erosion.

Given these f ndings, White and his partners think that adding composts to contaminated soils could help stabilize postmining sites. "The results strongly suggest that available soil carbon—which we were able to provide with the compost—may be a critical variable in establishing and maintaining a healthy microbial population in soils contaminated by similar mine wastes," White says.

The team published their f ndings in *Applied Soil Ecology* in 2011.—By **Ann Perry,** ARS.

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

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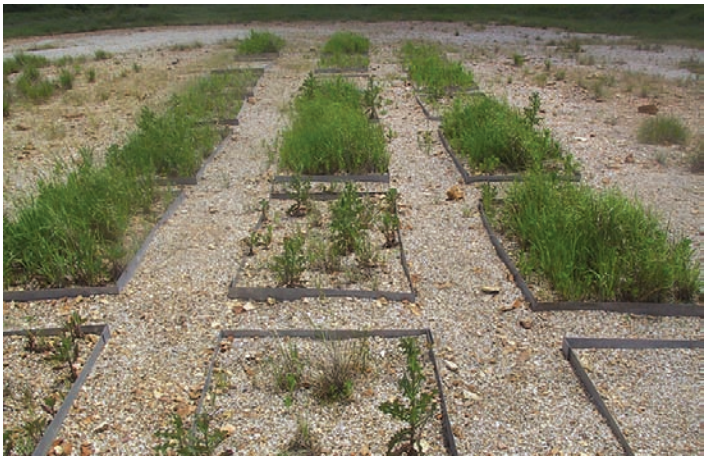
A study plot after beef cattle manure compost was added to soils degraded by mining. Compost can increase soil pH, plant-available phosphorus, total nitrogen, carbon, available water to support plant establishment and growth.

LUKE BAKER, BROOKSIDE LABORATORIES, INC. (D2678-2)

A plot showing varying vegetation that occurred after different levels of beef cattle manure compost amendments. Plant growth suffered when no compost was used but excelled when high amounts were used.



LUKE BAKER, BROOKSIDE LABORATORIES, INC. (D2678-1)

DALE SPURGEON (D2663-1)

Trapping Weevils and Saving Monarchs

KIM KAPLAN (D2664-1)

Above: A monarch butterfly. These beautiful insects feed on milkweed, a plant under attack from milkweed stem weevils.

Left: A boll weevil trap baited with an experimental formulation of pheromone. During tests to determine the pheromone's ability to lure boll weevils, ARS scientists found that it also attracted milkweed stem weevils.

Widely admired for its eye-catching wings and transcontinental migrations, the monarch butterfly, *Danaus plexippus*, depends on milkweed plants to survive. The monarch's eastern population migrates each year between the Midwest and the mountains of central Mexico. Concern about loss of milkweed habitat has prompted conservationists to recommend milkweed plantings in yards and gardens so that monarchs can keep making their long-distance trips.

Now, an unexpected finding by Charles Suh and colleagues at the Agricultural Research Service's Areawide Pest Management Research Unit in College Station, Texas, could help save milkweed habitat and preserve one of North America's most admired insects. They have found a formula for a lure that can trap a major milkweed pest.

The discovery stems from research originally designed to help the Texas Boll Weevil Eradication Foundation (TBWEF), which uses traps to monitor and detect boll weevil populations. Captures of weevils in traps are also used to help eradication-program managers decide whether to spray insecticides against boll weevils in particular fields.

But the traps haven't always been reliable for detecting incipient weevil populations. At some field locations, for example, no weevils were captured in traps, but substantial weevil infestations were later found in nearby fields.

In 2009, TBWEF asked Suh to investigate. Though the boll weevil pheromone has a specific ratio of four component chemicals, commercial lures are formulated with a different ratio of the four pheromone components because the lures are easier and less expensive to manufacture that way. Suh and ARS colleague John Westbrook asked the manufacturer to produce a lure that replicated the ratio of the natural components, and they compared that lure with the standard-blend lure in field trials in Mexico and South Texas.

