



United States  
Department  
of Agriculture

Agricultural  
Research  
Service

# South Atlantic Area Fiscal Year 2009-2011 Research Highlights

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United States Department of Agriculture  
Agricultural Research Service



## Foreword

In the following pages you will find articles about the work of the Agricultural Research Service (ARS) in its South Atlantic Area. You may not be familiar with us, so let us take this opportunity to introduce you to ARS, the principal in-house research agency of the U.S. Department of Agriculture (USDA).

Congress first authorized federally supported agricultural research in the Organic Act of 1862, establishing what is now USDA. That statute directed the Commissioner of Agriculture “to acquire and preserve in his Department all information he can obtain by means of books and correspondence, and by practical and scientific experiments.”

The scope of USDA’s agricultural research programs extends far beyond Congress’s vision of 1862. Today, agricultural research has a direct impact on nearly all aspects of modern life. Our scientists not only study crops and livestock to improve quality and quantity but they also devise new ways of using those crops to add value to our lives, increase exports, improve human health, and protect the environment.

We in ARS, like all Americans, are very concerned about safe and nutritious food. We study the bugs that cause foodborne illness and find ways to reduce or eliminate unwanted pests from the food supply. If agricultural problems arise, such as a new disease of crops or livestock, we have the capacity to respond rapidly to find safe and appropriate solutions.

We care deeply about the environment. Extensive programs in the ARS South Atlantic Area focus on preserving and improving soil, air, and water quality. We are finding new and innovative ways to manage animal wastes, prevent soil erosion, and eliminate pesticides from surface water and groundwater.

These examples represent only a few of the ARS research programs dedicated to maintaining and enhancing the economic strength of U.S. agriculture, while improving the quality of life for every American. In this volume you will find some facts about the South Atlantic Area, a list of the research units, where they are located, and the people you can contact for further information. We are delighted that you are reviewing this volume and hope that you will find the articles useful and interesting. Please let us know if we can be of service to you.

**Deborah L. Brennan, Area Director**  
**S. Karl Narang, Associate Area Director**

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Cover: The broad scope of scientific efforts in the South Atlantic Area includes research on parasitic wasps, composting, wheat, weevils, peaches, caterpillars, pigs, citrus greening, soybean roots, Iris plants, and peanuts.

# *Nasonia* Wasp Genome Completed

## Advance important to parasitoid research

**T**he genome of *Nasonia*, a parasitoid wasp used for genetic research since the 1950s, has been completed. This is big news in the genetics research community both domestically and around the globe, since the wasp is emerging as a new model system in genetics.

Scientists at the U.S. Horticultural Research Laboratory (USHRL) in Fort Pierce, Florida, played key roles in the efforts to unravel the genome.

The *Nasonia* genus consists of three closely related wasps: *N. vitripennis*, *N. giraulti*, and *N. longicornis*. These small insects seek out flies—notably blow flies, flesh flies, and house flies—then sting and lay their eggs in the fly pupae.

*Nasonia* are easy to work with in the laboratory, produce large families, and have a short generation time, which is the average age at which a female produces offspring. Visible and molecular markers are available in *Nasonia*, as well as additional genetic resources, and hosts for the wasps are available commercially and are easy to rear.

The project's genesis was in 2003, when ARS held a genetics symposium hosted by national program leader Kevin Hackett. In that meeting it was identified that additional work on parasitoid genomics was needed. Through some fact checking, USHRL entomologist Wayne Hunter found that University of Rochester (New York) genetics professor Jack Werren had already begun *Nasonia* genomics work. Hunter helped to produce genetic libraries and sequence some 10,000 expressed sequence tags of the genome. Werren

used this data and that of the Institute for Genomic Research to complete the *Nasonia* genome, which is expected to be released this year (2009).

"*Nasonia* are excellent organisms for research and teaching," says Hunter. "They've been the subject of genetic, ecological, evolutionary, and developmental research for more than 40 years."

Parasitic wasps, such as *Nasonia*, are extremely important regulators of agricultural pests. Biological control programs using parasitoid wasps save about \$20 billion annually by preventing crop losses to invasive species in the United States. These wasps have been a major benefit to food production for humans by reducing the amount of food crops destroyed by pests and reducing the need for pesticides.

For all these reasons, *Nasonia* was the logical first choice for sequencing of a parasitoid genome. Information from the genome is being used to identify

important genes in parasitoid biology, and there is broad interest in using the *Nasonia* genome to identify and annotate genes involved in important biological processes—such as sense of smell, behavior, and even enzymatic pathways.

More information on the *Nasonia* Genome Project and the genome sequencing can be found at [www.rochester.edu/College/BIO/labs/WerrenLab/nasonia/genomeprojectindex.html](http://www.rochester.edu/College/BIO/labs/WerrenLab/nasonia/genomeprojectindex.html).—By **Alfredo Flores**, ARS.

*This research is part of Crop Protection and Quarantine, an ARS national program (#304) described on the World Wide Web at [www.nps.ars.usda.gov](http://www.nps.ars.usda.gov).*

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JACK WERREN (D1479-1)



Parasitic wasp, *Nasonia vitripennis*.

# A Better Way To Spot Eggshell Cracks

**N**obody wants to buy a carton of eggs only to find that some are cracked. So naturally cracks are a key factor in processing and grading table eggs. But some cracks, called “microcracks,” are so small that even an experienced human grader’s eye can miss them at the processing plant. Unfortunately, the microcracks grow over time and are often easily visible by the time they reach consumers at market.

Cracks are a food-safety concern because they can allow contamination of the egg by pathogens, such as *Salmonella*. Fortunately, safety procedures for eggs begin long before the consumer buys a carton.

The USDA’s Agricultural Marketing Service (AMS), which regulates eggs, needed a more objective method to detect microcracks—one that is simple and inexpensive and works in a batch system. They turned to Agricultural Research Service (ARS) scientists for a solution.

The ARS team included food technologist Deana Jones, at the Egg Safety and Quality Research Unit, and engineers Kurt Lawrence, Seung Chul Yoon, and Bosoon Park, image analyst Jerry Heitschmidt, and technician Allan Savage, at the Quality and Safety Assessment Research Unit. They designed and built a device that helps find those hard-to-see cracks. Both research units are part of the Richard B. Russell Research Center in Athens, Georgia. A patent application for the technology has been filed.

## The Old Way’s the Hard Way

Currently, many high-speed egg-processing plants use high-frequency analysis to “listen” for cracks, says Lawrence. Other plants still do it the old-fashioned way, with human graders visually inspecting eggs with a bright light source in a low-light environment.

It is impossible to detect all cracks, so a few are always missed; but the problem is that no method picks up the microcracks. The result is that some cartons—by the time they get to market—may have eggs with too many cracks. Another problem is that current methods may unnecessarily remove eggs that are thought to be cracked but really aren’t—what scientists call a “false positive.”

Advances in modern egg-grading machines have resulted in the processing of up to 180,000 table eggs per hour. Processing plants that operate under USDA grading certification practices are required to have a certain portion of their eggs graded by AMS human graders. In the largest plants, the limiting factor for processing speed is the grader—so there’s a need for a second person to handle the volume.

With this in mind, the ARS researchers developed a new technology that allows tiny cracks to be more obvious. It can also spot other blemishes on an egg that are assumed to be cracks but really aren’t. The technology uses a pressure chamber and an imaging system that makes even the tiniest fractures more apparent.

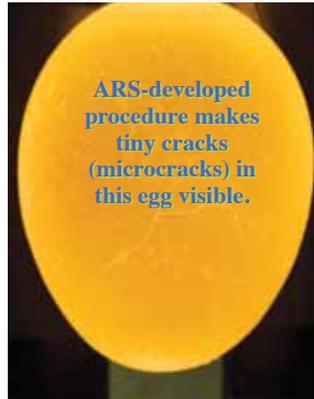
## Technology Emulates Human Graders

The idea is based on one of the basic methods human graders use to identify egg cracks: They gently tap two eggs together and listen for a dull sound. “This technique relies on the acoustic properties of the egg. In other words, a cracked egg makes a different sound than an intact one,” says Jones.

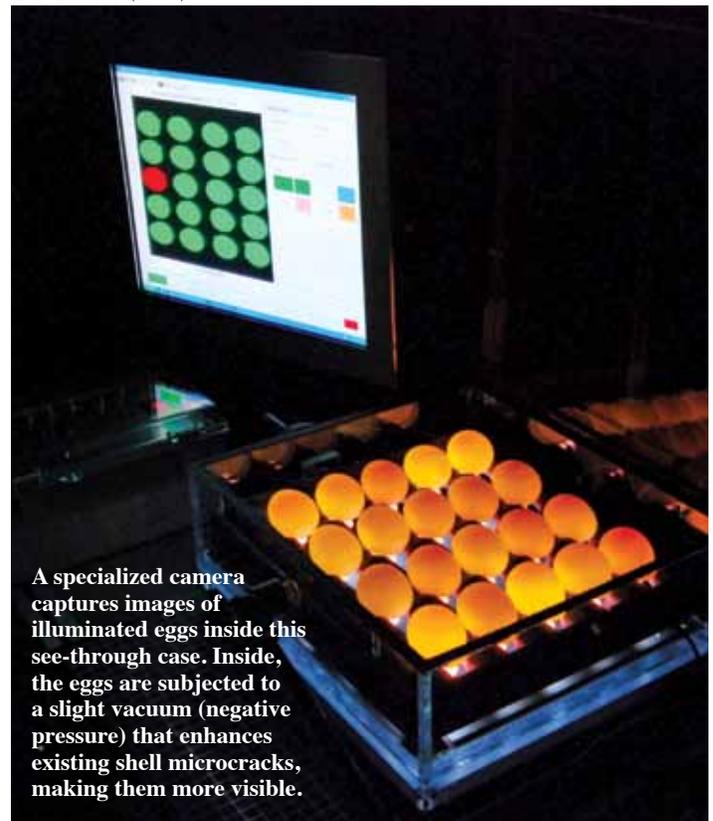
The second method is simply to visually inspect the egg for a crack. “If there’s a feature that looks like it might be a crack or if the grader hears the indicative sound of a cracked egg, they’ll press and/or squeeze the egg to help confirm the presence of a crack,” Jones explains.

Initial research with an imaging system tried to emulate the visual inspection. But too many noncrack shell features were perceived as cracks, leading to

STEPHEN AUSMUS (D1309-7)



JERRY HEITSCHMIDT (D1314-1)



**Food technologist Deana Jones (foreground) and hyperspectral imaging specialist Jerry Heitschmidt examine eggs for shell quality defects as biological science aide Vicky Broussard uses a pressure device to test eggs for cracks.**

STEPHEN AUSMUS (D1310-7)



numerous false positives. So the researchers needed a method to enhance cracks—similar to the way human graders squeeze the egg along the crack to see if it opens. “The question for us then became how to automatically press along a crack that can be located anywhere on an egg shell and in any orientation,” says Lawrence.

Further engineering research was in order. It was at that point the scientists realized that the wrong question was being asked—or rather, the answer didn’t fit the question. The detection system could not press or squeeze the egg like the graders did, but what would happen if they could pull on the egg with a slight negative pressure, or vacuum?

It was like a lightning bolt of inspiration—and that’s what a crack looks like to the camera when it opens under pressure. The first prototype chamber was built for a single egg, but the scientist quickly expanded it to a 20-egg chamber.

To test the chamber, 1,000 white-shell table eggs were obtained from a nearby egg-processing facility, transported to the laboratory, and brought to room temperature to simulate processing conditions. Many of the eggs were manipulated to cause microcracks and were immediately examined by AMS graders and scored as either intact or cracked. The eggs were then subjected to the negative pressure/imaging system and regraded.

The results were surprising. “The system detected 99.4 percent of the eggshell cracks while recording almost no false positives—0.3 percent—for an overall accuracy of 99.6 percent. In comparison, the professional human graders had 85.8 percent crack detection and 1.2 percent false positives, with an overall accuracy of 94.2 percent on these hard-to-see microcracked eggs,” Lawrence says. “The system’s results are much better than anyone had achieved earlier.

“This could very well provide a tool that egg graders can use to consistently identify cracked eggs and improve the quality of the eggs that reach the consumer.”—By **Sharon Durham, ARS.**

*This research is part of Food Safety, an ARS national program (#108) described on the World Wide Web at [www.nps.ars.usda.gov](http://www.nps.ars.usda.gov).*

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# Growing Premium Seafood—Inland!

**P**eople who know seafood know that cobia and Florida pompano are among the world's best. Found in the warm waters off the Atlantic and Gulf coasts, these saltwater superstars are prized for both commercial and sport fishing. Pompano (pronounced POM-puh-no) and cobia (COE-bee-uh) have firm, mostly white flesh that's perfect for grilling, pan-frying, or baking. Some people find that pompano has a pleasing, slightly sweet note.

Pompano is flat and silvery, and looks something like sunfish. Cobia is long and sleek, with a silver stripe on its dark-grey sides. Both of these scrumptious seafarers command premium prices—sometimes as much as \$30 a pound filleted.

Someday, pompano, cobia, and other marine finfish might be commonly raised inland, hundreds of miles from the nearest ocean or bay. They'd be reared in huge tanks of water—fresh or slightly salty—in what's known as a “recirculating aquaculture system” (RAS).

These systems, in which water is cleaned and used again and again, are referred to as “closed” because they're almost

into and out of tanks every year. What's more, RAS-based fish farms could provide an alternative to today's practice of raising marine fish in net cages or pens in the ocean. RAS production of seafood could diminish unwanted spread of nutrients from feed or fish wastes, reduce risk of diseases being transmitted from domesticated fish to their seagoing brethren or vice versa, and avoid unplanned breeding.

But before these low-salinity farms for seafood can become a reality, much more remains to be discovered about the needs of the saltwater fish that would be reared in them. In addition to figuring out the biological realities of raising these fish, there's a myriad of engineering details to be worked out. After all, the RAS technology was designed for freshwater—not saltwater—fish farms. For example, some of today's RAS filtering technology is about 30 percent less efficient in handling seawater than freshwater.

A team of ARS aquaculture researchers in the southeast Florida city of Fort Pierce are tackling all these issues. Fish

STEPHEN AUSMUS (D1341-10)



**Agricultural engineer Timothy Pfeiffer uses a hand-held meter to measure water quality in a moving bed biofilter.**

completely self-contained. They offer the potential to reduce discharge of everyday fish-farm effluent to as little as 3 percent—or less—of the total amount of water used each day. All the effluent could be treated to make it suitable to apply on land (if it's freshwater) or to artificial saltwater marshes (if it's low-salinity wastewater).

The fish waste and unused food collected in the system's filters could be used as nutrient-rich compost or perhaps for generating methane.

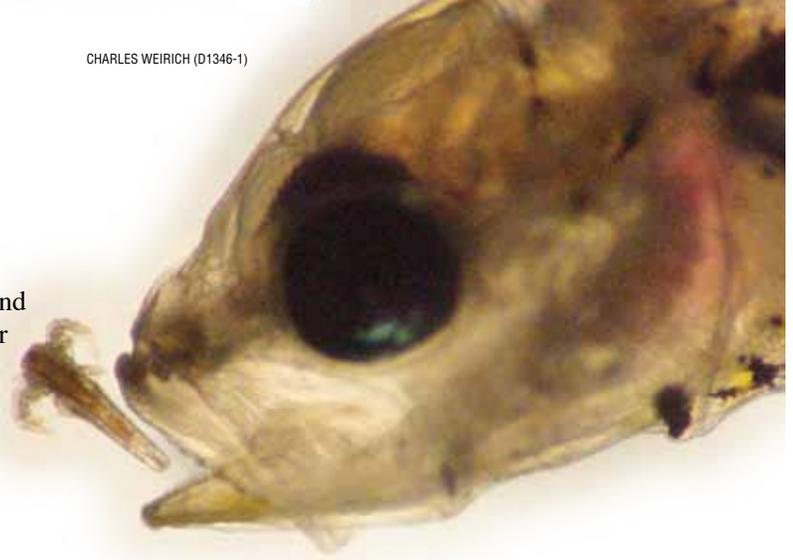
These water-sparing systems present an alternative to coastside flow-through operations that run millions of gallons of seawater

STEPHEN AUSMUS (D1228-11)



**Fish nutritionist Marty Riche feeds juvenile Florida pompano during studies to determine appropriate feeds and feeding-management practices for profitable inland production of saltwater fish.**

biologist Chuck Weirich, fish nutritionist Marty Riche, and agricultural engineer Tim Pfeiffer are based at the Harbor Branch Oceanographic Institute. Part of Florida Atlantic University, the institute is one of many organizations with which the team collaborates. The ARS research is managed through the Arkansas-based Harry K. Dupree Stuttgart National Aquaculture Research Center.



**Fishery biologist Charles Weirich uses image analysis to determine optimal sizes of live prey, such as this brine shrimp (left), for fish larvae to consume. (Magnified about 30x.)**

### Spawning Success

To make the systems a commercial and environmental success requires taking a close look at every aspect of the lives of the fish—from spawning by the parents, or broodstock, to the needs of the larvae, or seedstock, that emerge from the tiny eggs, to the requirements of the fingerlings, the juvenile fish that end up as delectable fillets or, in some cases, broodstock.

Every detail—from how to coax broodstock to spawn all year round to how much ammonia and nitrate buildup fingerlings can tolerate in their recirculating tanks—has to be tested. With this new knowledge, growers who invest in the RAS technology should have a better chance of making their new-age fish farms profitable.

In an experiment with 2,400 juvenile pompano, the scientists showed that it's indeed possible to raise this species in water that's only slightly salty. In this case, the water had a salinity of only 5 parts per thousand as compared to the 35 parts per thousand in most of the world's oceans.

The accomplishment is a remarkable scientific first. It provides important proof that the concept of raising saltwater species in nearly fresh water—an alien environment to which the animals are *not* adapted—can be done for short periods of time. In this case, the pompano that the researchers reared from juveniles to healthy, harvest-ready adults lived in the low-salinity water of the “grow-out tanks,” as they are called, for only 110 days. What remains is to make the system practical, profitable, and energy efficient for not just the grow-out phase but for the broodstock and hatchery phases, as well.

### Food, Water, and Room To Grow

What to feed the saltwater fish during the various stages of their captive lives is, of course, one of the most pressing questions. In a study with 50,000 pompano larvae, fish biologist Weirich and colleagues learned more about the feeding habits of these baby fish. For the study, the researchers used digital photography and image-analysis software to observe and record the larvae, which, in their earliest days, are transparent and look something like tadpoles.

The scientists determined the optimum size classes of two kinds of live prey—brine shrimp and tiny organisms called “rotifers.” At various stages of their growth, the little fish are capable of catching and eating these snacks as they swim by. During this growth phase, the upper and lower jaws of the larvae,

and their miniature digestive systems, are still developing. The fish are born with their mouths closed, and the size of the opening, or “gape,” that develops determines the size of the food—in their case, rotifers and brine shrimp—that they're able to nab and swallow.

In new studies, Weirich and colleague Riche are determining how to wean the larvae off of these live feeds more quickly. That's because cultivating live feeds for the infant fish is more costly and labor-intensive than simply giving them dry feeds. Those feeds won't appear on their menu until the fish have made the transition from larvae to juvenile.

Moving juveniles from the nursery tanks to the world of the grow-out tanks—where they'll develop to market-ready weights—poses the question of how many of these young fish can be put into a tank without overcrowding them.

Overstocking can cause stress and may increase risk of disease, reduce growth rates, and create other problems that can bite into profits. Weirich, Harbor Branch Oceanographic Institute colleague Paul Wills, and Riche found that pompano stocked at the rate of 200 fish per tank weighed about 1.5 pounds at harvest, making them about 10 percent heavier than pompano stocked at a rate of 400 per tank.

But a cobia study by Weirich and Wills showed no significant difference in their survival, or weight at harvest, regardless of whether the fish were stocked at a low (35 fish per tank), medium (70 fish), or high (106 fish) density. At the end of the study, all the cobia weighed about the same. Each yielded two generously sized fillets.

No matter what the stocking rate, finfish won't flourish in recirculating tanks if there are life-threatening problems with the quality of the water. To keep the recirculating water clean and to limit risk of disease, the researchers are determining the most cost-effective use of what are known as “biofilters.” These devices contain bacteria that convert the toxic ammonia in fish waste into less-toxic forms (nitrite and nitrate). In other work, they're evaluating alternative methods to efficiently remove solids—such as waste and uneaten feed—from the system.

STEPHEN AUSMUS (D1226-34)



Marty Riche and technician Patrick Tracy harvest market-size Florida pompano from grow-out tanks at the Harbor Branch Oceanographic Institute (HBOI) to determine growth rates during feed nutrition studies.

STEPHEN AUSMUS (D1230-5)



Using tissue and enzyme assays and growth data, Marty Riche develops feeds for marine fish grown in low-salinity water.

STEPHEN AUSMUS (D1227-2)



Assisted by HBOI technician Terri Breeden (left) and ARS technician Todd Lenger (center), ARS fish biologist Charles Weirich measures and weighs cobia grown in recirculating aquaculture systems.

Pfeiffer is leading studies of what is known as “airlift technology” to power the system’s water-treatment components. Airlift systems can cut energy costs because they use less electricity than conventional centrifugal pumps for moving water.

There’s an additional twist to the engineering and design research: The water-quality requirements of each species may differ, and what works to keep one species healthy and fast growing might not succeed with another. That means the components, or combinations of components, may have to be customized.

Much remains to be learned about the fish and the recirculating systems. And even when the technology is ready to use, no one can predict how long it will then take before inland farming of seafood—in these recirculating tanks—becomes as common as on-land farming of rainbow trout or catfish. For people living far from the sea, who’d like to be able to buy fresh, locally raised seafood at their neighborhood supermarket, environmentally responsible inland mariculture simply can’t happen soon enough.—By **Marcia Wood, ARS.**

*This research is part of Aquaculture, an ARS national program (#106) described on the World Wide Web at [www.nps.ars.usda.gov](http://www.nps.ars.usda.gov).*

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STEPHEN AUSMUS (D1229-5)



**Right:** Charles Weirich (left), HBOI biological scientist Fernando Cavalin (center), and HBOI technician Meghan Anderson study a sample of hundreds of pompano larvae.

# Luring Varroa Mites to Their Doom

The *Varroa* mite, *Varroa destructor*, is only about one-sixteenth of an inch long. But that hasn't stopped the eight-legged, blood-sucking parasite from becoming the single worst pest of honey bees since first being detected in Florida in the 1980s.

Any threat to honey bees is a threat to American agriculture. Without them, the yield and quality of many flowering crops would suffer—almonds, apples, blueberries, cantaloupe, cranberries, and zucchini, to name just a few. Indeed, as the chief pollinator of these crops, the honey bee's contributions are considered a \$14 billion asset to our economy—and that's not even counting the honey and beeswax the insect produces.

So it is with quite a bit of urgency that researchers nationwide are seeking new ways to control *Varroa*, particularly methods that will diminish reliance on the chemical controls—fluvalinate and coumaphos—now used. At the ARS Chemistry Research Unit in Gainesville, Florida, research leader Peter E.A. Teal is testing a bait-and-kill approach using sticky boards dosed with natural chemical attractants, called “semiochemicals.”

For patenting reasons, Teal won't reveal what the specific compounds are, other than to say they're naturally produced by honey bees and highly attractive to *Varroa* mites.

In nature, *Varroa* mites rely on the semiochemicals to locate—and then feed on the bloodlike hemolymph of—both adult bees and their brood, weakening or killing them. Severe infestations can decimate an affected hive within several months—and rob the beekeeper of profits from honey or pollinating services. But in this case, the mites encounter a more heady bouquet of honey bee odors that lure them away from their intended hosts and onto the sticky boards, where they starve.

Preliminary tests of the attractant have been promising. “For example, we are able to induce 35 to 50 percent of mites to drop off of bees when we present them with either of the two attractants, and more than 60 percent of free mites are attracted to these chemicals in biological tests,” Teal reports. Moreover, it doesn't appear that the extra dose of semiochemicals wafting through the hive interferes with the honey bees' normal behavior or activity to any significant degree, adds Teal, who, along with postdoctoral associate Adrian Duehl and University of Florida collaborator Mark J. Carroll, reported the results this past January at the 2009 North American Beekeeping Conference in Reno, Nevada.

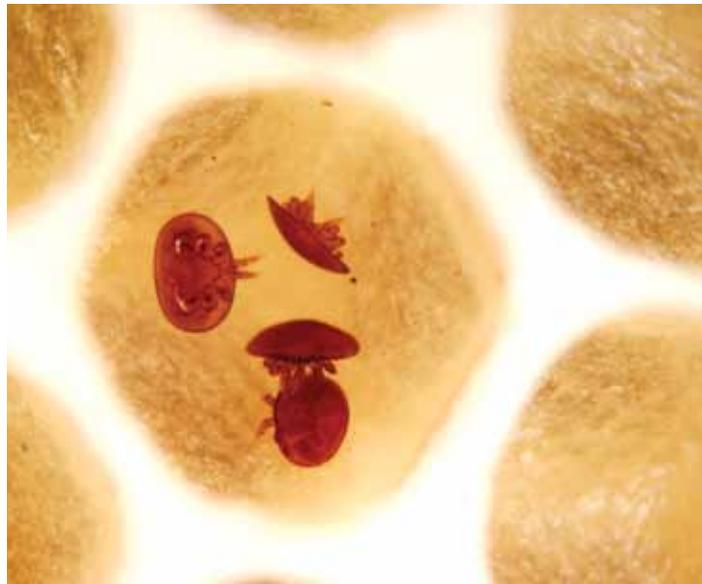
The researchers hope ARS's patenting of the *Varroa* attractants will encourage an industrial partner to develop the technology

further for use by beekeepers as both a monitoring tool and an alternative to chemical controls.—By **Jan Suszkiw**, ARS.

*This research is part of Crop Protection and Quarantine, an ARS national program (#304) described on the World Wide Web at [www.nps.ars.usda.gov](http://www.nps.ars.usda.gov).*

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ADRIAN DUEHL (D1506-1)



**Varroa mites in a cell of a honey bee comb that has been treated with attractants identified by chemist Peter Teal and collaborators. The other cells (with no mites) are control cells (no attractants).**

## Controlling Fire Ants Takes a Group Effort



In studies to determine whether fire ants dominate other ants in South America, worker ants of *Crematogaster* and *Pheidole*, two competitors of the red imported fire ant, try to dominate a tuna bait.

DAVID OI (D1491-1)



Spores of the microsporidian pathogen *Kneallhazia solenopsae*, a potential biocontrol of fire ants.

**R**ed imported fire ants (RIFA), native to South America, accidentally landed in the United States in Mobile, Alabama, during the 1930s and advanced across the southern region. For more than 20 years, two ARS labs—one in Argentina and the other in Florida—have been collaborating in studies of RIFA biology and biocontrol in hopes of controlling the pest's numbers in the United States.

The hunt for biological control of imported fire ants began in the 1970s in Brazil. The program at ARS's South American Biological Control Laboratory (SABCL) in Hurlingham, Argentina, started in 1987. Staff there have since conducted well over 340 field trips in Argentina, Bolivia, Brazil, Chile, Paraguay, and Uruguay for exploration and monitoring of fire ants and their natural enemies. During this time, 350,000 kilometers were covered, 15,000 fire ant colonies were collected or examined in the field, and some 150 fire ant colonies were hand carried to ARS's Center for Medical, Agricultural, and Veterinary Entomology's (CMAVE) quarantine facility in Gainesville, Florida.

### Fire Ants Dominate Other Ants in South America

Scientists at SABCL have studied interactions between RIFA (*Solenopsis invicta*) and other aboveground foraging ants in two habitats in northeastern Argentina. A combination of pitfall traps and baits were used to study day-to-day activity in ant communities to see how the ants interact with one another. Each pitfall trap consisted of a 50-milliliter plastic tube buried in the ground and half-filled with soapy water, giving the scientists a measure of the diversity and abundance of the ant species at the site. The bait consisted of 1 gram of commercial canned tuna placed on a 5-centimeter-diameter plastic card near the trap. Observations of ants competing for the bait revealed the dominance abilities of each ant species at that site.

Some 28 ant species coexisted with *S. invicta* in an open area of forest along a watercourse, whereas only 10 species coexisted with *S. invicta* in dry forest grassland.

"We found the highest numbers of fire ants in the area near the water," says Luis Calcaterra, a biologist at SABCL who works closely with the lab's director Juan Briano. "But we were surprised to see it perform better as a discoverer and a dominator in the dry habitat, despite the lack of resources there."

A more recent, larger scale study of more than 100 ant species in natural and modified environments in northern Argentina revealed that RIFA is the most numerically dominant species in other habitat types as well, says Calcaterra.

Before these studies, it was thought that the fire ant was not dominant in its native land. But it proved to be the most numerically and behaviorally dominant, winning 78 percent of interactions with other ants, mainly against its most fierce competitor, *Pheidole obscurithorax*, an ant of northern Argentina and Paraguay. In a battle with the Argentine ant, *Linepithema humile*, the fire ant won 80 percent of the time.

"A quick mass recruitment was the main reason the fire ants were able to dominate the baits," says Calcaterra. "They organize well."

Though the fire ants were ecologically dominant, the study revealed that other species were able to coexist. "It was possible because of the relatively poor ability of *S. invicta* to discover the

food resources, but it is also due to the higher ant diversity and competitive pressure in its native range compared to its area of introduction,” explains Calcaterra. The presence of phorid flies affected the foraging rates of fire ants but had little effect on fire ant interactions with other ants.

**Biological Control of Red Imported Fire Ants**

The technology transfer between CMAVE and SABCL has been mutually beneficial. Overall, 36 shipments were made to CMAVE from SABCL, and they included 397 fire ant colonies (204 infected with diseases, 136 parasitized with phorid flies or parasitic ants, and 57 healthy). The lab continues to look at the biocontrol potential of key candidates—the microsporidia *Kneallhazia solenopsae* and *Vairimorpha invictae*, phorid flies in the genus *Pseudacteon*, the parasitic ant *Solenopsis daguerrei*, wasps in the genus *Orasema*, and the nematode *Allomermis solenopsii*.

“Pathogens like *K. solenopsae* and *V. invictae*—alone or in combination—are associated with localized declines of 53-100 percent in fire ant populations in Argentina,” says CMAVE entomologist David Oi.

And phorid flies, which parasitize and kill fire ants, are now in use in the United States to reduce RIFA foraging and help control some populations.

Now, CMAVE and SABCL scientists are looking to add a new scientific wrinkle—using the phorid fly as a vector for infecting the fire ant population with the microsporidia.

Though preliminary data showed that *V. invictae* could not be successfully transmitted to phorid flies effectively, *K. solenopsae* could be. Oi, CMAVE entomologists Sanford Porter and Steven Valles, and SABCL’s Briano and Calcaterra were able to infect phorid flies with *K. solenopsae* without harming the flies. The next step is to determine whether infected flies are able to infect RIFA with the microsporidia—providing another mechanism for transmission between RIFA colonies.

“*K. solenopsae* not only reduces fire ant colony size, it also reduces the amount of sexual brood—or reproducing ants, affects queen survival, and increases the death rate of colonies,” says Oi.

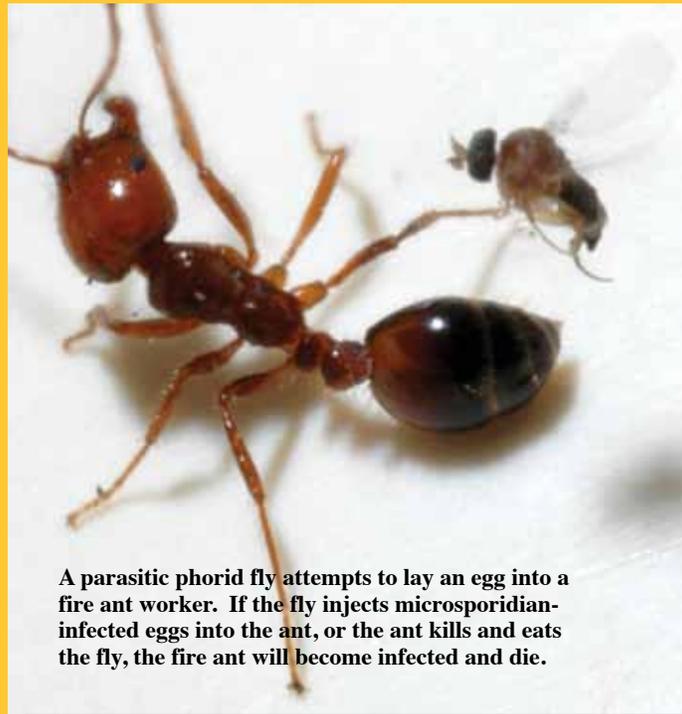
Other cooperative research between CMAVE and SABCL on biological control of fire ants evaluates ecological interactions of fire ants and phorid flies in different habitats in Argentina. Genetic studies also are being performed at CMAVE with material collected in South America to characterize the population structure of the parasitic ant *S. daguerrei* and to determine the source population of the fire ants present in the United States and other countries.

“Our work continues in order to develop novel technologies and strategies to control red fire ant populations and mitigate their damage,” says Oi.—By **Sharon Durham** and **Alfredo Flores**, ARS.

*This research is part of Crop Protection and Quarantine (#304) and Veterinary, Medical, and Urban Entomology (#104), two ARS national programs described on the World Wide Web at [www.nps.ars.usda.gov](http://www.nps.ars.usda.gov).*

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SANFORD PORTER (D1505-1)



**A parasitic phorid fly attempts to lay an egg into a fire ant worker. If the fly injects microsporidian-infected eggs into the ant, or the ant kills and eats the fly, the fire ant will become infected and die.**

SANFORD PORTER (D1505-2)



**A newly hatched phorid fly emerges from the head of a red imported fire ant that has been parasitized and killed.**



ARS scientists are evaluating U.S. wheat lines for rust resistance in hopes of giving U.S. wheat and barley breeders a head start towards protecting new varieties from Ug99, a deadly wheat stem rust in eastern Africa.

# Collectively Facing an Ugly Rust

**W**hen the threat to a food staple like wheat is worldwide, the best way to counter it is to enlist the world's experts in a research coalition. That's just what has been done to answer the very real threat of Ug99, a new stem rust to which most of the wheat and barley grown in the United States and the rest of the world has no resistance.

As part of that global response, the Agricultural Research Service became a founding partner in the Borlaug Global Rust Initiative (BGRI), in alliance with the International Maize and Wheat Improvement Center (CIMMYT), the International Center for Agricultural Research in the Dry Areas (ICARDA), the Food

In 1999, a new strain of cereal stem rust was first reported in Uganda, hence the name "Ug99." It was found in Kenya in 2001 and in Ethiopia in 2003. Now it has spread to Yemen, Sudan, and Iran.

Stem rust is favored by hot days and mild nights with adequate moisture for nighttime dews, and it is spread by the wind—conditions that can occur anywhere wheat grows. Historically, stem rust has been the most destructive disease of wheat and barley. ARS has found that nearly all spring wheat varieties grown in the United States are susceptible to Ug99, as are more than 75 percent of U.S. winter wheat and most of the commercial barley varieties.

YUE JIN (K11192-1)



Close-up of stem rust on wheat.

and Agriculture Organization of the United Nations, and Cornell University. The BGRI is chaired by Nobel Peace Prize winner Norman E. Borlaug.

"To reduce the vulnerability of U.S. and global wheat crops to Ug99 requires an international partnership of scientists and institutions with diverse expertise and facilities. We are fortunate that ARS was one of the first institutions to respond to the call from Dr. Borlaug," says Ravi Singh, BGRI's chief wheat scientist.

"ARS has several of the world's small pool of experts in cereal rust research," explains Kay Simmons, ARS national program leader for plant genetics and grain crops. "We have several collaborative research projects under way that are feeding critical information into BGRI, and these partnerships are the best way to leverage everybody's resources to combat Ug99."

ARS is partnering with CIMMYT and the Kenya Agricultural Research Institute (KARI), to screen wheat and barley lines from breeders from all over the United States for Ug99 resistance.

Kenya has had Ug99 since 2001, so having use of the nursery there provides a way for U.S. breeders to find out which of their new varieties and lines may be able to stand up to the rust without

bringing the pathogen into the United States and without each wheat- or barley-breeding project trying to start its own nursery overseas.

More than 5,000 U.S. lines have been evaluated through this program so far.

“Everybody wins. Not only does the United States benefit from this nursery, but ARS is also sharing all the information from these screenings with the other members of BGRI, and they are sharing their results with us,” Simmons says.

In fact, researchers from other BRGI members—such as Agriculture and Agri-Food Canada, KARI, and CIMMYT—help score the wheats at various points in the growing season alongside ARS scientists such as plant pathologist/geneticist David Marshall, research leader of the ARS Plant Science Research Unit in Raleigh, North Carolina.

“Results from the 2005-2007 screenings showed that Ug99 has overcome even more major resistance genes than previously believed,” Marshall says. “This only emphasizes how important it is to find new ways for wheat to deal with Ug99.”

ARS has also used the test nursery to screen a significant portion of the small-grains germplasm collection in search of new sources of resistance.