In a field study, they set up 80 pairs of traps along county roads and highways in Atascosa and Frio counties in Texas, with each pair spaced at least 50 meters apart and traps within each pair spaced about 25 meters apart. They baited one trap in each pair with a lure containing the standard blend and baited the other with the experimental blend. Traps were checked once a week from mid-May to mid-June, and lures were replaced every other week.

In the first week, the researchers found that the traps were capturing a type of weevil distinctly different from the boll weevils they expected. The mysterious strangers were quickly identified as milkweed stem weevils, *Rhyssomatus lineaticollis*, a major pest of milkweed. They initially discounted the number of milkweed stem weevils being lured into the traps as irrelevant, but by the second week, it became obvious that more milkweed stem weevils were being captured than boll weevils and that the milkweed weevils were increasingly attracted to the experimental lure. The results showed that while the boll weevils were no more attracted to the experimental lures than to the standard lures, the milkweed weevils were more attracted. Overall, four times more milkweed weevils were captured in traps baited with experimental lures than in traps baited with standard lures.

The discovery, reported in *Southwestern Entomologist*, could be used to develop a trap-based system for detecting milkweed weevil and monitoring their dispersal and movements across landscapes, Suh says. Such a system could also help conserve a rare type of milkweed. The number of milkweed species attacked by the stem weevil includes Mead's milkweed *(Asclepias meadii)*, which is listed as a threatened species of plant (a risk level just below endangered) and is the focus of a federal recovery plan by the U.S. Fish and Wildlife Service.—By **Dennis O'Brien,** ARS.

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

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(K1681-15)

New Tool Predicts Piglet's Nursing Ability

A sow nursing her litter of piglets.

Unlike humans, when pigs are born, they enter the world without any immunity against foreign elements like disease-causing pathogens. Their chance for survival relies heavily on getting enough colostrom—a milk-like substance produced by mammals after giving birth.

Newborns that fail to nurse and receive colostrum from the sow within the f rst 24 hours usually die. That's because piglets are born with limited energy stores, and colostrum also provides the energy they need to stay alive.

For the swine industry, preweaning mortality has long been a major problem, costing an estimated $1.6 billion each year. Now, a new tool may help give these at-risk animals a second chance.

To improve neonatal piglet survival, Agricultural Research Service physiologists Jeffrey Vallet, Jeremy Miles, and Lea Rempel at the U.S. Meat Animal Research Center (USMARC) in Clay Center, Nebraska, have developed a measuring technique referred to as the "immunocrit" that can determine whether neonatal piglets have received adequate colostrum from the sow.

Colostrum contains immunoglobulins, which are antibodies made by the sow's immune system to protect against bacteria, viruses, and other foreign substances. Humans receive these antibodies in their mother's womb, but pigs and other livestock rely on passive transfer through nursing after birth, says Vallet, research leader of USMARC's Reproduction Unit. Thus, piglets are born with no immunoglobulin, and piglet serum immunoglobulin ref ects their colostrum intake.

"Colostrum gives piglets their f rst antibodies so that they can have some immunological protection during the f rst couple of days of life," Miles says. "If they don't suckle, they don't have any immunoglobulins."

The Immunocrit at Work

The immunocrit, which measures newborn piglet serum immunoglobulin, is simple, inexpensive, rapid, and accurate. It is similar to the hematocrit, used for years by doctors to measure the volume of blood cells and determine whether a patient is anemic, Vallet says.

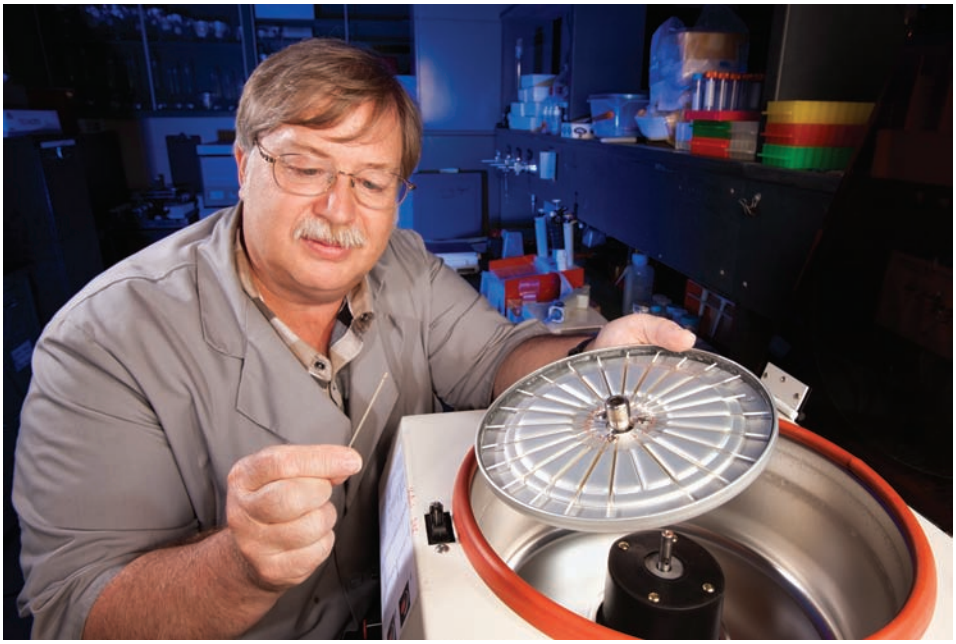
Blood samples are taken from piglets on day one after birth, mixed with ammonia sulfate to precipitate immunoglobulin, put into a microcapillary tube, and spun so the precipitated immunoglobulin settles to the bottom. The volume of the precipitated immunoglobulin is then measured and divided by the total volume in the tube.

PEGGY GREB (D2658-1)

Physiologist Jeffrey Vallet collects a blood sample from a 1-day-old piglet. The sample will be used to run a new immunoglobulin immunocrit technique that will tell whether the piglet received sufficient colostrum from its mother.

"We can go through a litter of piglets and take blood samples quickly and eas-

PEGGY GREB (D2660-1)



Technician Mike Judy loads immunoglobulin immunocrit tubes into a centrifuge. After centrifugation, the immunocrit measurement is easily made with a metric ruler.

ily, and the assay itself is very simple to use," Vallet says.

Scientists have demonstrated that immunocrit measurements are predictive of piglets' mortality and nursing ability and that the average immunocrit of piglets in a litter ref ects the sow's colostrum production capability. Because the test is so rapid, it is possible to identify compromised piglets and take steps to rescue them, Vallet says.

Help for the Smallest

The immunocrit is good at identifying piglets within a litter that haven't eaten at all or haven't had the opportunity to nurse, Miles says. In one experiment, scientists used the immunocrit to assess colostrum intake in a group of piglets—the smallest from each litter—and then measured the contents of each piglet's stomach. They found that some piglets' stomachs were nearly empty. Those same piglets had an immunocrit measurement of nearly zero, validating that the immunocrit accurately detects piglets that receive no colostrum within a 24-hour period.

Immunocrit results correlated well with results from a more complicated and expensive traditional method—protein A sepharose combined with electrophoresis—in detecting piglets that had not nursed at all.

In another study, using more than 2,000 piglets, researchers found that the immunocrit could predict preweaning survival. They also noted a connection between immunocrit measurements and piglet weight: Heavier piglets were more likely to survive the challenge of not getting colostrum within the critical time frame.

Enhancing Management Practices

The immunocrit can be used to test management practices, such as split suckling, and other strategies used by swine producers to help prevent colostrum def ciency, Vallet says.

Split suckling is a labor-intensive method that involves marking the f rst-born group of piglets, putting them aside, and then allowing the last piglets born uninhibited access to the sow. The practice is designed to improve access to colostrum for later-born piglets, because studies have shown that there is some inf uence of birth order on colostrum intake.