The information from this screening has helped jump-start a cooperative stem rust-resistance breeding program at Oklahoma State University. ARS geneticist Michael Pumphrey at the Plant Science and Entomology Research Unit, Manhattan, Kansas, transferred new resistance genes into germplasm that university professor Brett Carver is now incorporating into locally adapted breeding populations.

“What ARS accomplished in 2 years would have taken us about 5 years, assuming we would have enjoyed the same success in crossing with some of the more cantankerous wild wheat relatives used in this project,” Carver says. “This partnership allows me to remain focused on the locally specific breeding objectives that already have my attention.”

### Opening a New Window

Recently, the Bill & Melinda Gates Foundation provided a \$26.8 million grant to Cornell University to create the Durable Rust Resistance in Wheat (DRRW) project, which will help bolster Ug99 research. With this grant, Cornell University has brought together 15 partnering institutions from all over the world, including ARS, with the goal of systematically reducing wheat’s vulnerability to rust diseases through an international collaboration of unprecedented scale and scope, according to Ronnie Coffman, DRRW’s principal investigator.

ARS is most heavily involved in three of the project’s objectives: tracking wheat rust pathogens, exploring whether rice offers any immunity to rust that might be

transferred to wheat, and discovering new sources of rust resistance in wild wheat and wild barley.

Because most of current Ug99-effective genes are derived from relatives of common and durum wheat, ARS is looking to wild relatives as a source of genes for new types of resistance. Preliminary studies led by plant pathologist Yue Jin at the ARS Cereal Disease Laboratory have found some resistance to Ug99 in einkorn, goatgrass, and sanduri wheat.

ARS will be joining with ICARDA and the University of Minnesota to screen other wild relatives of wheat and barley.

“Wheat stem rust is borne by the wind across every political boundary where wheat is grown. To monitor it and defeat it, international partnerships have always been essential,” Coffman says. “Since the beginning of rust research, ARS scientists have been good leaders and good partners, working at the forefront of science on behalf of the world community. Without their essential participation, the DRRW project would not be possible.”

More information on DRRW can be found at [www.wheatrust.cornell.edu](http://www.wheatrust.cornell.edu).—By **J. Kim Kaplan**.

*This research is part of Plant Diseases, an ARS national program (#303) described on the World Wide Web at [www.nps.ars.usda.gov](http://www.nps.ars.usda.gov).*

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KAY SIMMONS (D516-1)



**Nobel Peace Prize winner Norman Borlaug (second from left) believes Ug99 is the most serious threat to wheat and barley in 50 years. He is shown here consulting with Kenyan and CIMMYT leaders near wheat plots in Kenya.**

# TRACKING *Salmonella's* EVOLUTION FROM INNOCUOUS TO VIRULENT

STEPHEN AUSMUS (D1335-16)



To better understand how bacteria evolved the ability to get into eggs, Jean Guard-Bouldin and technician Cesar Morales analyze how many colony types are present in the organs of chickens infected with *Salmonella*.

In forensic science, DNA is often the last word. But an ARS scientist has found that even having one full DNA sequence may not be the definitive answer in the case of *Salmonella* microbes.

A team of researchers led by veterinary medical officer Jean Guard-Bouldin at ARS's Egg Safety and Quality Research Unit in Athens, Georgia, has found *S. enteritidis* strains to be so similar genetically that they appear identical—yet they may behave differently inside the hen.

To distinguish between the apparently identical genomes, researchers must use a technique called “whole-genome mutational mapping” to analyze multiple strains. It’s a way of comparing the genomes to find small differences in DNA—called “polymorphisms”—among them.

In food safety and public health, *S. enteritidis* is problematic.

It is the only *Salmonella* pathogen that is found inside the egg, is present in healthy hens, and is able to cause illness in humans.

“To reduce current levels of infection, we’re studying how *S. enteritidis* evolves and infects hens on the farm,” says Guard-Bouldin. “Using mutational changes in the *Salmonella* genome as a sort of ‘breadcrumb trail,’ we’ve tried to determine the first time this bacterium became capable of getting inside the egg from hen reproductive organs.”

## Mapping Mutations

The mutational maps for *S. enteritidis* were obtained by using a database from the Sanger Institute to generate a set of primers that “tile” the DNA of the test strains. Tiling keeps fluorescent-labeled DNA from producing light as a signal. If a signal is produced, that means that a mismatch is present in the test strain. All potential mismatches, or polymorphisms, are sequenced multiple times in multiple strains to identify the exact location and nature of the change. To date, 447 polymorphisms have been detected, and about half of these have been confirmed by sequencing.

## Down the Genome Path

“*S. enteritidis*, like a lot of other bacteria, is able to reproduce very quickly—in optimal conditions, every 20 minutes,” says Guard-Bouldin. “Such a fast pace allows the organism to accumulate polymorphisms. Only healthy competitors go on to reproduce, survive, and then thrive in the infection pathway to the egg.”

Once her mutational maps were complete, Guard-Bouldin could apply a mathematical approach that compares the number of polymorphisms seen today between strains that have been recently isolated to come up with a rate of evolution. This has led to a theory that a large-scale swap of DNA about 36 years ago occurred between strains in association with the emergence of egg contamination. Evolution beginning hundreds of years ago, however, probably laid the footing for today’s problem.

While the hybrid strain that recently emerged had the ability to contaminate the internal contents of eggs, it also had a problem: It was carrying incompatible viruses within its genome.

As a result, says Guard-Bouldin, “The hybrid strain split very quickly into two lineages, each carrying one virus. Except for the different viruses, the two strains had identical genomes, and both contributed to the beginning of the pandemic. Both of the newly split lineages continued to evolve by accumulating small changes in their genomes. They eventually began to vary in their ability to contaminate eggs, to survive on the farm, and to challenge our ability to understand their association with chickens.”

### Searching for Small Changes

Paula Cray, research leader of ARS's Bacterial Epidemiology and Antimicrobial Resistance Research Unit, also in Athens, was able to detect a few of these changes using a more conventional method—pulse field gel electrophoresis (PFGE). This method provided a baseline for estimating the frequency at which DNA changes were being missed. Whereas mutational mapping found 447 polymorphisms, 12 of which disrupted genes, PFGE found only 2 differences between the *Salmonella* strains that varied in the ability to contaminate eggs.

“In fact, in a comparison of PFGE patterns, every band difference could account for about 200 polymorphisms going undetected,” says Guard-Bouldin. Cray's research is a good example of why food safety agencies are continuously testing which methods are best for detecting evolutionary trends in bacteria that threaten public health.

### The New Horizon

The data from this research is being entered into a publicly available database by the National Center for Biotechnology Information, part of the National Institutes of Health.

STEPHEN AUSMUS (D1337-11)



An important intermediate step in the analysis of evolution is to determine which methods are effective for detecting differences between strains. Using a Bio Rad imager and pulse field gel electrophoresis, veterinary medical officer Jean Guard-Bouldin (left) and technicians Carolina Hall (center) and Cheryl Pearson-Gresham classify strains that are being used to produce mutational maps.

STEPHEN AUSMUS (D1336-6)



A typical egg contamination experiment often involves as many as 1,000 eggs. Here, Jean Guard-Bouldin removes the contents from eggs, which are then added to liquid media and cultured for the presence of *Salmonella*.

“This information about differences between genomes could help streamline the process of finding out how human disease organisms evolve to become more virulent,” says Guard-Bouldin. “The main focus for us now is to continue sequencing entire genomes and searching for more genetic changes that help us understand the *Salmonella* organism.

“Up until recently, genomic techniques for delving this deeply into the genetic code of multiple *Salmonella* strains weren't available or cost effective.

“If we can understand how *Salmonella* evolved to become pathogenic, perhaps we can apply the same principles to other foodborne pathogens and begin to study foodborne illness the way influenza is being monitored—with equal emphasis on the importance of small, as well as large, genetic changes.”

According to the Centers for Disease Control and Prevention, about 40,000 cases of salmonellosis are reported in the United States every year. Most result in diarrhea, fever, and abdominal cramps lasting 4 to 7 days, but severe cases caused about 70 deaths in 2000. Adequate cooking eliminates the risk of infection from eggs.—By **Sharon Durham, ARS.**

*This research is part of Food Safety, an ARS national program (#108) described on the World Wide Web at [www.nps.ars.usda.gov](http://www.nps.ars.usda.gov).*

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## Tiny Moth Tackles Old World Climbing Fern

ANTHONY BOUGHTON (D1349-1)



A research plot in Jonathan Dickinson State Park, with defoliated and browned clumps of Old World climbing fern, *Lygodium microphyllum*. The damage was caused by biocontrol caterpillars of the moth *Neomusotima conspurcatalis*, or Neo. Undamaged native ferns are shown in the foreground.

For more than 12 years, ARS scientists and cooperators have been on a quest to control Old World climbing fern, one of the most invasive plants in all of Florida and a large threat to the prized Florida Everglades.

The fern, *Lygodium microphyllum*, can reach lengths of over 90 feet. It forms thick blankets of light-green vegetation that can completely cover other plants, blocking sunlight crucial to their survival. *Lygodium* also allows wildfires that would normally only scar cypress tree trunks to race up the tree and set the canopy afire.

If left uncontrolled, this aggressive invader could soon cover one-third of south Florida and cost \$8 million annually in control expenses.

ARS entomologists Bob Pemberton and Anthony Boughton, with the Invasive Plant Research Laboratory in Fort Lauderdale, Florida, have been working closely with scientists from the Brisbane-based ARS Australian Biological Control Laboratory—a cooperative venture with Australia’s Commonwealth Scientific and Industrial Research Organization, or CSIRO—to find natural enemies that will stop climbing fern’s spread. Additional cooperators include the South Florida Water Management District, the Florida Fish and Wildlife Conservation Commission, and the Florida Department of Environmental Protection’s Division of Recreation and Parks.

Now, they may have found a solution.

### Small Insect, Big Job

A little moth known as *Neomusotima conspurcatalis*—nicknamed “Neo”—is currently the most successful of all the biocontrol agents that have been tested by the Fort Lauderdale and Brisbane scientists.

“Since its initial release in January 2008, Neo has rapidly expanded its populations,” says Pemberton. “There are hundreds of thousands of moths.”

Pemberton was the first to discover the biocontrol potential of *Neomusotima* insects. He found insects of this genus feeding on *L. microphyllum* near Hong Kong during a 1997 expedition.

ANTHONY BOUGHTON (D1455-1)

Neo caterpillar feeding on a leaflet of *L. microphyllum*.





**In Jonathan Dickinson State Park, entomologists Bob Pemberton (left) and Anthony Boughton inspect *L. microphyllum* vines that have been partly defoliated by Neo caterpillars.**

Pioneering research by the Brisbane scientists pinpointed stem borers as potential biocontrols in 1999. Later expeditions taken by Purcell; CSIRO colleagues Tony Wright, Jeff Makinson, Bradley Brown, and Ryan Zonneveld; and former Brisbane director Goolsby encompassed Australia, Singapore, Thailand, Indonesia, Malaysia, and Hong Kong and other parts of China. These fern forays have yielded stem borers from several different species of *Lygodium*.

Preliminary determinations by M. Alma Solis, an expert on fern-feeding moths and research leader of the ARS Systematic Entomology Laboratory in Beltsville, Maryland, indicate that borers collected in Hong Kong, Singapore, and Thailand are actually new to science.

Notes Purcell, “We’re the most excited about the stem borer from Hong Kong because we’ve been able to raise it successfully in captivity in cages of potted *Lygodium microphyllum* plants.”

That hasn’t been the case, for instance, with the Singapore stem borer specimens. “They like thicker-stemmed plants which, at present, can’t be provided for them in the lab,” Purcell says. “That makes rearing the Singapore borers near impossible, for now.”

Robust laboratory colonies are a must. They’re essential for the “host-specificity tests” in which scientists determine whether stem borers can survive and reproduce on climbing fern exclusively, instead of being able to prosper on other plants—such as Florida’s many native ferns. And bustling lab colonies are of course needed to stock fern-infested Florida sites and to replenish those outdoor colonies until they become self-sustaining.

With further research and requisite federal and state approvals, stem borers may one day work in harmony with Neo to halt climbing fern’s advance. Their prospective new home, Florida’s fragile Everglades ecosystem, is an irreplaceable national treasure. If they help save the ’Glades, these insects will surely become a national treasure, too.—By **Stephanie Yao** and **Marcia Wood**, ARS.

*This research is part of Crop Protection and Quarantine, an ARS national program (#304) described on the World Wide Web at [www.nps.ars.usda.gov](http://www.nps.ars.usda.gov).*

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Later work by Brisbane scientists Matt Purcell, Tony Wright, former Brisbane director John Goolsby—now with ARS at Weslaco, Texas—and John Molden of the Department of Agriculture and Food, Western Australia, highlighted Neo’s potential. Rigorous testing by both labs led to Neo’s 2008 outdoor release in Florida.

Despite its small size, Neo is formidable. The moth reproduces quickly, generating large numbers of offspring almost every month. Neo’s larvae feed on *Lygodium* leaves and, in large numbers, can defoliate entire plants. The larvae also eat the later regrowth.

Today, Neo’s thriving colonies—direct descendants of the insects imported from Australia—prosper at three test sites in Florida’s Jonathan Dickinson State Park. Neo has browned and defoliated *Lygodium* at all three sites, according to Boughton, and is now expanding to adjacent *Lygodium*-infested areas in the park.

“Neo’s impact is incredible,” says Fort Lauderdale research leader Ted Center. “The involvement of the Australian lab was critical and shows the advantage of having ARS foreign labs.”

The push continues to find new insects—moths, mites, beetles, and more—to help hardworking Neo.

Why the need for more recruits?

Explains Pemberton, “Using a diverse array of organisms—some feeding on the same parts of the fern and others feeding on different portions—provides a better attack on this weed.”

**Stem Borers: Deadly Insiders**

One of the most promising of the insects that might someday join the fern-fighting force in Florida is a small brown moth known as a “stem borer.” As its name implies, this biocontrol’s bailiwick is the elongated stems of climbing fern that extend upright from the plant’s dense, ground-hugging mats.

The female moth, which measures about a half-inch from wingtip to wingtip, lays eggs that hatch into unusually long, skinny, cream-colored larvae, explains entomologist Purcell, who is acting director at the Australian lab.

The larvae bore into the fern’s stems and, by eating the stem innards, or pith, create a snug home for themselves.

“This can be catastrophic for the plant,” says Purcell. “The stem borer’s tunneling and feeding can kill all of the fern’s growth above the larva’s entry point. That means, essentially, that a half-inch-long larva that bores a tunnel a few feet long has the potential to kill 40 feet or more of fern. That’s a remarkably powerful punch for so small an insect.”

**Neo adults perched on leaflets of *L. microphyllum*.**



# Flu Fighters

## Identifying, Treating, and Preventing the Flu With Help From ARS Research

**T**he first reports of a novel swine-origin influenza outbreak surfaced in the U.S. media in April 2009. The U.S. Centers for Disease Control and Prevention (CDC) first detected human infection in mid-April. Over the next 6 weeks, human infections were detected in more than 40 countries and nearly every U.S. state.

Early media reports referred to the virus—now known as “2009 influenza A (H1N1)” —as “swine flu,” a potentially confusing term for the public. Scientists initially concluded that the virus came from pigs because its genetic material was most similar to that of swine influenza virus. We now know that the 2009 H1N1 virus is a “triple reassortant virus,” meaning that it contains genetic material from swine, avian, and human influenza viruses—a mix that may help the virus spread quickly and pass between humans and pigs.

For some researchers, the story of a new influenza virus infecting pigs and people was all too familiar. As early as 2007, researchers from USDA’s Agricultural Research Service (ARS) and Animal and Plant Health Inspection Service (APHIS) had been monitoring a strain of influenza that could spread between pig and human populations.

In August 2007, several people developed flulike symptoms after exhibiting their swine at a county fair in Ohio. Many of the pigs became sick as well. Tests revealed the source of the illness: a triple reassortant influenza A (H1N1) very similar to viruses that are endemic in U.S. pig herds.

Scientists from ARS and APHIS characterized the virus and found that in pigs it was slightly more virulent than average. In light of the virus’s characteristics and its documented ability to spread from pigs to humans, the scientists advocated close monitoring of influenza in swine, birds, and other species.

In September 2008, ARS, APHIS, and CDC launched a collaborative effort funded by CDC to develop a national

JAMES FOSSE (D1611-1)



ARS veterinary medical officers Kelly Lager (left) and Amy Vincent collect a nasal swab from a piglet to test for novel H1N1 influenza virus.

swine influenza virus (SIV) surveillance pilot program. The goal is to better understand the epidemiology of SIV infections and to improve diagnostic tests, preventive management, and vaccines for swine and humans. This program has been instrumental in implementing surveillance for the 2009 outbreak, but it’s not the first time ARS influenza work has had major benefits. ARS scientists have been at the forefront of avian and swine influenza research for decades.

Agency scientists have developed tests to rapidly identify poultry infected with avian influenza and vaccines to protect against the disease. They’ve also developed and tested vaccines for swine. And ARS scientists have contributed to public health by identifying flu strains, assessing vaccines, and developing international

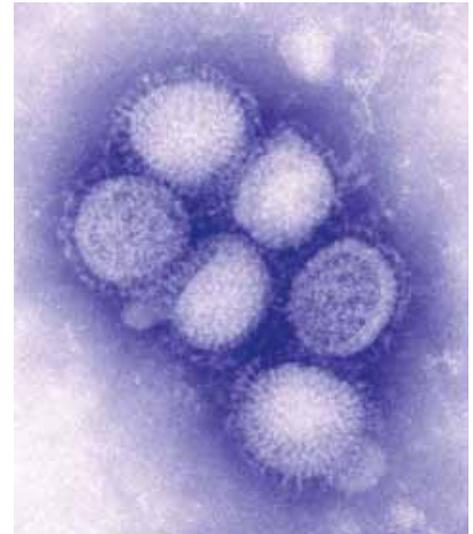
standards for inactivating flu virus in cooked products.

### Swine Surveillance

A 2008 paper co-written by ARS veterinary medical officer Amy Vincent states that swine influenza has undergone dramatic changes in the past decade, after nearly 80 years of relative stability. The increase in new subtypes and variants has complicated efforts to control the disease and increased the need for innovative strategies to fight it.

One such strategy is the pilot SIV surveillance program, in which APHIS coordinates with the National Animal Health Laboratory Network to screen potential SIV cases

CDC INFLUENZA LABORATORY (D1610-1)



Transmission electron micrograph of the H1N1 virus.



**In studies to evaluate swine flu vaccines, cells are observed for signs indicating they are infected by live swine influenza A virus.**

submitted from veterinary diagnostic laboratories. ARS then characterizes virus samples of special interest. The agencies also share virus isolates.

“We hope the pilot program will eventually become a permanent fixture,” Vincent says. “Having an established program would enable us to track not only where SIV occurs, but whether the virus undergoes significant mutations. This information could be essential in the event of an outbreak.”

The virus behind the current outbreak—like the virus from the Ohio county fair—is a type of influenza A known as “H1N1.” Though these two viruses have some substantial genetic differences, they both have surface proteins from swine influenza viruses. The surface proteins of these viruses are very different from those

of human seasonal influenza A (H1N1) viruses, which means that most humans do not have any cross-reactive antibody against them. Other viruses that have caused human illness, but originated in animals, include H5N1 and H7 viruses from poultry.

At the beginning of the 2009 H1N1 outbreak in humans, pork producers had two important questions: Is the virus capable of infecting pigs? And if so, how will they respond? To find out, Vincent and her colleagues challenged young pigs with the virus in a biosafety level 3 facility at the ARS National Animal Disease Center (NADC) in Ames, Iowa. They observed that the pigs developed clinical signs of influenza consistent with those seen in endemic SIV infections. The scientists are also examining antisera and existing vaccines to determine whether the immunity that develops in response to infection or vaccination will protect pigs against the current outbreak.

### **Pigs, Poultry, and Pathogenicity**

Because the new 2009 H1N1 virus contains genetic material from avian influenza viruses, two more questions must still be addressed: Is the virus capable of infecting avian species? And are poultry and other birds potential vectors for influenza in humans? Though the answers to these questions are still unknown, ARS scientists have a strong foundation of avian influenza research on which to build.

Scientists at the ARS Southeast Poultry Research Laboratory (SEPRL) in Athens, Georgia, have developed and evaluated avian influenza vaccines, helped assess public health threats, evaluated virus virulence, and helped develop protocols for inactivating flu viruses in food products. More recently, they’ve been evaluating how specific viruses pass from one animal to another.

Domestic pigs generally catch influenza from other pigs, but they’re also susceptible to infection from humans and birds. Fortunately, several highly pathogenic

strains of avian influenza virus don’t appear to cause significant disease in pigs. That’s the conclusion of a study led by Aleksandr Lipatov (now with the CDC). The research team also included Yong Kuk Kwon, Luciana Sarmiento, Erica Spackman, David Suarez, and David Swayne at SEPRL, and Kelly Lager at NADC.

“The finding is significant because pigs are thought to be vessels for mixing avian influenza and human influenza to allow genetic reassortment that could set the stage for pandemic avian influenza,” says Swayne.

In the studies, piglets were exposed to four different strains of highly pathogenic H5N1 avian influenza viruses. Two swine influenza viruses were used as controls. The studies show that none of the H5N1 virus strains caused significant or fatal disease in pigs, but they did cause mild to moderate inflammation in the lungs.

Infection with either swine influenza virus resulted in much more severe symptoms and pneumonia.

In related research, scientists at NADC and SEPRL have collaborated to develop two sets of diagnostic tests to quickly and accurately differentiate the new 2009 H1N1 virus from endemic forms of swine and avian influenza viruses already circulating in the United States.

Research efforts like these are essential to ensuring the health and safety of people and livestock in the United States and around the world.—By **Laura McGinnis** and **Sharon Durham**, ARS.

*This research is part of Animal Health, an ARS national program (#103) described on the World Wide Web at [www.nps.ars.usda.gov](http://www.nps.ars.usda.gov).*

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# Closing In on a Citrus Killer

*Huanglongbing, the Citrus Greening Disease*

**O**ranges seem to have everything you'd ever want from an orchard-fresh fruit. This superstar citrus—one of America's top-10 favorite fruits—boasts a pleasing flavor, a cheerful color, and a health-imparting dose of vitamin C.

But a pair of powerful natural enemies is now making life difficult in some of the nation's orange groves. One is a bacterium that causes a devastating, exotic disease known as Huanglongbing (HLB). The

of citrus—are conducting investigations that may yield new, affordable, eco-friendly ways to thwart this invasive disease and the psyllids that spread it.

## Profile of a Pathogen and Its Vector

HLB causes afflicted citrus trees to weaken and eventually die. Death can result either from HLB itself or from other diseases to which the infection renders the citrus more susceptible.

As the disease progresses, leaves mottle

feed on the sap of plants infected with the bacteria. However, the bacteria are harmless to the psyllids, as well as to people, pets, and livestock.

Growers can, of course, spray their orchards to kill the psyllids and can also destroy infected trees. But neither tactic is guaranteed to stop or wipe out the disease.

First detected in the United States in Florida in 2005, HLB has already infected millions of citrus trees in that state. The disease has recently shown up in Louisiana, and discovery of Asian citrus psyllids in Texas and California puts groves in these states at risk, too.

## To Help Save Orchards, Test Psyllids!

Growers, nursery owners, and backyard gardeners who want to protect their prized citrus trees are hindered by the fact that there's no way to reliably detect the HLB microbe early—well before the trees begin to show obvious symptoms.

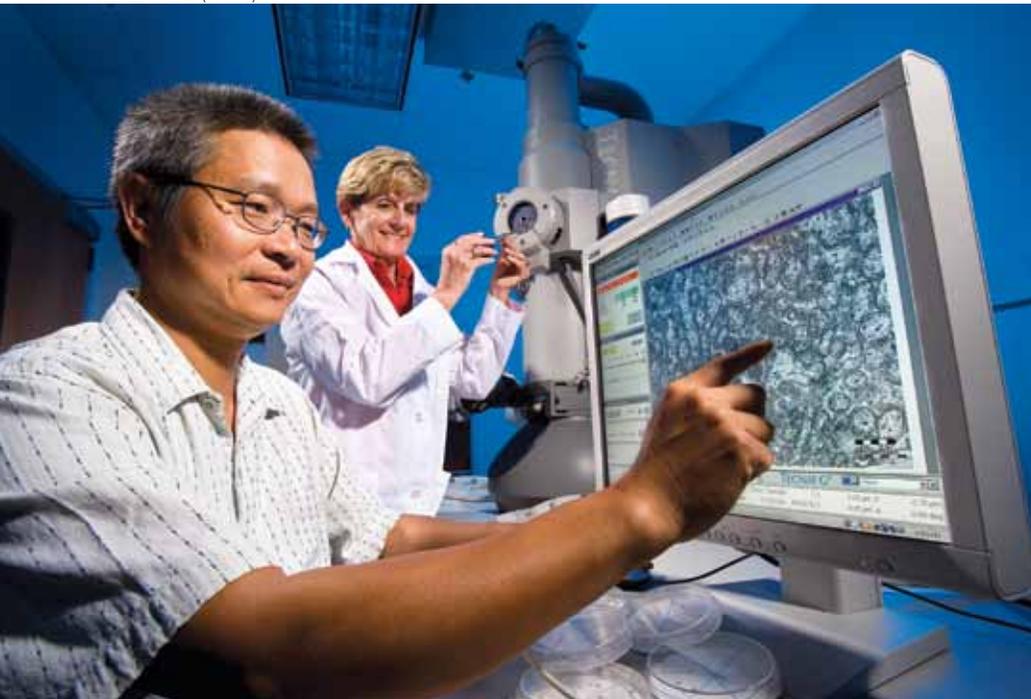
But some diagnostic tests, though imperfect, have been developed. ARS plant pathologists Richard F. Lee and Keremane L. Manjunath and colleagues have taken one such test—intended for pinpointing the microbe in plants—and have adapted it to find the pathogen in psyllids. Lee and Manjunath are based at the ARS National Clonal Germplasm Repository for Citrus and Dates in Riverside, California.

Their test, though not the first for detecting *Candidatus* L. asiaticus in psyllids, is apparently one of the newest of its kind to be based on what's known as “real-time qPCR” (quantitative polymerase chain reaction) technology.

The assay is comparatively quick and relatively inexpensive and can be performed by technicians working at any of the hundreds of labs across the country that already handle other PCR-based tests.

The scientists have used the test to analyze some 3,000 psyllids collected from orchards, backyards, retail nurseries, and other venues where citrus plants are grown or sold in Florida. The study showed, for the first time, that discount garden centers were likely involved in the inadvertent,

STEPHEN AUSMUS (D1436-2)



With the use of a transmission electron microscope, molecular biologist Jianchi Chen and technician Darlene Hoffmann, both with ARS in California, look for bacteria associated with citrus greening disease.

other is a tiny insect, a psyllid (pronounced *SILL*-id), that spreads the bacterium.

Oranges and many citrus relatives are extremely vulnerable to HLB. Also known as “citrus greening,” because infected oranges may remain green instead of ripening naturally, HLB is regarded as the world's most destructive disease of citrus.

And HLB is incurable—at least for now. But ARS scientists in California and Florida—states that are leading producers

and yellow, and tree growth is stunted. Infected trees produce either no harvest or low yields. The fruit may be lopsided, hard, bitter, and unmarketable.

HLB is generally thought to be caused by any of three different species of bacteria, namely, *Candidatus* Liberibacter africanus, americanus, or asiaticus. Psyllids such as the Asian citrus psyllid, *Diaphorina citri*, can become lifetime carriers of the disease soon after they

widespread, and rapid progression of the disease throughout Florida in recent years. In particular, sale and transport of orange jasmine, or *Murraya paniculata*—a popular landscaping plant and citrus relative—may have played a surprisingly important role in the spread of the disease.

The research also demonstrated that assaying psyllids may provide a way to detect HLB as many as 2 years before disease symptoms become apparent in afflicted plants. Early detection gives plant-health officials, and others, a helpful heads up. No one wants a repeat of what happened in Brazil and Florida, where HLB apparently had a 5- to 10-year head start before it was detected.

### Unmasking the Microbe's Genetic Secrets

Meanwhile, other ARS scientists are working with collaborators in the United States and abroad to sleuth the genetic makeup, or genome, of *Candidatus L. asiaticus*. Their explorations may yield new clues to undermining the pathogen.

The work is particularly difficult because the microbe can't be grown in pure culture in the laboratory. Such culturing would make it much easier for researchers to extract, evaluate, and decipher the sequence of the microbe's genetic material, or DNA.

ARS plant physiologist Hong Lin, who is with the ARS San Joaquin Valley Agricultural Sciences Center near Parlier, California, has modified a technique known as "genome walking" to obtain the likely sequence of the microbe's DNA. The approach has allowed his team to determine what sequences might occur on either the "upstream" or "downstream" ends of the very few stretches of *Candidatus L. asiaticus* DNA that have already been sequenced by researchers in Europe. Lin is using samples of DNA from HLB-diseased pummelo and other citrus in China.

In the south Florida city of Fort Pierce, plant pathologist Yong-Ping Duan and entomologist David Hall, both with the ARS

U.S. Horticultural Research Laboratory, are using a different approach. Instead of studying DNA from infected citrus trees, they are studying DNA from psyllids that test positive for the microbe.

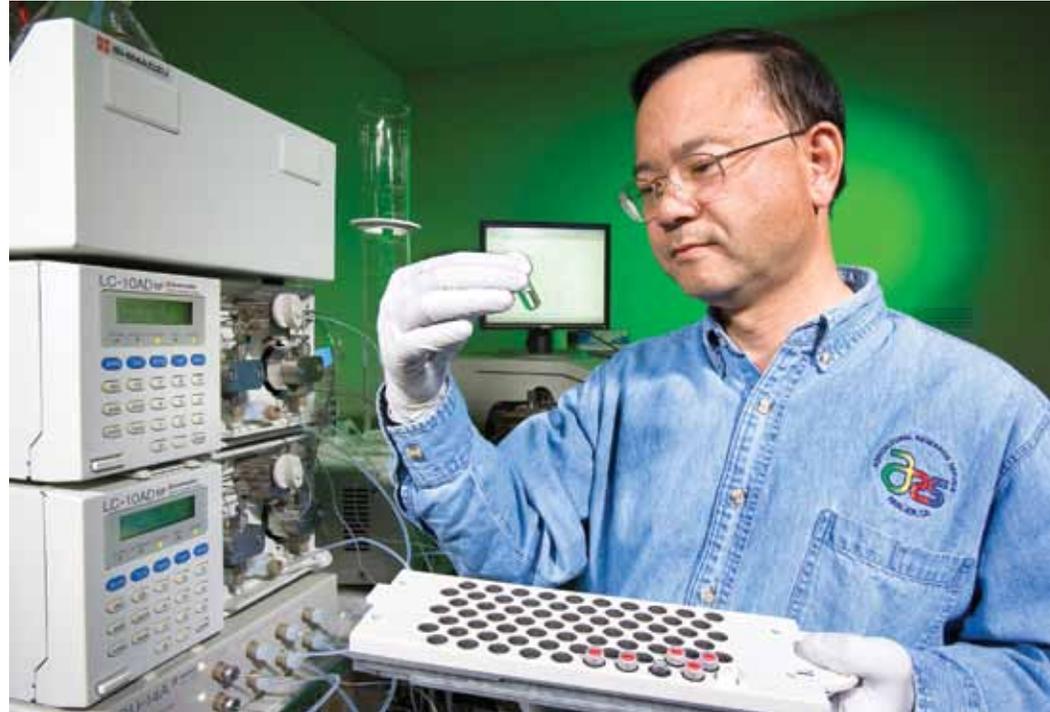
In comparing some of the predicted sequences from Duan's research with those from the Parlier work, Lin has found a 99-percent correlation. That's impressive, given that the researchers are using different sources for the DNA, from different parts of the world, and are using different

knowledge of the little-known, HLB-associated *Candidatus* species.

In all, the citrus greening research is putting a powerful squeeze on microbe and psyllid alike.—By **Marcia Wood, ARS.**

*This research is part of Plant Diseases, an ARS national program (#303) described on the World Wide Web at [www.nps.ars.usda.gov](http://www.nps.ars.usda.gov).*

STEPHEN AUSMUS (D1437-27)



**Using a protein mass spectrometer, plant physiologist Hong Lin measures host plant gene expression in response to pathogen infection.**

analytical techniques to sequence them.

Duan, who leads the *Candidatus L. asiaticus* genome sequencing work for ARS, has collaborated with Lin's team in grouping the sequences into lengthy stretches. This piecing together has yielded a map of the complete genome of the microbe.

In California, Lin's preliminary analyses have revealed interesting information about the microbe's evolution and its relatedness to several other bacteria. These findings help broaden researchers'

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# Soybean & Wheat Response to Climate Change

**G**lobal greenhouse gas emissions are projected to rise dramatically in the next 40 years, with increased outputs of carbon dioxide (CO<sub>2</sub>) being the main culprit. In light of our changing environment, Agricultural Research Service scientists in Urbana, Illinois, and Raleigh, North Carolina, are examining how the increase in greenhouse gases, particularly CO<sub>2</sub> and ozone, will affect two of the world's most widely planted crops: soybeans and wheat.

## Open-Top Chambers Offer Insight

At the Plant Science Research Unit in Raleigh, ARS plant physiologists Fitzgerald Booker, Kent Burkey, and Ed Fiscus are assessing how climate change will affect soybean and wheat growth rates, crop yield, and soil chemistry by exposing the crops to the elevated levels of CO<sub>2</sub> and ozone projected for the year 2050.

Soybeans, wheat, and other crops grow more when CO<sub>2</sub> levels are elevated, because there is more carbon captured during photosynthesis for the plants to use. But those same plants are damaged and stunted by elevated levels of ground-level



PEGGY GREB (D1548-1)

ozone, a gas created when sunlight “cooks” automotive and industrial pollutants that originate from combustion of carbon-based fuels.

Levels of both gases are rising. The Intergovernmental Panel on Climate Change, an international panel of highly regarded scientists, estimates that CO<sub>2</sub> levels could be about 1.5 times greater than the current 380 parts per million

by 2050. The panel also estimates that daytime ozone in the summertime, now at about 50-55 parts per billion, may rise 20 percent over the same period.

Besides assessing the effects of future air concentrations on both crops, the researchers are conducting a 5-year project to determine whether a widely accepted no-till cropping system will improve soil quality and sequester carbon when levels of the gases rise.

“We know no-till increases carbon sequestration in the soil. The question is, What is going to happen with elevated levels of CO<sub>2</sub>, and how are changes in other atmospheric gases, namely ozone, going to affect that?” Booker says.

The Raleigh researchers have 16 open-top chambers, divided into 4 treatments: 4 with elevated ozone, 4 with elevated CO<sub>2</sub>, 4 with both gases elevated, and 4 controls. They are pumping the chamber air with up to 40 percent more ozone and CO<sub>2</sub> than what is found in today's ambient air. They are also charcoal-filtering the air so they can reduce ozone in the control chambers and tease out the impact that different ozone and CO<sub>2</sub> levels, by themselves, have on the plants.

In addition, the researchers are putting the postharvest residues, such as plant stems, empty pods, and dead leaves, back into the chambers, essentially

INSTITUTE FOR GENOMIC BIOLOGY/UNIVERSITY OF ILLINOIS (D1546-2)



ARS scientists Carl Bernacchi (left), Don Ort (center), and Lisa Ainsworth in a plot of soybeans treated with elevated carbon dioxide (CO<sub>2</sub>) at the SoyFACE Global Change Research Facility at the University of Illinois Research Farm in Urbana. In the foreground is an automatic retractable awning used to capture rainfall in order to investigate the interaction of drought and elevated CO<sub>2</sub> on soybean. Behind the scientists is an array of infrared heaters used to raise the temperature of a portion of the plot to investigate interactions with anticipated global warming.



**Plant physiologist Fitzgerald Booker prepares to place an optical scanner into a tube that will be positioned in the soil to photograph soybean roots. The photos will be analyzed to determine changes in root length and distribution caused by exposure to elevated carbon dioxide and ozone, which are pumped into the open-top field chambers.**

making them small, self-contained plots that mimic conditions found in no-till systems. To measure changes in the soil's carbon and nitrogen content makeup, the researchers take samples twice a year. The CO<sub>2</sub> pumped into the chambers has a specific isotope marker so they can track it from the air through the plants and into the soil. They are analyzing the soil cores by depth to determine where composition changes may occur and also the amount of bacteria and fungi found in each layer to see whether microorganism populations or communities change.

After completing 3 years of the project, preliminary results show a trend for higher levels of soil carbon in chambers with elevated CO<sub>2</sub> but not in chambers with elevated ozone. Elevating CO<sub>2</sub> also reduced protein levels in wheat flour by 7 percent to 11 percent, but it had no effect on soybean seed protein.

### **SoyFACE: New Technology, New Understanding**

Researchers in Urbana and cooperators with the University of Illinois at Urbana-Champaign have been working on a project called "SoyFACE"—short for Soybean Free Air Concentration Enrichment—that also measures how the projected increases in CO<sub>2</sub> and ozone will affect soybean production.