"The immunocrit can be performed 24 hours after the split suckling procedure to f nd out if progress is being made in improving colostrum in different piglets," Vallet says. "Producers can also use the immunocrit as a monitoring device for day-one piglet care. For example, they can randomly select piglets and benchmark how those piglets are doing."

The new technique isn't just for pigs. It could also f t well into management practices of cattle producers. The immunocrit was successfully used to monitor colostrum intake of 96 calves 24 hours after birth.

Taking a Genetic Approach

"Another strategy is to use genomics to modify the colostrum-piglet-mother interaction during that f rst 24-hour window," Vallet says. "We should be able to use the immunocrit to get some idea of the sow's ability to produce colostrum and then genetically select for colostrum production."

Preliminary research conducted by Gary Rohrer, a geneticist at USMARC, suggests that individual immunocrit values are heritable, presumably because nursing ability is heritable. From analyses of piglets and their mothers, Rohrer found the most signif cant portion of the variation—50 percent—is accounted for by the piglet's genetics. The mother is responsible for 20 percent of the variation.

Immunocrit data collected from 500 litters—about 5,000 piglets—provide a valid sample for genomic research, Vallet says. Data from each individual piglet is an indicator of its nursing ability, but the average across all piglets gives some indication of the sow's colostrum-production ability.

"When it comes to genetic associations, the more numbers, the better," Rohrer says. "Not only do we have a much higher heritability for the actual piglet's ability or potential, we also have a lot more records."

Rohrer plans to group DNA from piglets with very high immunocrit values and compare it with DNA collected from piglets with very low values.

"We can eff ciently genotype those pools of DNA, estimate frequencies, and hopefully identify regions of the genome that are affecting the pig's ability to acquire and absorb colostrum," he says.

If successful, researchers would be able to recommend genetic markers that allow pork producers to identify and breed sows that ably produce colostrum and piglets with improved neonatal nursing abilities—an outcome that would help reduce the odds of preweaning mortality.—By **Sandra Avant,** ARS.

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

Jeffrey Vallet is with the USDA-ARS U.S. Meat Animal Research Center, Spur 18D, Clay Center, NE 68933; (402) 762-4187, jeff.vallet@ars.usda.gov.

Beneficial Fungus Formulated Into

Af atoxins are highly toxic substances produced by several species of *Aspergillus* fungi. But not all *Aspergillus* produce af atoxins. Some, in fact, are considered benef cial. One such strain, K49, is now being recruited to battle its harmful *Aspergillus* relatives, preventing them from contaminating host crops, like corn, with the carcinogen.

In collaboration with Italy's University of Bologna (UB) microbiologist Cesare Accinelli, ARS plant pathologist Hamed Abbas and ARS soil microbiologist Bob Zablotowicz (now retired) devised a new method of formulating K49 as a f rst-line defense against af atoxin contamination of corn, which costs an estimated $200 million annually in U.S. losses alone. So potent is the af atoxin that U.S. law prohibits the sale of corn or any grain destined for human consumption if it contains more than 20 parts per billion.

K49 is known as nontoxigenic (or atoxigenic) because of its inability to produce af atoxin, explains Abbas, who is with ARS's Biological Control of Pests Research Unit in Stoneville, Mississippi. But the fungus, or mold, is quite adept at excluding its toxin-producing cousins (*A. f avus* and *A. parasiticus*) from ecological niches and resources that both need to survive. Exploiting this rivalry, called "biocompetitive exclusion," offers an effective way to diminish af atoxin levels in both soil and corn kernels, says Abbas, who coauthored a 2011 *Crop Protection* paper with Accinelli and his colleagues at UB's Department of Agro-Environmental Science and Technology in Bologna, Italy.

Wheat and barley grains are typically used as carriers to apply commercial strains of biocompetitive *Aspergillus* molds. But instead of using those grains, the ARS-UB team encapsulated K49 in a bioplastic product, called "Mater-Bi," made of cornstarch, vegetable oil, and other constituents.