FACE technology, which was first used for crop research by ARS soil scientist Bruce Kimball, ARS plant physiologist Jack Mauney (now retired), and scientists from the U.S. Department of Energy's Brookhaven National Laboratory, allows testing of plants in open-air field conditions. ARS scientists Don Ort, Lisa Ainsworth, and Carl Bernacchi, in the Photosynthesis Research Unit, and Randall Nelson, in the Soybean/Maize Germplasm, Pathology, and Genetics Research Unit, use the technology to produce atmospheric conditions predicted for the year 2050.

Horizontal pipes arranged in an octagon about 70 feet in diameter surround each test plot. A computer measures wind direction and speed, then releases concentrated amounts of CO<sub>2</sub> and ozone. The wind carries the gases across the soybean crop.

"At the start of the project, we sought to understand how CO<sub>2</sub> and ozone affect soybean independently," says Ort. "We found soybean yield increases by about 12 percent at the elevated CO<sub>2</sub> concentrations predicted for 2050, half of what previous studies estimated. We also found that increased ozone is quite harmful to yield, reducing it by about 20 percent. In fact, our study showed current levels of ozone are already suppressing soybean yield by up to 15 percent."

The results of the individual studies led the scientists to examine the combined effects of CO<sub>2</sub> and ozone on soybean. They found that elevated CO<sub>2</sub> partially offsets the ozone damage. These findings with SoyFACE confirm general results obtained with open-top chamber studies of ozone and CO<sub>2</sub> effects on crop yield conducted at Raleigh and other locations. But the ability of SoyFACE technology to test these principles in the open air, without the environmental modifications caused by the chambers themselves, means greater confidence in our understanding of how plants respond in the real world, including the actual estimates of impact on crop yields. Furthermore, there is much to be learned about how other interacting factors

that affect ozone uptake may come into play by mid-century.

The scientists recently began studying how combined factors will affect soybean production. They are looking at drought and increased temperature, drought and increased CO<sub>2</sub>, and elevated temperature and CO<sub>2</sub>. They'll also be analyzing how microbial communities and soil carbon storage are affected by these changes.

These projects provide valuable information that will help breeders develop soybean cultivars better adapted to an ever-changing climate.—By **Stephanie Yao and Dennis O'Brien, ARS.**

*This research is part of Air Quality (#203), Global Change (#204), and Plant Biological and Molecular Processes (#302), three ARS national programs described on the World Wide Web at [www.nps.ars.usda.gov](http://www.nps.ars.usda.gov).*

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PEGGY GREB (D1550-1)



**To assess oxidative stress in soybean grown at SoyFACE under elevated ozone concentrations, ARS molecular biologist Lisa Ainsworth (back) and graduate student Kelly Gillespie use a liquid-handling robot to perform a high-throughput assay.**

# Why Watermelons Just Grow and Grow

PEGGY GREB (D1432-1)

It takes around 5 weeks for a tiny watermelon flower to grow into a ready-to-eat watermelon. Though ripe watermelons typically weigh anywhere from 5 to 40 pounds, record-breakers have tipped the scales at 250 pounds or more.

“Watermelon is a fruit that grows so fast, and it can get so big,” says ARS plant geneticist Amnon Levi. “We know there must be some very interesting genes at work.”

Levi works at the ARS U.S. Vegetable Laboratory in Charleston, South Carolina. He teamed up with plant pathologist Pat Wechter and plant geneticist Karen Harris on the first-ever study that identified and characterized key genes regulating watermelon growth and development.

Over a 3-year period, plant geneticist Angela Davis, who works at the ARS South Central Agricultural Research Laboratory in Lane, Oklahoma, grew watermelons in the field for the research to ensure that the genes would be responding to actual field conditions, such as pathogens and weather extremes.

“One year it was so dry that we had to stop watering because water restrictions had been imposed,” Davis notes. “The other 2 years it rained so much that the plants became stressed from too much water.”

Davis extracted RNA from watermelon fruit at three stages of growth and ripening: at 12 days after pollination (DAP), when the flesh was white; at 24 DAP, when the flesh was pink; and at 36 DAP, when the flesh was red. Then she sent RNA from the tissue samples to Charleston for analysis.

## Genes Prompt Plus-Size Fruit

Levi used this collection of RNA to develop a library of 832 expressed sequence tags (ESTs), which are unique gene segments that direct different aspects of development and metabolism. Then he worked with Wechter and Harris to decipher how the ESTs regulated plant growth and development.



Plant pathologist Pat Wechter (left) and plant geneticist Amnon Levi examine watermelon for fruit quality. They are studying gene expression in the three stages of watermelon ripening.

The team found that these ESTs were active in metabolism, cell growth, cell development, and transporting nutrients and other substances across cell walls. They also came into play in cell division, cellular communication, DNA copying,

plant defense, and stress response.

The Charleston researchers then identified significantly different levels of EST expression during the early, middle, and late stages of fruit growth and ripening. They found 335 ESTs that had at least

a twofold increase or decrease in copy number in at least one of the three stages. Of these 335 ESTs, 239 were very similar to ESTs found in other plants, while the remaining 96 had not been previously identified in any sequenced plant species.

“Most of these genes regulate targeted activities,” Wechter says. “They are very specific genes that do certain things at a certain time during growth and ripening. Many of them are involved in the development of the watermelon vascular system, which is a crucial component for growth and ripening.”

“The vascular system in a watermelon forms very fast because it is building the infrastructure for fruit, like building a highway before building a city,” Levi says. “This system is how fluids transport sugars from the leaves into the fruit.”

### Ethylene Enters the Picture

But some of the results were unexpected—and may someday give breeders and producers an edge in moving watermelons to market.

In some types of fruit, ethylene gas is produced, and it is responsible for many ripening processes. These fruits are referred to as “climacteric fruits.” In fact, producers often harvest climacteric fruits—such as tomatoes—before they are ripe, and then promote ripening by exposing the harvested fruit to ethylene.

But ethylene has not been linked to ripening of nonclimacteric fruits. Scientists consider watermelons and other cucurbits—along with grapes, citrus, and strawberries—to be nonclimacteric fruits. So Wechter and the others were very surprised to see differences in expression levels of genes involved in ethylene production in watermelon fruit. They then measured the amount of ethylene produced by developing and ripening fruit. They found a burst of ethylene production during the white-fruit stage and lesser amounts produced during the later stages.

“We just didn’t think ethylene had any role in the ripening of watermelon,” Wechter says. “Now we know it could be a

central component of the ripening process. And if it’s important in watermelon, it could be important in other nonclimacteric fruit as well.”

Jim Giovannoni is a molecular biologist who works at the ARS Robert W. Holley Center for Agriculture and Health in Ithaca, New York. He maintains the Cucurbit Genomics Database ([www.icugi.org](http://www.icugi.org)), a gene bank for the Cucurbitaceae family that is used by researchers around the world. He helped the Charleston team sort through the watermelon ESTs.

“Finding ethylene activity in watermelon is significant because it shows the existence of the same type of genes that are also found in tomato and *Arabidopsis*—our model plant,” Giovannoni says. “This shows that genes involved in model systems are also seen in crop systems, which validates the models we use. In addition, we have identified ESTs that could be used to develop molecular markers for ethylene response in watermelon.”

### Enigmatic ESTs Remain

Though the ESTs linked to ethylene activity were a surprise, the team was able to pinpoint their function. But the team also found 96 ESTs that remain somewhat of a mystery.

“They appear to be active all the time, which suggests they regulate more generalized, basic functions for plant survival,” Wechter says. “But we can’t match them up with any other known plant ESTs. We’re trying to confirm they are unique to watermelon.”

In 2008, U.S. watermelon crops valued at \$492 million were planted on just over 134,000 acres. But pathogens—including watermelon vine decline and *Phytophthora* blight—continue to threaten production. Finding sources of genetic resistance to these threats is essential to the success of watermelon crops across the southern United States.

“Cultivated watermelons are not genetically diverse at all, which makes them much more vulnerable to pathogens and environmental stresses,” Harris notes. “It’s

difficult to find genetic differences in such a narrow selection of cultivars.”

“I want to do more studies looking at the interaction of watermelon genes when the plant is challenged by pathogens,” Wechter adds. “Our EST study is a good start. Now we need to build on this information and find ways to develop more resistant melons to meet the challenges in field production.”—By **Ann Perry, ARS.**

*This research is part of Plant Genetic Resources, Genomics, and Genetic Improvement (#301) and Plant Diseases (#303), two ARS national programs described on the World Wide Web at [www.nps.ars.usda.gov](http://www.nps.ars.usda.gov).*

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PEGGY GREB (D1433-1)



**Plant geneticist Karen Harris removes watermelon flesh for RNA extraction.**

# Using Floating Vegetated Mats To Treat Fishery Wastewater

**S**aving water is an environmentally responsible goal, especially in the southeastern parts of the country, where droughts are fairly frequent. To that end, Agricultural Research Service scientists in Tifton, Georgia, are testing the feasibility of using floating vegetation to remove nutrients from fishery wastewater. The long-term goal is to develop a system to treat fishery wastewater, return it to ponds for reuse, and use the nutrients to produce biomass, or plant material.

The study participants are soil scientist Robert K. Hubbard, at the ARS Southeast Watershed Research Laboratory; plant geneticist William Anderson and plant pathologist Jeffrey P. Wilson, at the ARS Crop Genetics and Breeding Research Unit, in Tifton; and University of Georgia animal science associate professors Gary Burtle and Larry Newton.

The current study is being conducted jointly by ARS and the University of Georgia's Aquaculture Unit in Tifton. "It builds on previous research showing that vegetation can be grown on floating mats in swine-wastewater lagoons," says Hubbard.

Wastewater from the fish-production ponds is pumped into 340-gallon aquaculture tanks. Each tank has a 10-foot-square floating mat on which the vegetation grows. "Our first objective is to find plant species that grow well in fishery wastewater," says Hubbard.

The researchers are currently testing 12 different plant species: St. Augustinegrass, Tifton 85 bermudagrass, common bermudagrass, canna lilies, iris, bamboo, bulrush, cattail, bordergrass, napiergrass, reeds, and maidencane. "So far, the iris is the best performer," says Hubbard.

In the second part of the study, beginning in spring 2009, researchers will determine the effects of the vegetation on water quality and the amount of nutrients removed when plant biomass is harvested, Hubbard says.

The plant material will be harvested on an as-needed basis and the plant tissue analyzed for nitrogen, phosphorus, and potassium. Harvested plant material has several potential uses. It can be transplanted, used as feedstock for energy production, or composted and used as a soil amendment.—By **Sharon Durham, ARS.**

*This research is part of Soil Resource Management (#202), and Manure and Byproduct Utilization (#206), two ARS national programs described on the World Wide Web at [www.nps.ars.usda.gov](http://www.nps.ars.usda.gov).*

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BOBBY SHIVER (D1291-1)



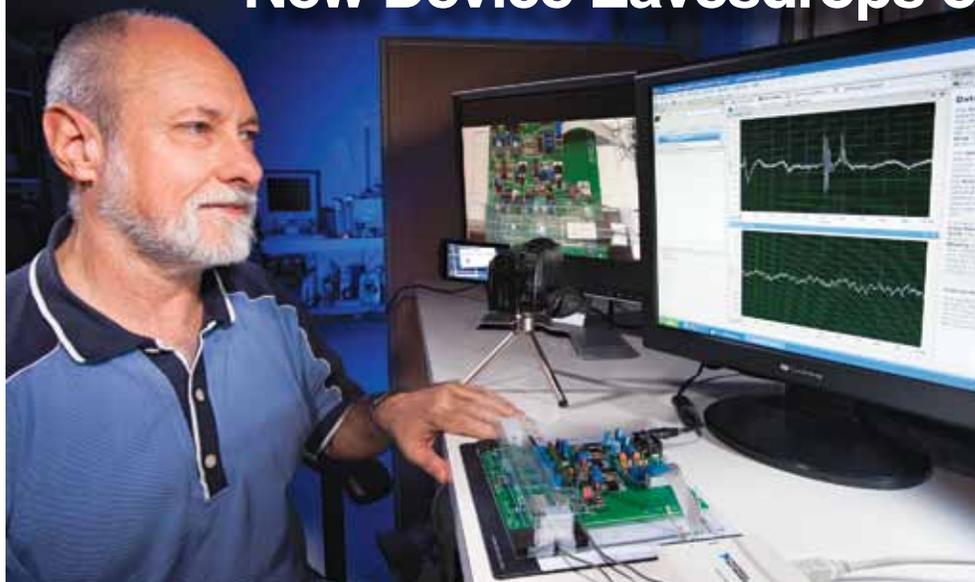
Soil scientist Robert Hubbard adjusts iris plants recently transplanted onto floating mats in aquaculture tanks. The tanks have been sprigged with different plant species to see which plants grow best in fishery wastewater. The leafy green plants in the back are canna lilies.

ROBERT HUBBARD (D1292-1)



Of the plant species tested on floating mats in fishery wastewater, iris plants, shown here, grew best.

# New Device Eavesdrops on Insect Pests



Entomologist Richard Mankin examines signals collected by an inexpensive prototype system (on the bench, at his fingertips) for automated insect detection and identification.

**G**rain elevators, warehouses, and food-processing facilities are a hungry insect's delight, thanks to ample food and climate-controlled conditions. Fortunately, there are many effective strategies for keeping stored-product insects in check—including targeting of problem areas with extra sanitation measures and baits. But determining pest whereabouts and numbers in order to effectively implement these strategies can be challenging.

Now, after 8 years of research, Agricultural Research Service entomologist Richard Mankin and colleagues may have some inexpensive help. Using commercially available parts, they've built a monitoring device that detects insects by the sounds they make.

Collaborating with Mankin are North Carolina State University researchers Ryan Hodges, Troy Nagel, and Coby Schal, all in Raleigh; and Roberto Pereira and Philip Koehler, both at the University of Florida in Gainesville.

The most likely application will be to automate routine monitoring of industrial-scale traps, especially those placed in hard-to-reach areas like crawl spaces or near food-processing equipment. "Automation could be useful in a situation where you

have a trap in place for a long time and you don't want to have to check it every week or two," says Mankin, at the ARS Center for Medical, Agricultural, and Veterinary Entomology in Gainesville.

Armed with automated traps, managers could improve the timing and placement of control measures or even avoid using them when there's no need. The researchers' insect intelligence gathering isn't restricted to stored-product pests, though. They're also targeting home intruders like *Blattella germanica* (the German cockroach) and *Cimex lectularius*, better known as the "bed bug."

Mankin says their objective was to create a device that would make automated insect monitoring not only affordable, but also easy to use and reliable. Toward that end, they integrated commercially available sensors (LEDs, microphones, and piezoelectric film) with high-gain amplifiers and laptop-run software for analyzing digital signals.

Their system uses the sensors to collect infrared, acoustic, and vibrational signals generated by three kinds of insect movements: wriggling, crawling, and scraping. The software analyzes the signals to create a profile of the target insect that distinguishes it from other species.

The researchers tested their device on three stored-product pests (rice weevil, red flour beetle, and drugstore beetle) and two household pests (German cockroach and bed bug). Individuals of each pest were placed inside small arenas where their signals could be collected and analyzed for differences in profile, magnitude, and duration. Although all five species generated all types of signals, red flour beetles mostly wriggled, German cockroaches typically ran or crawled, and bed bugs mostly scraped.

Mankin envisions users placing the devices in traps in or near infested structures and monitoring them remotely via laptop computer. "You would probably receive an alarm when a potential target insect was detected," he says. "The information could also be saved in a spreadsheet, with the time of occurrence and probability that this was a target insect."—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](http://www.nps.ars.usda.gov).*

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# Producers and Pigs Profit From Manure

PEGGY GREB (D1774-1)



ARS soil scientists Matias Vanotti (left) and Ariel Szogi (right) and Lewis Fetterman, the CEO of Terra Blue, Inc., examine swine wastewater before (dark liquid) and after (clear liquid) treatment in the second-generation system they invented.

For almost 10 years, Agricultural Research Service (ARS) soil scientists Matias Vanotti and Ariel Szogi have worked with farmers, state agencies, and businesses to improve swine manure management practices. Now the scientists have developed a streamlined system that delivers a winning trifecta—healthier pigs, healthier profits, and a healthier environment.

A key factor in this success? “We paid lots of attention to what farmers and industry were telling us,” says Vanotti, who works with Szogi at the ARS Coastal Plains Soil, Water, and Plant Research Center in Florence, South Carolina.

Manure can be used to fertilize crops, but excess nitrogen and phosphorus that is not taken up by plants can be carried away by water and end up polluting nearby streams, lakes, and groundwater. Controlling pathogens, odors, and emissions of ammonia and greenhouse gases is also a concern with livestock production. So swine producers in North Carolina—who in 2008 sent close to 20 million pigs to market—are always on the lookout for ways to reduce the environmental impact of their facilities.

Vanotti and Szogi have been essential partners in this search. In 2005 they unveiled a first-generation swine manure management system created in partnership with Terra Blue, Inc., a private business based in Clinton, North Carolina. (See “Blue Lagoons on Pig Farms?” *Agricultural Research*, March 2005.)

## Taking It to the Next Level

When Vanotti and Szogi started out, the technology available to them resulted in a highly effective first-generation treatment system. But the results from their farm-scale trials and new discoveries suggested that they could design an even more economical and simple process.

An added incentive: In 2007, the State of North Carolina started a statewide Lagoon Conversion Program (LCP) that provided financial support to livestock farmers who installed new manure management technologies that improved water and air qual

# Management Process

ity. The LCP set clearly defined targets for techniques that would meet their criteria of an Environmentally Superior Technology (EST) for manure management.

An EST needed to eliminate discharge of animal waste to surface waters and groundwater. It also needed to “substantially eliminate” emissions of ammonia and odors detectable beyond the production facility. An EST would also substantially eliminate the release of disease-transmitting vectors and pathogens as well as nutrient and heavy metal contamination of soil and groundwater. And an EST would need to run economically and efficiently.

The scientists’ system already met the environmental targets. “We needed to demonstrate that we could cut costs while maintaining the efficiency of the first-generation system,” Szogi says.

So Vanotti and Szogi went back to the drawing table and worked with Lewis Fetterman, the chief executive officer of Terra Blue, Inc., to redesign and fine-tune the process. In the end, the team made 24 changes to the first-generation system to lower its installation and operating costs and improve its reliability.

A U.S. Patent was granted for this new system (U.S. Patent 7,674,379, March 9, 2010).

The on-farm system used solid-liquid separation and nitrogen and phosphorus removal processes. In the right combination, these systems replaced traditional anaerobic lagoon systems with a process that produced clean, deodorized, and disinfected effluent.

After processing, the nutrient-rich solids were transported off-site to a central composting facility that produced class A composted materials for making organic plant fertilizer and plant growth media.

The team reduced the solid-liquid separation operation from 7 days a week to just 2. Several other adjustments were also made to this process, including simplifying the equipment used in the dewatering process and the production of drier solids. This improved solids handling, lowered

transportation costs, and made the solids more suitable for composting. It also increased their potential for use as fuel for on-farm energy generation by thermal technologies.

To cut costs further, Vanotti and Szogi removed two tanks from the biological nitrogen removal process and incorporated a high-performing nitrifying bacterial sludge (HPNS), which they developed over 10 years in the laboratory. The new HPNS was well adapted to operate efficiently in both high-ammonia wastewater and cold temperatures, which translated into a smaller plant footprint. It also reduced the cost of ammonia removal.

A U.S. Patent Application was filed for the HPNS (Serial No. 12/495,958, July 1, 2009).

Finally, the scientists invented a simplified process to simultaneously separate the phosphorus and the manure solids. This innovation required fewer polymers than the previous separation system and also reduced equipment costs.

The revamped system was tested for 15 months on a 5,145-head swine farm over 4 growing cycles. For this full-scale project, they collaborated with microbiologist Patricia Millner, who works at the ARS Environmental Microbial and Food Safety Laboratory in Beltsville, Maryland,



Close-up of piglet and sow.

and chemist John Loughrin, who works at the ARS Animal Waste Management Research Unit in Bowling Green, Kentucky.

The results: the revamped system met EST standards at one-third the cost of the previous version. In cleaning up manure wastewater, the system removed almost 100 percent of pathogens and odor-causing components, 95 percent of total phosphorus, 97 percent of ammonia, and more than 99 percent of heavy metals copper and zinc.

The new system also cut emissions of methane and nitrous oxide—powerful greenhouse gases—by 97 percent. In addition, the system transformed the old lagoon into an aerobic reservoir that reduced 90 percent of the ammonia emissions.

The second-generation system used less energy to boot—power consumption dropped by 44 percent. The newer system was also more labor efficient and easier to operate than the first system, in part because of the development of operation practices that integrated automation with simple operator input.



PEGGY GREB (D1777-1)

Using a deep sampling probe, Ariel Szogi inspects the amount of calcium phosphate produced in a phosphorus-separation module that is part of the new system.



PEGGY GREB (D1778-1)

After processing, the nutrient-rich solids are transported off-site to a centralized composting facility. The composting process results in materials with an earthy scent and rich texture that can be used in organic fertilizers, soil amendments, and potting soils.

### Payback Time

All told, the second-generation technology was two-thirds less expensive to build and operate than the first system. What Vanotti and Szogi didn't anticipate is how well the animals would respond.

But Billy Tyndall, the farmer who owned the facility where the second-generation system was being tested, began to notice an improvement in his pigs immediately after the system was installed. "The animals were heavier," Szogi says. "And they were showing a better conversion of feed."

The scientists and Tyndall were so impressed that they extended the project—originally planned for 5 months—to run for another year and a half to see if the results stayed consistent over five growing cycles. At the end of the study, the scientists tallied up the surprising results.

Daily weight gain increased 6.1 percent, and feed conversion improved 5.1 percent. Animal mortality decreased 47 percent, and cull weight was reduced 80 percent. The farmer sold an average of 5,265 pigs per growing cycle, which resulted in a 1,138,247-pound net gain per cycle. Using the second-generation system instead of the lagoon system, the farmer sold 61,400 pounds more hogs—a 5.8 percent increase—per growing cycle.

"Participating in the program took my average farm to the top of the field," Tyndall declares.

The scientists attribute the productivity improvement in part to the improved air quality and health conditions in the barns. Since the recycled wastewater was mostly ammonia free, ambient ammonia levels in the barns dropped an average of 75 percent.

"Improvement in animal production is important to farmers because that's their bottom line," Vanotti says.

Fetterman, their Terra Blue, Inc., partner, sees it from another angle. "If you treat the pigs well, they will pay you back," he says.

### Sharing the Success

The second-generation system was discussed in a chapter of "Manufacturing Climate Solutions: Carbon-Reducing Technologies and U.S. Jobs," published in 2008 by Duke University. The report was commissioned by the AFL-CIO and the Environmental Defense Fund and featured technologies that could help reduce greenhouse gas emissions and create green jobs in the United States.

The authors concluded that the second-generation system could help swine-producing states protect existing jobs and keep the door open for future job expansion.

In 2009, the USDA Natural Resources Conservation Service's Environmental Quality Incentives Program began a 5-year initiative with additional funding for North



PEGGY GREB (D1778-1)

Matias Vanotti and C. Ray Campbell, vice president of research and development, Terra Blue, Inc., examine a compost sample.

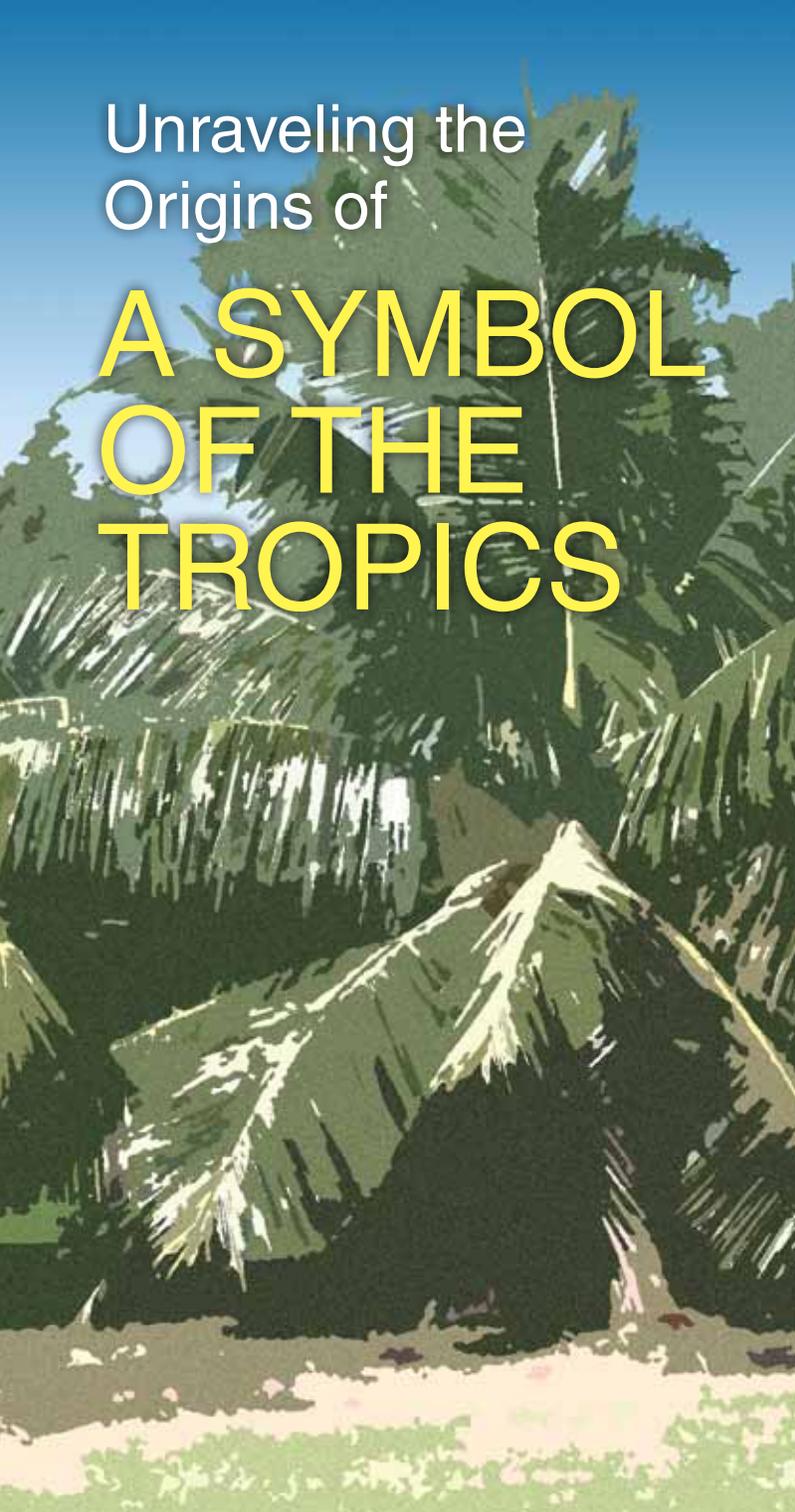
Carolina livestock farmers who participate in the LCP. At the time the program was established, only the second-generation system met all the LCP qualifications for funding, which will pay for up to 90 percent of the costs involved in installing an EST for manure management.

"When one considers the many direct and indirect benefits of the cleaner hog-waste technology, farmers and society may not be able to afford not to convert to the new technologies," Fetterman observes.

"We learned our lessons and tried to make a new system as simple to operate and economical as possible," Vanotti adds. "Now we need to install these systems in a sufficient number of farms to confirm their environmental benefits at a regional scale and to facilitate the development of markets for co-products from recovered manure nutrients."—By **Ann Perry**, ARS.

*This research is part of Manure and Byproduct Utilization (#206), Water Availability and Watershed Management (211), and Food Safety (#108), three ARS national programs described at [www.nps.ars.usda.gov](http://www.nps.ars.usda.gov).*

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Unraveling the  
Origins of

# A SYMBOL OF THE TROPICS

The coconut variety Niu Leka, or Fiji Dwarf (see back cover), from the South Pacific may represent the earliest lineage in the coconut's domestication.

The coconut tree is a symbol of the Tropics, and as a source of fiber, food, fuel, soap, and cooking oil, it is sometimes called “the tree of life.” But one aspect of the tree has remained a mystery—its origins. Scientists have debated the tree’s genealogical roots for decades.

Using genetic markers found in coconuts and other palm trees from around the world, Alan Meerow, a geneticist with the USDA-ARS Subtropical Horticulture Research Station in Miami, Florida, has completed a phylogenetic analysis of a large group

of palm species (the Attaleinae subtribe) that provides the most comprehensive look yet at the coconut tree’s family history. The results suggest that the ancestors of the coconut tree originated in South America, that the tree’s closest living relatives are a modern genus of American palms (the genus *Syagrus*), and that it diverged from them about 35 million years ago. The genus *Syagrus* includes another popular Florida ornamental, the queen palm.

Meerow and colleagues also found that the modern coconut tree probably evolved about 11 million years ago, perhaps in the South Pacific. The Fiji Dwarf, a variety of coconut tree now grown in the United States, shares its South Pacific ancestry with many of today’s other coconut varieties, according to Meerow. “The Fiji Dwarf is more distantly related to all these other varieties,” he says.

The work, published in *PLoS One*, is more than an academic exercise. Five of the 80 known varieties of coconut tree are major ornamentals in Florida. Identifying their closest relatives will help in the search for genes with traits capable of resisting diseases, insect pests, and other threats. An epidemic of lethal yellowing phytoplasma in the early 1980s destroyed an estimated 100,000 coconut palms in South Florida. Bud rot, caused by several fungal pathogens, threatens coconut production around the world.

“The more we know about it, the easier it will be to address future threats,” Meerow says.

Patterns of differences in DNA can open a window into a plant’s evolutionary past, revealing when it diverged from its ancestors. Meerow and colleagues looked for patterns among a family of genes developed as markers by ARS researchers studying cacao (chocolate) plants. Known as “WRKY genes,” they are valuable “clocks” for dating the occurrence of important evolutionary events.

With these molecular clocks and evidence from fossil palms, Meerow traced the coconut tree’s ancestry back more than 40 million years to palms that grew in both Madagascar and eastern Brazil. He also found that milestones in the coconut’s early “family tree” coincide with major geological events in South America, making it likely they played a role in how these palms evolved. A group of palm species in southern Brazil, for example, split off from a relative in Chile about 14 million years ago, around the time when geological events gave rise to the Andes Mountains. The extensions of oceans into the western Amazon region that lasted from 25 to 30 million years turned dry areas into wetlands and probably altered the evolution of coconut ancestors growing at the time across South America, Meerow says.—By **Dennis O’Brien, ARS.**

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

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# New Cultivars and Farming Strategies

**P**eanuts are an important crop, contributing more than \$4 billion to the country's economy each year. But rapid growth in cities along with water level declines in aquifers throughout the South have resulted in fewer acres for farming and less water available for irrigation. To meet future food-supply demands, crop production will have to increase, but it must do so under the constraints of less water and, most likely, less farm land.

Agricultural Research Service scientists with the Plant Stress and Germplasm Development Research Unit in Lubbock, Texas, and the National Peanut Research Laboratory (NPRL) in Dawson, Georgia, are working with cooperators to help peanut farmers maintain and improve their production in a changing environment.

## Managing Abiotic Stresses

At the Lubbock laboratory, plant physiologist Paxton Payton and postdoctoral research associate Rao Kottapalli are examining molecular mechanisms involved in peanut response to abiotic stress. Abiotic stress is the negative effect that nonliving factors, such as drought and heat, have on living organisms.

The researchers recently conducted groundbreaking work aimed at linking responses at the molecular level to the physiology and yields in peanut plants. They examined the proteins, particularly their expression, that control drought stress in peanut.

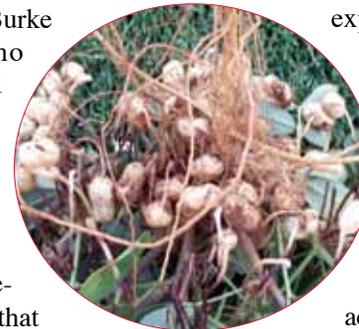
To examine diverse peanut germplasm more efficiently, Payton and Kottapalli performed greenhouse and molecular screening of 70 genotypes from the U.S. peanut mini-core collection and 7 additional cultivars representing varieties commonly grown in the southern United States and semiarid regions in Asia. The mini-core collection—developed by ARS plant breeder Corley Holbrook in Tifton, Georgia—consists of 112 peanut accessions that represent most of the variation present in the larger collection of peanut germplasm.

Field screening of the accessions was performed by New Mexico State University scientist Naveen Puppala and a team of Texas AgriLife Research and Texas Tech University scientists led by Mark Burow. Photosynthetic measurements of the field-grown plants by Payton and Kottapalli helped narrow down the group to two accessions that are highly tolerant to heat and drought and two that are highly susceptible.

This follows previous work by the AgriLife and Texas Tech scientists and ARS scientists John Burke and Gloria Burow, who demonstrated heat and drought tolerance in a smaller set of peanut germplasm. They are now examining the inheritance of abiotic stress tolerance and hope to develop molecular markers that can be useful in breeding.



PAXTON PAYTON (D1559-1)



PAXTON PAYTON (D1560-1)

“Identifying drought responses in the mini-core collection can help us determine potential genetic components involved in abiotic stress tolerance,” says Payton. “Our study identifies potential targets for breeding and genetic engineering of abiotic stress tolerance in peanut crop plants.”

Perhaps most importantly, the phenotypes Payton and Kottapalli identified in the screening process were confirmed by field trials under stress-inducing conditions. Puppala and Burow are using the results of this and other screening experiments to make crosses to improve abiotic stress tolerance in peanut.

Payton and the researchers are also testing peanut's response to other abiotic stresses. In recent tests, they found that the most heat-tolerant accessions were also the most drought tolerant. They also plan

**Above: Drought-tolerant peanut yield from full irrigation (top) and deficit irrigation (bottom). Even with less water, drought-tolerant peanuts still yielded fairly well.**

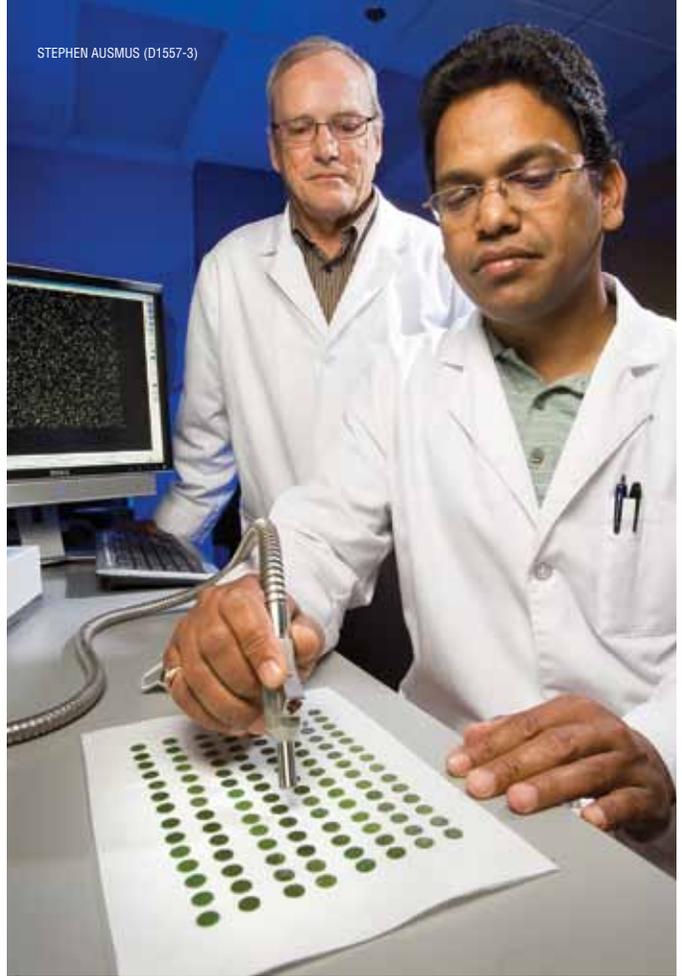
**Below: Drought-sensitive peanut yield from full irrigation (left) and deficit irrigation (right). With less water, the sensitive peanuts yielded about 90 percent less.**

PAXTON PAYTON (D1561-1)



PAXTON PAYTON (D1562-1)





to build on previous salt-stress work by AgriLife and Texas Tech scientists and conduct low-temperature screenings and tests in the coming year.

### Developing New Farming Techniques

NPRL agronomist Wilson Faircloth and plant stress physiologist Diane Rowland (formerly with NPRL and now with Texas AgriLife Research) conducted field trials for 5 years in west Texas to determine the effects of deficit irrigation and, more recently, conservation tillage on peanut performance.

“Under deficit-irrigation management early in the season, plants appeared to reach maturity sooner than under late-season or other water deficits, and they maintained their yields,” says Faircloth. “Exposure to induced early-season drought may acclimate the crop to the drought stress that commonly occurs during late-season growth.”

It is also during this latter part of the growing season that growers typically experience water deficits due to reduced pumping capacity, further increasing the risk of yield loss.

“We wanted to find out whether conservation tillage with a cover crop could be used as an additional drought-mitigation tool,” says Faircloth. “To do this, we did a field study to compare traditional, high-intensity tillage to conservation tillage with a rye cover crop.”