These bullet-shaped granules in which K49 is encapsulated offer several advantages, says Abbas. Chief among these is improved storage life and viability of the mold, once applied. The formulation's starch component also provides nourishment to the mold. And because actual wheat and barley grains aren't used, seed-hungry animals like rats and birds aren't likely to eat the formulation, giving K49 a chance to release spores for dispersal to corn plants via wind or insect feeding. The bioplastic is also a readily available resource that

PEGGY GREB (D2671-1)

Plant pathologist Hamed Abbas applies the nontoxigenic *Aspergillus flavus* formulated in bioplastic granules to knee-high corn.

PEGGY GREB (D2673-1)

Following application, some of the nontoxigenic *A. flavus* bioplastic granules end up between the leaves of the developing corn plant. Most of the granules fall to the ground.

Bioplastic "Bullets"

PEGGY GREB (D2674-1)

Hamed Abbas and technician Caleb Chambers prepare bioplastic granules for field trials to control aflatoxin in corn.

biopesticide makers can use, and it safely degrades in the environment, Abbas notes.

During f eld tests in the Mississippi Delta from 2001 to 2004, inoculation of grains with K49 dramatically reduced af atoxin levels in corn (see "Protecting Corn From Af atoxin," *Agricultural Research*, September 2010, pp. 8-10). In the latest round of trials, conducted by Accinelli's group near Bologna from 2009 to 2010, bioplastic formulations of K49 netted similar af atoxin reductions (65 to 97 percent, depending on where applied).

"We f nd you get better results from applying K49 when corn is knee-high—or at what's called the '8-10 leaf stage'—than when it's at the tassling or silking stage," says Abbas.

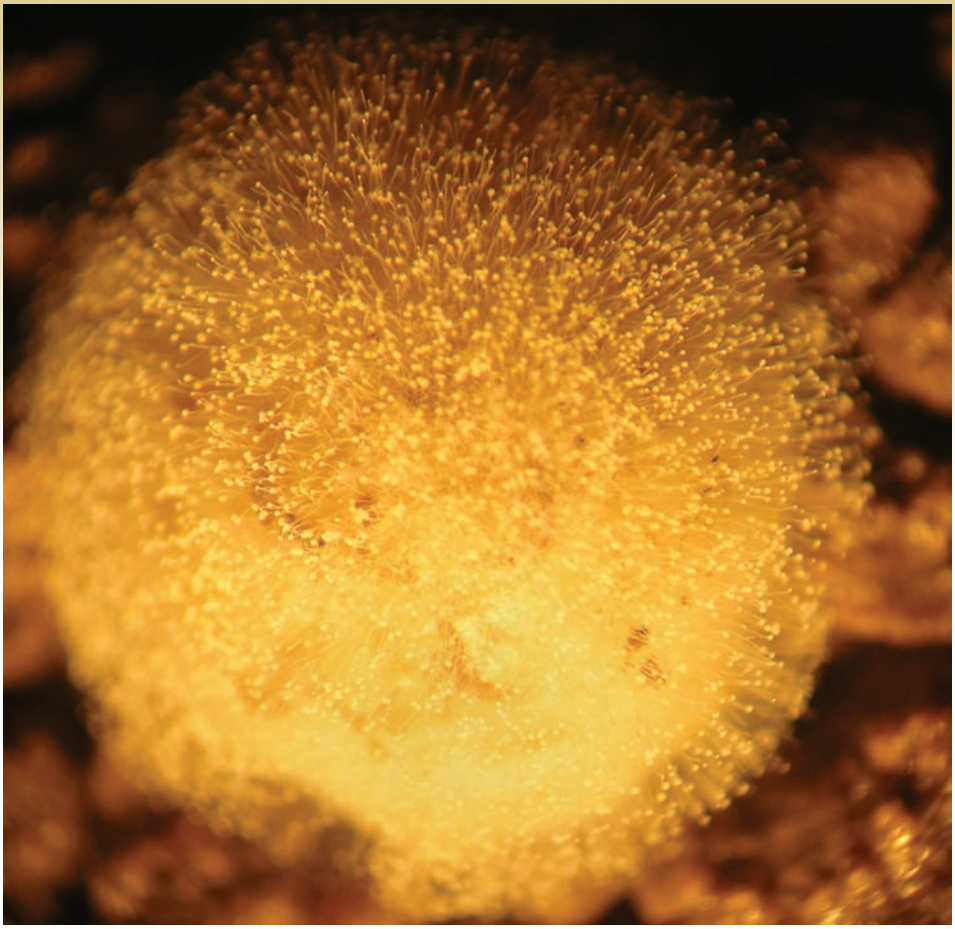
A patent has been f led on the bioplastic formulation, and the researchers hope that its issuance will encourage a biopesticide manufacturer to license the technology and develop it commercially.

Meanwhile, they're comparing different granule sizes to determine which works best with airplane-mounted sprayers, which may make applying K49 more practical for growers. They're also evaluating mixtures of K49 and certain chemical pesticides, as well as other biocompetitive strains, for possible synergistic effects that could improve performance or save on f eld application costs. In addition, the unique bioplastic matrix may also be effective in delivering other soil-applied fungi for controlling crop diseases. For example, the bioplastic has been shown to be an attractive method of incorporating benef cial *Trichoderma* fungi into potting mix to protect f owers such as impatiens from fungi that cause damping-off disease.

"There's really no one good way of controlling aflatoxins by traditional means—certainly not with chemical fungicides," says Abbas. But combined with resistant cultivars and other measures, a new biocompetitive strain could give corn farmers an added tool to use in fortifying crops against outbreaks that can lead to contamination and costly losses.—By **Jan Suszkiw,** ARS.

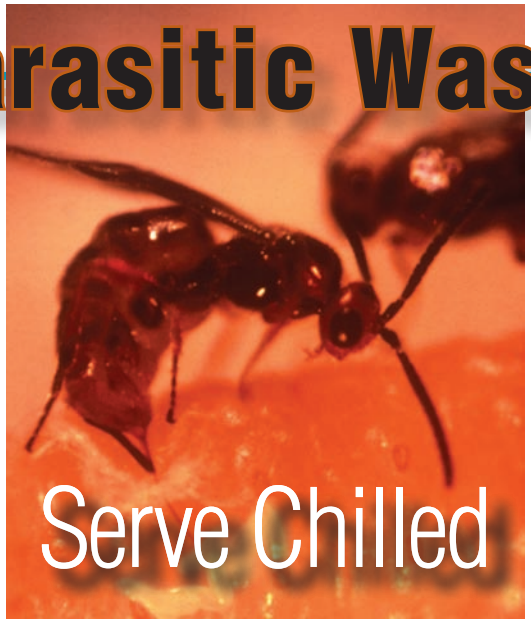
This research is part of Plant Diseases, an ARS national program (#303) described at www.nps.ars.usda.gov.

Hamed K. Abbas is in the USDA-ARS Biological Control of Pests Research Unit, National Biological Control Laboratory, 59 Lee Rd., P.O. Box 67, Stoneville, MS 38776; (662) 686-5313, hamed.abbas@ars.usda.gov.



After a bioplastic granule is applied to the soil, the nontoxigenic *A. flavus* fungus grows and sporulates as shown.

Parasitic Wasps Serve Chilled



JOHN BROWER (D2662-1)

Small parasitoid wasps could become important allies in the f ght to reduce multi-billion-dollar losses caused by stored-product pests, offering warehouse and retail managers a biological alternative to chemical controls, which can be costly and environmentally worrisome to use.

The top recruit is *Habrobracon hebetor,* a 2- to 3-millimeter-long, reddish-brown wasp that seeks out the caterpillar offspring of hosts like the Indianmeal moth and immobilizes them with a paralyzing sting. The wasp then deposits several eggs on her quarry. Within a few days, her maggotlike brood emerge to suck out their host's juices. Afterwards, they spin a cocoon and pupate before emerging as adult wasps, ready to start the cycle over.