To be sure the research would hold up under grower conditions, the scientists tested it in one half of a center-pivot irrigation system located in Lubbock, Texas. They subdivided it into six half-acre sections and applied varying amounts of water to peanut and cotton planted in either conservation or conventional tillage.

“We used varying amounts of water to simulate a range of conditions—from normal season-long irrigation to early-season drought to late-season drought,” says Faircloth. Irrigation treatments were done in the traditional manner (meeting 100 percent of the water need) and to simulate early-season drought (50 percent

**ARS physiologist John Burke and postdoctoral researcher Rao Kottapalli perform a chlorophyll fluorescence assay on peanut leaf samples from irrigated and drought-stressed plants. This assay is one of several that are used to screen germplasm for variability in abiotic stress tolerance. The monitor in the background shows an image of a peanut DNA microarray that is used to identify genes involved in stress response and to compare contrasting germplasm.**

of the full amount for the first 45 days after crop emergence) and late-season drought (50 percent of the full amount for the 45 days before harvest). Soil conditions and plant physiological responses were intensively monitored in the test areas. These included soil water content, plant photosynthetic rates, metabolic fitness, soil and canopy temperatures, rooting patterns, and tissue collections for genomic expression.

“Conservation tillage in the form of strip tillage was successfully demonstrated in this series of field tests, a first for peanut in this region,” says Faircloth. Additionally, under deficit irrigation, strip tillage increased yield when compared to conventional tillage. “This yield increase was attributed to increased water-holding capacity of the soil and changes in peanut rooting patterns. The combination of conservation tillage and deficit irrigation promotes conservation of water during the early season, and if implemented

over a large geographic area such as the Southern High Plains, it has potential to significantly affect late-season water issues by reducing the amount of water needed for peanut irrigation.”

With more research, ARS will be able to help peanut farmers prepare for changes in the environment, which will help keep this delicious and nutritious legume available for generations to come.—By **Stephanie Yao** and **Sharon Durham**, ARS.

*This research is part of Agricultural System Competitiveness and Sustainability (#216), Plant Biological and Molecular Processes (#302), and Quality and Utilization of Agricultural Products (#306), three ARS national programs described at [www.nps.ars.usda.gov](http://www.nps.ars.usda.gov).*

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# Opening Up a New World of **Maize**



Steve Pigozzo, a North Carolina State University research assistant, pollinates a corn plant.

Maize, or corn, is the most diverse crop species known. On average, two maize lines can be as genetically different as a human is from a chimpanzee, and such diversity can make sorting out the genetic basis of key traits particularly challenging. But a specially designed collection of maize lines is so large, diverse, and well-characterized that it has become a powerful and widely used genetic tool for researchers around the world who search for genes to enhance the crop's desirable traits.

This collection of maize lines, called the “Nested Association Mapping” (NAM) population, has been developed by Agricultural Research Service scientists over the past several years by crossing a commonly studied corn variety (B73) with 25 diverse lines and repeatedly self-fertilizing the offspring to create 5,000 inbred lines, each with a unique combination of the parental lines' genetic traits. The resulting plants, grown at ARS sites near Ithaca, New York; Raleigh, North Carolina; and Columbia, Missouri, are tagged in the

field with bar codes, and their growth rates and other characteristics are carefully measured.

The project team includes Ed Buckler, a plant geneticist at the ARS Robert W. Holley Center for Agriculture and Health in Ithaca; plant geneticist Jim Holland in the ARS Plant Science Research Unit in Raleigh; plant geneticists Michael McMullen and Sherry Flint-Garcia in the ARS Plant Genetics Research Unit in Columbia; and Stephen Kresovich, a former Cornell University plant geneticist who is now vice president for research and graduate education at the University of South Carolina.

The NAM strategy consists of compiling two data sets—detailed genome-sequencing data from the parent plants and a broader glimpse of the genetic details in the larger population—that can be cross-referenced to provide detailed genetic descriptions of all 5,000 lines. “If you want to look at the diversity of maize, with the 25 lines we chose as parents, this is as good as it gets,” says Buckler, a cofounder of the project.

Buckler, Holland, McMullen, Flint-Garcia and others are using the NAM lines to search for quantitative trait loci (QTLs) and other signposts along the corn genome that can be linked to desirable qualities and traits. QTLs are stretches of DNA that might encode different combinations of traits.

The collection of NAM lines has proved invaluable not only to ARS scientists, but also to researchers at a dozen universities and a number of seed companies that use the lines to study a range of traits, including drought tolerance, disease resistance, and dozens of features related to nutritional quality and biofuel development. The work is addressing both practical and basic scientific questions, such as whether a few genes produce major effects or many genes produce minor effects, or whether some combination of both occurs. “It’s one of the first systematic ways to organize and map the genetic diversity of maize from around the world,” Buckler says.

# Traits



KEITH WELLER (K7743-13)

Maize has tremendous genetic diversity. The nested association mapping (NAM) population is beginning to tap into some of this diversity to help improve corn.



PEGGY GREB (D1919-1)

Geneticist Sherry Flint-Garcia collects corn leaf tissue for DNA genotyping while Michael McMullen, also a geneticist, records field observations with a hand-held field computer.

## Taking a Deeper Look at Photoperiod Sensitivity

Having developed the NAM population, Holland, Buckler, and their colleagues are now taking a closer look at some of the genes that control flowering time and a related characteristic known as “photoperiod sensitivity,” or sensitivity to day length.

The origins of maize can be traced to tropical Latin America. There, maize plants flower as the days grow shorter. When maize was carried from the tropical regions to temperate regions of the Americas, it had to adapt to the longer day lengths found during the summer in the temperate regions. Today, this adaptation is a major genetic difference between tropical and temperate maize.

“Tropical maize possesses genetic diversity that could be used to improve maize in temperate regions, such as the United States,” explains Holland. “But because tropical maize flowers very late when grown under long day lengths, undesirable traits such as poor yield can mask other favorable traits such as disease resistance. Our goal is to better understand the mechanisms behind photoperiod sensitivity so we can access the genetic diversity of tropical maize more easily.”

Buckler also says that understanding flowering time in corn would help in understanding it in other plant species. For example, if researchers want to move genetic material around within a plant’s genome to improve another trait, they need to know where flowering is controlled in the genome. “We don’t want to move a gene variant for disease resistance and inadvertently affect flowering time,” Buckler says.

In findings published last year in *Science*, the team reported that the large differences in flowering time among different lines of maize are caused by the cumulative effects of up to 56 QTLs, but that overall, each QTL has a relatively small effect.

“For an outcrossing species like maize, there are generally lots of genes with little



Michael McMullen records plant height with a hand-held field computer.

effects. But if you line up these little effects together, you can do some very big things,” Buckler says.

### Tropical-Temperate Crosses

In a separate project, Holland’s team crossed two tropical, photosensitive maize lines with two temperate maize lines. The tropical lines—one from Mexico, the other from Thailand—were chosen because they flower very late under the long day lengths of the U.S. Corn Belt. Including them in the study also helped Holland compare photoperiod response in tropical maize from different regions. The temperate lines represented the two main breeding pools found in the United States.

Through genetic mapping, the team sufficiently narrowed the maize genome to pinpoint four QTLs that are associated with photoperiod sensitivity. These QTLs—named “*ZmPRI-4*” by the researchers—represent 2 percent of the genetic map.

To genetically characterize their lines, Holland’s team used molecular markers originally developed by Buckler and McMullen for the NAM population. Although Holland’s lines were developed

independently of the NAM population, they are now being integrated as an extension of the NAM population, because both populations were mapped using the same molecular markers.

“We also compared the QTLs responsible for photoperiod sensitivity with similar ones found in model plant species, such as *Arabidopsis* and rice,” says Holland. “We found related genes in maize and the model species—but not in the same important genomic regions. Some of the known photoperiod regulators in *Arabidopsis* and rice do not appear to be major photoperiod regulators in maize.”

Holland is currently confirming that the QTLs found in the tropical-temperate crosses are also important in the NAM population. “If we can focus on a few genome regions to select for genes that permit earlier flowering under long day lengths, the offspring will be better adapted to the long day lengths of temperate regions. Breeders can then focus on incorporating desired traits, such as increased yield and disease resistance, into U.S. maize varieties.”

### Analyzing Recombination Effects

In Columbia, McMullen and collaborators genotyped 4,699 lines of the NAM population and were able to analyze 136,000 “recombination events,” areas where the parental genes recombined. McMullen is a co-principal investigator of the project and leads genetic map construction of the NAM population—conducting gene sequencing, population development, genetic assays, and measuring plant traits in the field.

McMullen’s genetic mapping work has revealed enormous differences among families of corn plants with regard to recombination rates, which are the frequencies of physical exchanges of chromosomal portions from the different parents of a cross. They found that recombination rates are lower in the center of the chromosomes than in the chromosome ends.

“This limits the possibilities for selecting a superior line. To be able to generate

the best combinations of beneficial genes in a plant, you need strong recombination rates,” says McMullen. “And that’s a problem over much of the corn genome.” The findings, also published in *Science* in 2009, highlight a challenge for the scientists: How to improve recombination rates in the central regions of the maize chromosomes.

Also in Columbia, Flint-Garcia is using NAM to understand the genetic control of kernel composition traits, such as starch, protein, and oil. She specializes in plant breeding, and her results could be used to genetically custom design corn kernels to fit the various oil-, starch-, and protein-content needs of farmers and end users.

“When designing maize for ethanol production, high starch is preferred,” says Flint-Garcia. “Poultry farmers prefer their maize feed to have high oil content.”—By **Dennis O’Brien** and **Stephanie Yao**, ARS, and **Alfredo Flores**, formerly with ARS.

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

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Sherry Flint-Garcia examines and shells seed of the NAM inbred line founders.

# Want To Know What a Gene Does? Use a Little MAGIC

Do you believe in MAGIC? Agricultural Research Service plant geneticist Peter Balint-Kurti and colleagues at Purdue University do, especially when it comes to testing gene function in maize plants. But these scientists aren't using card tricks or pulling rabbits out of hats. For them, MAGIC is an acronym that stands for "mutant-assisted gene identification and characterization."

"MAGIC is a gene-centered approach that uses mutant genes or other genetic variants controlling a trait of interest as 'reporters' to identify novel genes and variants for that trait," explains Balint-Kurti, who is in the ARS Plant Science Research Unit in Raleigh, North Carolina. He teamed up with Guri Johal and Cliff Weil at Purdue to create MAGIC.

MAGIC offers researchers a new way of sifting through the large amount of natural genetic variation present in most plant species to identify and map versions of genes important for the control of specific agriculturally useful traits. MAGIC starts out with a clearly defined mutation affecting a trait of interest and then identifies other genomic regions that affect the same trait. By using a mutation, the trait in question can be exaggerated, making it easier to spot other genes affecting the trait that would not otherwise be readily detectable. The process involves crossing the mutant gene into plants with different genetic backgrounds and then evaluating the offspring for the strength of the mutant phenotype.

PETER BALINT-KURTI (D1933-1)



Two leaves from sibling plants. The plant on the left has the *Rp1-D21* gene, which causes hypersensitive response (the mottled yellow appearance). The plant on the right doesn't have this gene.



JOE BUNDY (D1934-1)

ARS plant geneticist Peter Balint-Kurti (right) and postdoctoral researcher Adisu Negeri examine mutant corn plants for hypersensitive response lesions.

Balint-Kurti and colleagues demonstrated MAGIC's usefulness when examining "hypersensitive response" in maize. Hypersensitive response is a defense mechanism used by all plants in which one or a few cells surrounding the site of pathogen attack essentially commit suicide to prevent further spread of the pathogen.

In a study published in *Genetics*, the researchers reported on mutant gene *Rp1-D21*, a partially dominant disease-resistance gene that causes hypersensitive-response lesions to form spontaneously all over the plant—whether the pathogen is present or not. They found that the gene's phenotype is profoundly affected by genetic background. The scientists crossed a plant containing *Rp1-D21* with offspring of B73 and Mo17, two popular maize inbred lines. B73 partially suppresses the *Rp1-D21* phenotype, and Mo17 partially enhances it. With this approach, the scientists identified a genomic location involved in the pathway controlling the start or local spread of hypersensitive response.

"By using the MAGIC technique, breeders can effectively screen the vast amount of genetic diversity present in maize—and in other plant species—for

lines and genomic locations that have genes useful for controlling important plant traits, such as drought tolerance, sugar accumulation, and aluminum tolerance," says Balint-Kurti. "In the present study, we are identifying variation controlling the plant defense response, which has been essentially invisible to us until now. This brings us one step closer to producing disease-tolerant cultivars."

More hypersensitive-response studies are under way. Funded by the National Science Foundation, the scientists are now using MAGIC to screen the 5,000-line Nested Association Mapping population—recently established by ARS scientists—for other genomic regions responsible for hypersensitive response. (See story on page 4 of this issue.)—By **Stephanie Yao, ARS.**

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

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# Protecting Corn Crops from Aflatoxin

*Aspergillus flavus* and *A. parasiticus* are naturally occurring soil fungi that are capable of invading food and feed crops and contaminating them with aflatoxin. Aflatoxin is a human carcinogen produced by the fungi and is also toxic to pets, livestock, and wildlife.

In addition to the safety hazard posed by aflatoxin contamination, the fungi place a significant economic burden on food and feed industries to ensure that contaminated products are prevented from reaching the food and feed supply.

Agricultural Research Service (ARS) researchers in Georgia, Mississippi, and Louisiana are looking at different approaches for control of aflatoxin contamination: One is “competitive exclusion”—using benign strains to impede colonization by harmful strains—and the other is developing corn germplasm that resists buildup of aflatoxin.

## Who’ll Win This Competition?

Recently retired microbiologist Joe Dorner at the National Peanut Research Laboratory in Dawson, Georgia, helped develop Afla-Guard, a biological control for *A. flavus* and *A. parasiticus* in peanuts.

Afla-Guard is composed of hulled barley coated with spores of a nontoxic strain of *A. flavus*. The nontoxic *Aspergillus* fungi successfully compete against the toxic species for the limited space and nutrients each needs to grow and thrive. In peanuts, Afla-Guard reduced aflatoxin by an average of 85 percent in farmers’ stock peanuts and up to 97 percent in shelled, edible-grade peanuts.

In light of this success, Dorner and other ARS scientists conducted a 2-year study of Afla-Guard in corn. They again found that it was effective in reducing aflatoxin levels—showing an overall reduction of 85 percent, when compared to the levels found in control fields.

Afla-Guard was applied to the corn crop in different ways: to soil when corn was less than a meter tall, in plant whorls prior to tassel formation, and as multiple sprays during silking.

“Afla-Guard has shown that it has a place in reducing aflatoxin in corn crops,” says Dorner. “After extensive study and research trials in Texas, Afla-Guard was registered by the U.S. Environmental Protection Agency (EPA) for use on corn, and that began with the 2009 crop.”

## Neutralizing Mycotoxin

ARS scientists in Stoneville, Mississippi, are also using competitive exclusion to manage aflatoxin in corn. Their studies use a benign *Aspergillus* strain dubbed “K49” to outcompete the harmful fungi. In 4 years of field trials, K49 applications to corn reduced aflatoxin by 60 to 94 percent.

To understand how K49 colonizes corn kernels, ARS plant pathologist Hamed Abbas used the pin-bar inoculation technique of applying the treatment. In this method, small finishing nails embedded in a wooden dowel are dipped in a K49 spore suspension and then used to penetrate corn husks in order to inoculate the kernels inside with the beneficial strain.

“This benign *Aspergillus* culture reduces both the toxigenic species and the mycotoxins they produce, which is very exciting,” says Abbas, who is in ARS’s Crop Genetics Research Unit in Stoneville. A cooperative research and development agreement was established this year with industrial partners to develop the technology, and large-scale, multi-location field trials are in progress, he says. “We are developing novel formulations of K49 and Afla-Guard to make foliar and aerial application feasible in the future.”

Other early research on competitive exclusion in cotton was done by ARS plant pathologist Peter Cotty, formerly in the ARS Food and Feed Safety Research Unit in New Orleans and now at the unit’s Tucson, Arizona, worksite. In 1996, ARS was awarded EPA approval to test one *Aspergillus* formulation, named “AF36,” in commercial cotton fields in Arizona. Successful testing paved the way for additional approvals and uses. EPA registration for use of AF36 on corn is pending.

## Corn Germplasm Lines Resist Aflatoxin

“The presence of aflatoxin in corn grain greatly reduces its value and marketability,” says ARS geneticist Paul Williams, who has worked on identifying and developing corn germplasm lines with genetic resistance to *A. flavus* infection and the subsequent accumulation of aflatoxin.

Corn, in particular, has been hit hard by aflatoxin, with annual losses to the corn industry estimated at \$192 million.

Williams, who is in the ARS Corn Host Plant Resistance Research Unit (CHPRRU) at Mississippi State, leads a multidisciplinary research team of five ARS scientists located at Mississippi State University and works with other university and ARS collaborators from several states.

Williams and his scientific team have developed and released germplasm lines that exhibit the highest known levels of resistance to *A. flavus*. In field trials conducted in Mississippi in 2008 and 2009, mean aflatoxin accumulation was about 95 percent lower in the hybrids produced by crossing the ARS-developed germplasm lines than in a group of commercial hybrids adapted to Mississippi.

In the 2008 field trials, germplasm lines Mp715 and Mp717 exhibited the highest levels of resistance to aflatoxin contamination. In 2009 the recently developed germplasm line Mp04:097 performed well in the trials: Hybrids produced by crossing Mp04:097 with other resistant lines exhibited the lowest levels of aflatoxin accumulation.

Williams, geneticist Marilyn Warburton, and plant pathologist Gary Windham are also mapping quantitative trait loci (QTLs) associated with resistance to aflatoxin accumulation in crosses between resistant lines (Mp715 and Mp717) and susceptible lines with good agronomic qualities. Their goal is to identify linked markers that can be used in marker-assisted breeding. Geneticist Matthew Krakowsky, in the ARS Plant Science Research Unit, Raleigh, North Carolina, has cooperated in these investigations.

## ARS researchers are working on different approaches to the problem of toxic *Aspergillus* fungi.

Williams says, “Aflatoxin accumulation is highly sensitive to environmental variations, and resistance is a highly quantitative trait, meaning that it’s controlled by multiple genes. This makes breeding for resistance a challenge. We believe that molecular markers could be the key to the production of corn hybrids with resistance to aflatoxin accumulation.”

The research team also found that the Mp715 and Mp717 lines are resistant not only to aflatoxin accumulation, but also to fumonisin accumulation. Fumonisin, like aflatoxin, is a mycotoxin—a toxic metabolite—and is produced by *F. verticillioides*. The toxin causes neurologic abnormalities in horses—such as weakness of the face and pharyngeal muscles, facial desensitization, and a tendency to lean to one side—after they’ve consumed infected corn.