Studies with packaged commodities, conducted by Agricultural Research Service scientists in the Stored Product Insect Research Unit (SPIRU) in Manhattan, Kansas, indicate that releasing *H. hebetor* wasps can reduce Indianmeal moth populations by 71 percent—and 97 percent if combined with the egg parasitoid *Trichogramma deion*. Sanitation and fumigation are among methods now used to control the pest, which causes harm directly, by feeding on grains and other edible stored goods, and indirectly, by leaving behind bits of cocoon, frass, and other debris.

"Indianmeal moths are very diff cult to control because they feed on almost any food material, but the parasitoids search them out very well," says SPIRU research leader James Throne. Their parasitism also cuts down on contaminants the pest leaves behind. And "once parasitoids get established, they provide a continual control program of their own doing," he adds.

For all its biocontrol promise, though, *H. hebetor* has yet to be used (in the United States, at least) as a frontline defense by warehouse or retail managers. In part, this is due to the diff culty commercial insectaries have had in mass-producing suff cient numbers of the wasp, whose eggs must be hatched and reared on living, captive hosts.

"There's interest by industry," Throne says, but no one company has taken the lead in commercializing the parasitoids.

Science, however, has pushed on.

Together with colleagues from Kansas State University-Manhattan and Huazhong Agricultural University (HAU) in Wuhan, China, Throne has focused attention on an insect resting state called "reproductive diapause."

In short, they found that they could artif cially induce the diapause state in *H. hebetor* wasps by "conditioning" them for prolonged refrigerated storage after rearing. In trials, the diapause-induced wasps generally survived cold storage for up to 2 months at 41˚F. But this depended on the set of conditioning temperatures and light-exposure periods used.

Throughout the experiments, the researchers compared the health, longevity, egg production, and other attributes of the diapause-induced wasps to a control group, which had not been conditioned. They found little signif cant difference between the two groups, aside from a lower percentage of female offspring from diapaused wasps than from non-diapaused ones (36 versus 52 percent).

Of the range of wasp-conditioning protocols the researchers evaluated, the optimal combination was 68˚F and a photoperiod exposure of 10 hours. "Cold storage provides f exibility and eff ciency in mass production and minimizes the costs of maintaining a colony during periods when releases are not required," reports the team, led by HAU graduate student Haoliang Chen, in a paper published in *Environmental Entomology.*

Small-scale tests in sheds simulating warehouse conditions are planned to evaluate the wasp's parasitism rates following release from cold storage.—By **Jan Suszkiw,** ARS.

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

James Throne is in the USDA-ARS Stored Product Insect Research Unit, 1515 College Ave., Manhattan, KS 66502; (785) 776-2796, james.throne@ars.usda.gov.

Above: Adult of the parasitoid wasp *Habrobracon hebetor* lays eggs in an Indianmeal moth larva, a major pest of processed cereal products in the United States. The larva will eventually be killed after the wasp's offspring feeds on it enough. Below, left: Larva of *Plodia interpunctella,* commonly known as the Indianmeal moth. Below, right: Adult Indianmeal moth.

SCOTT BAUER (K10313-1)

SCOTT BAUER (K10313-2)

Locations Featured in This Magazine Issue

Locations listed west to east.

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

Cropping Systems Research Laboratory, Lubbock, Texas
4 research units ■ 113 employees

Roman L. Hruska U.S. Meat Animal Research Center, Clay Center, Nebraska
6 research units ■ 113 employees

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

Southern Plains Agricultural Research Center, College Station, Texas
4 research units ■ 127 employees

Ames, Iowa
8 research units ■ 535 employees

St. Paul, Minnesota
3 research units ■ 76 employees

Madison, Wisconsin
5 research units ■ 129 employees

Jamie Whitten Delta States Research Center, Stoneville, Mississippi
7 research units ■ 323 employees

Houma, Louisiana
1 research unit ■ 48 employees

Columbus, Ohio
1 research unit ■ 15 employees

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

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

Subtropical Horticulture Research Station, Miami, Florida
1 research unit ■ 45 employees

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