“These lines should be useful in developing corn lines and hybrids with resistance to both fumonisin and aflatoxin accumulation in grain,” says Williams. The lines have been widely requested and used in plant breeding programs in state, federal, and international research institutions plus three major commercial seed corn companies and several smaller companies.

The CHPRRU scientists have also developed and released corn germplasm lines with resistance to fall armyworm and southwestern corn borer, thus reducing the devastating leaf feeding by the two pests. Williams, along with CHPRRU agronomist Paul Buckley, tested 20 single-cross corn hybrids in laboratory bioassays: Larvae of both fall armyworm and southwestern corn borer weighed significantly less when fed the ARS-developed corn leaf tissue than when fed the susceptible hybrids. The CHPRRU scientists have demonstrated that growing hybrids that sustain less damage from such ear-feeding insects as fall armyworm, southwestern corn borer, and corn earworm results in reduced aflatoxin contamination.

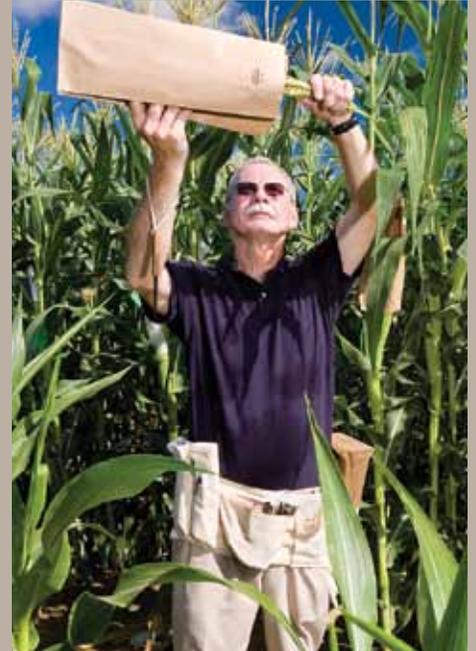
In related research, by comparing aflatoxin-susceptible with aflatoxin-

STEPHEN AUSMUS (D1897-23)



In Stoneville, Mississippi, plant pathologist Hamed Abbas uses the pin-bar inoculation technique to evaluate the colonization of corn by the biological control agent K49.

STEPHEN AUSMUS (D1903-6)



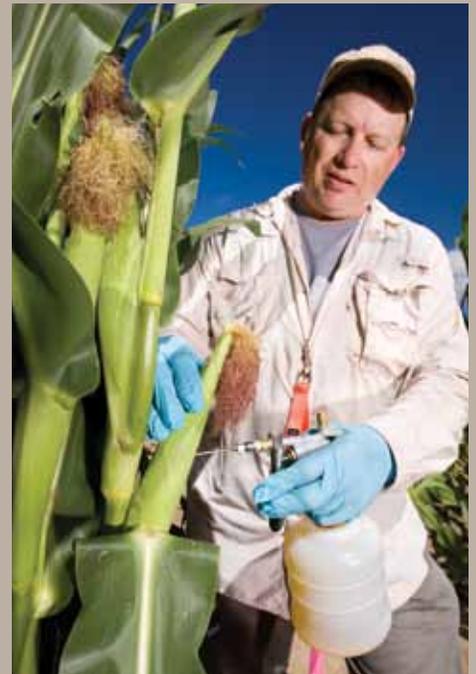
In Starkville, Mississippi, geneticist Paul Williams places bags over the tassels of corn plants with the desired molecular markers to collect pollen.

STEPHEN AUSMUS (D1901-11)



At Starkville, molecular geneticist Marilyn Warburton harvests leaf matter for DNA genotyping. The goal is to identify plants with markers for resistance to *Aspergillus flavus* and aflatoxin accumulation.

STEPHEN AUSMUS (D1904-11)



To determine the resistance of corn lines to aflatoxin accumulation, plant pathologist Gary Windham injects *Aspergillus flavus* spores into corn ears grown in research plots at Starkville.



STEPHEN AUSMUS (D1896-6)



In Stoneville, Mississippi, biologist Bobbie Johnson adds ammonium hydroxide to *Aspergillus* cultures to differentiate aflatoxin-producing strains (yellow and red) from nonproducers (off-white).

resistant corn lines (some developed by Williams), ARS plant pathologist Robert Brown in New Orleans identified and characterized a number of proteins that may be important in imparting resistance to corn. (See “Hardy New Corn Lines Resist Toxic Fungi,” *Agricultural Research*, Oct. 2009, p. 14.) In the collaboration with Warburton mentioned above, the genes for these proteins have been mapped to chromosomal regions containing QTLs previously linked to aflatoxin resistance in the corn genome. These specific resistance-associated proteins can serve as targets for the marker-assisted breeding being carried out in Mississippi.

Expanding our horizons in corn breeding, Brown, with financial support from the USDA Foreign Agricultural Service, the United States Agency for International Development, and the ARS Office of International Programs, established a collaborative breeding program with the International Institute of Tropical Agriculture in Ibadan, Nigeria, which recently released six aflatoxin-resistant corn lines. These lines, the product of up to 10 generations of selection and inbreeding, have

STEPHEN AUSMUS (D1902-5)



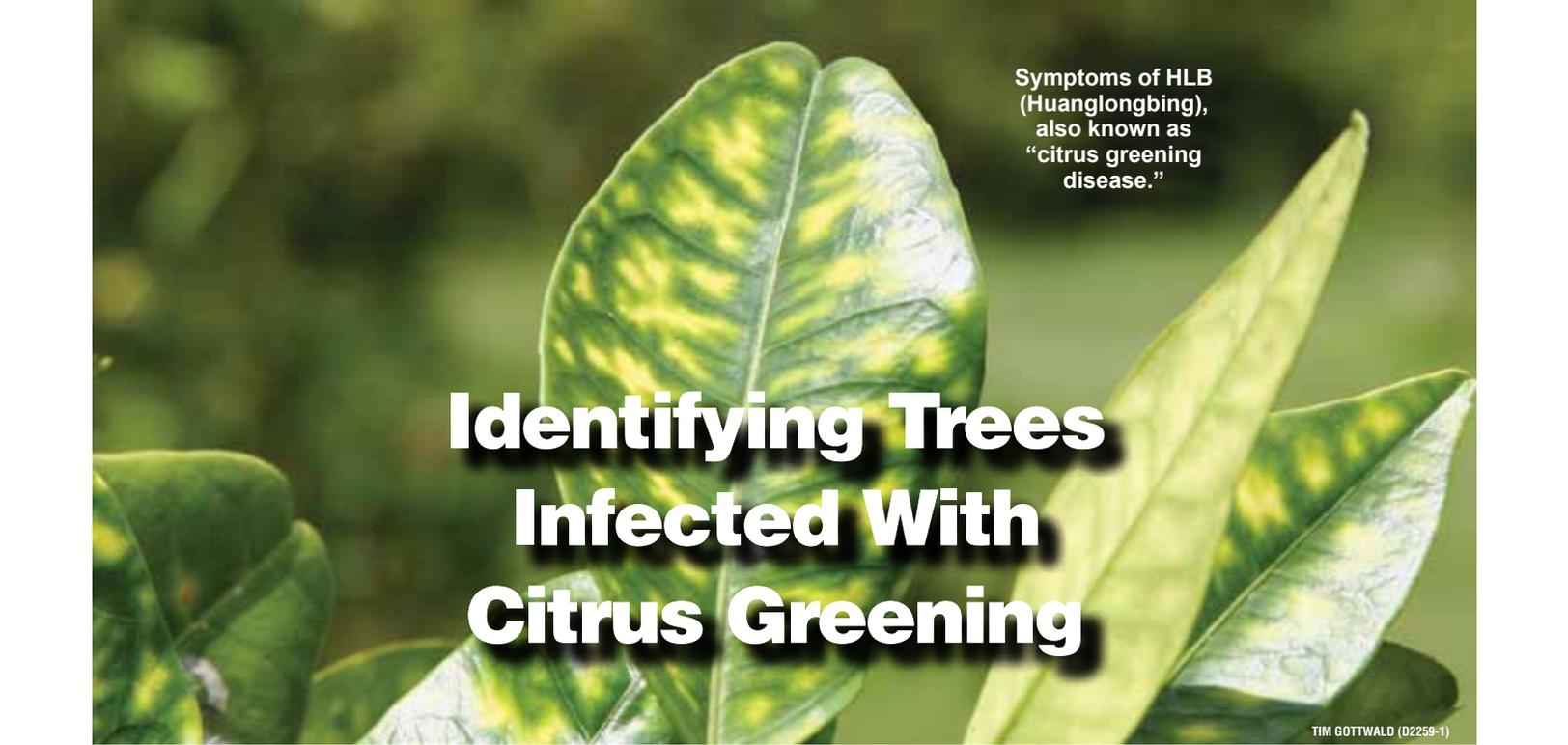
Marilyn Warburton and geneticist Paul Williams tag corn plants identified in the lab as having molecular markers associated with resistance to *Aspergillus flavus* and aflatoxin accumulation. These tagged plants will be crossed with other breeding lines to develop resistant corn hybrids.

passed quarantine and are now available in the United States from the ARS North Central Regional Plant Introduction Station, Ames, Iowa.—By **Sharon Durham** and **Jan Suszkiw**, ARS, and **Alfredo Flores**, formerly with ARS.

*This research is part of Food Safety (#108), Plant Genetic Resources, Genomics, and Genetic Improvement (#301),*

*Plant Diseases (#303), and Crop Production (#305), four ARS national programs described at [www.nps.ars.usda.gov](http://www.nps.ars.usda.gov).*

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Symptoms of HLB (Huanglongbing), also known as “citrus greening disease.”

# Identifying Trees Infected With Citrus Greening

TIM GOTTWALD (D2259-1)

**Citrus plants** are highly susceptible to a disease commonly called “HLB” (Huanglongbing), also known as “citrus greening disease.” Agricultural Research Service scientists in the Quality and Safety Assessment Research Unit in Athens, Georgia, and the Subtropical Plant Pathology Research Unit in Fort Pierce, Florida, joined forces to use a technology known as “Fourier transform infrared-attenuated total reflection” (FTIR-ATR) spectroscopy to identify citrus greening-infected plant leaves with 95-percent accuracy.

Citrus greening was discovered in Florida in 2005 and is rapidly spreading in the citrus-growing regions of the state. The disease is transmitted by the Asian psyllid, an insect found throughout Florida. Fruit from infected trees drops prematurely or fails to ripen.

A 2007-2008 survey conducted by the University of Florida, in collaboration with USDA’s National Agricultural Statistics Service and the Florida Department of Agriculture and Consumer Services, included 153,000 net acres of oranges and 17,676,000 orange trees. The survey revealed that growers—to prevent spread of disease—removed 847,208 infected trees during the survey period. Plants can be infected for up to several years before showing symptoms, and during this period the psyllid can transmit the disease agent to nearby plants.

Currently, the best method for detecting citrus greening-infected trees is a type of DNA testing called PCR (polymerase chain reaction), which is both costly and time consuming.

FTIR uses light to identify chemicals and reactions in a sample. This technology has the potential to detect the disease before visible symptoms occur and is cheaper and faster. The mid-infrared region of the electromagnetic spectrum reveals dramatic changes that occur in leaves of infected trees compared to leaves from noninfected trees. The results from this technique’s application were published in the journal *Applied Spectroscopy* in 2010.

To create the new technology, experts from both labs were needed. “ARS is great in this respect because it has so many experts with which to collaborate,” says ARS researcher Gavin Poole in the Fort Pierce unit. “Research leader Tim Gottwald and I had an idea to use spectroscopic methods to identify citrus greening before symptoms develop in the field, and our colleagues in Georgia were able to use their expertise to help us attack this problem.”

The Athens team included chemist Samantha Hawkins, engineer Bosoon Park, physiologist William Windham, and research leader Kurt Lawrence. Hawkins used an FTIR spectrometer with an ATR crystal accessory to test for the presence of citrus greening.

“We used a leaf removed from the citrus tree, dried it out in a microwave, and ground it into a powder—a simple protocol developed by Windham,” says Hawkins. A very small sample—just 0.1 milligrams—of the leaf powder was placed on top of an ATR plate. The system clearly discriminated HLB-infected leaves from healthy leaves. More work will be needed to discriminate HLB from other citrus diseases.

“This is a great method because the sample preparation is faster and easier than PCR, which is typically used to get a value of how much disease is in the leaves,” says Hawkins. “Growers currently send leaf samples out for PCR analysis that costs about \$6 to \$8 per sample and takes several days. The FTIR technique is done in seconds.”—By **Sharon Durham**, ARS.

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

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# “Fire Gel” Protects Beneficial Nematodes From Sun

PEGGY GREB (D2080-1)

The *Steinernema carpocapsae* nematode is a little worm that can protect peach and other stone fruit trees by attacking devastating borer pests. Some nematodes are pests, but these are beneficial because they can act as biological controls of the borers and other insect pests.

Trouble is, this nematode is sensitive to the sun’s harmful ultraviolet rays and heat, both of which can cause desiccation—the state of extreme dryness. In time this can cause death, so the nematodes could benefit from some sort of protection when exposed to these elements.

Agricultural Research Service scientists in Byron, Georgia, are trying a novel approach to help the tiny worms. They’re testing the same type of “fire gel” that has been used to help prevent the spread of fire to residential and commercial structures to see whether the gel, after it is sprayed onto fruit trees, can help nematodes avoid desiccation.

The gel creates a barrier between the fire and the structure it’s protecting, preventing the spread of fire. This barrier property could also serve as a moisture “blanket” for nematodes, allowing them to attack pests above ground without being harmed by the sun.

There are two species of borers that attack peaches—lesser peachtree borers,



Peaches grown at the USDA-ARS Southeastern Fruit and Tree Nut Research Laboratory.

which attack the aboveground portions of the peach tree, and peachtree borers (also known as greater peachtree borers), which attack the roots of the tree. In laboratory settings, *S. carpocapsae* nematodes proved effective in killing both borer species.

The nematodes are efficient at controlling the underground-dwelling peachtree borer but lose their effectiveness above ground. That’s because the nematodes’ chances of survival are far greater below ground—where the soil’s moisture helps them stave off drying—than above ground, where they’re exposed to sun and heat.

According to entomologist David Shapiro-Ilan, with ARS’s Southeastern Fruit and Tree Nut Research Laboratory in Byron, “If fire gels can protect a house, they may be able to protect nematodes for a few days so that they can kill the lesser peachtree borer.”

And he’s shown that the gel does just that. Shapiro-Ilan, along with fellow Byron entomologist Ted Cottrell, tested different formulations aimed at protecting the nematodes during aboveground application, including the fire gel.

After 2 years of testing, a nontoxic, environmentally friendly brand of fire gel (Barricade) was the most effective treatment. The best part was that the gel-nematode combination left only 30 percent of the lesser peachtree borers alive in 2008 (from 100 percent initial survival), and none survived in 2009.

The scientists believe the sprayable gel could be used to protect other beneficial



Entomologist David Shapiro-Ilan (left) sprays a gel formulation onto a peach tree limb while technician Wanda Evans prepares the nematode application. The gel is being used to protect beneficial nematodes from damage due to extreme drying and UV radiation. With the protective formulation, the nematodes go to work killing harmful insect pests, such as the lesser peachtree borer.

The scientific team plans to test the fire gel on grower orchards. Horton, who also serves as an extension specialist, will be instrumental in finding these grower cooperators. Horton is developing an instructional video offering farmers tips on how best to apply the gel. Mizell will conduct field trials in Quincy, Florida, to determine the best time of year to apply the gel and to see whether Shapiro-Ilan's technology is applicable in that region.

Shapiro-Ilan is also working with ARS collaborators Bob Behle and Chris Dunlap in Peoria, Illinois, as well as Lerry Lacey in Wapato, Washington. Behle, an entomologist, and Dunlap, a chemist, specialize in formulation of microbial agents and have provided Shapiro-Ilan with some of the ingredients needed for his field trials.

Lacey, an entomologist, has developed a foam formulation that consists of wood

Fire-gel sprayed onto peach tree limbs.



species besides *S. carpocapsae*. The gel might be used in combination with other beneficial nematodes to control a wide range of pests in trees or other crops above ground.

Shapiro-Ilan and Cottrell received funding from a U.S. Department of Agriculture Pest Management Alternatives Program and collaborated with entomologists Dan Horton at the University of Georgia and Russ Mizell at the University of Florida.

fiber, wood flour, and starch. This formulation forms a protective crust, whereas the gel is sticky.

Shapiro-Ilan has tested the two formulations—gel and foam—and found that the gel worked best for borers in the southeast, possibly due to the hot, dry climate. Lacey found that the foam worked better in the wet, cool northwest, protecting nematodes that attack the codling moth, a destructive pest of apples and pears. Shapiro-Ilan and Lacey will continue to test the formulations under a variety of conditions.

The scientific team is researching how to further optimize application rates and timing and to perform large farm-scale tests using air-blast sprayers to apply the fire gel. The scientists will also test air handgun sprayers, which efficiently disperse the fire gel in a uniform spray pattern. Additional laboratory tests are being conducted in search of superior nematode strains.

“We’re going to test how much and how often we’d like to disperse the fire gel through these means,” said Shapiro-Ilan, who plans to work with small and organic growers first and continue with large-scale growers in the next few years. “We’d like to make this process as efficient and economical as possible so we can get the most bang for our buck. Growers are savvy enough that if something makes economic sense, they’re going to do it.”—  
By **Alfredo Flores**, formerly with ARS.

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

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Inset: A lesser peachtree borer larva (*Synanthedon pictipes*) emerging from a wound it made in a peach tree.

Right: A wound in a peach limb made by lesser peachtree borer (*Synanthedon pictipes*).



Entomologists Ted Cottrell (above left) and David Shapiro-Ilan observe beneficial nematodes (*Steinernema carpocapsae*) that are used to control the lesser peachtree borer. Shapiro-Ilan and Cottrell developed a sprayable gel as a novel method for protecting the nematodes from desiccation (extreme dryness) and UV radiation.



# Pickle Spoilage Culprit

## May Give the Environment a Helping Hand

If the next jar of pickles you reach for looks a little red—stop! From time to time pickled cucumber products can get a red coloration indicative of benign spoilage bacteria. Though this doesn't happen often, it can still be an unpleasant experience. ARS scientists in the Food Science Research Unit (FSRU) in Raleigh, North Carolina, have found its cause, how to prevent it, and more.

According to ARS microbiologist Ilenys Pérez-Díaz and her colleagues, some species of *Lactobacilli*—food-related microorganisms—can cause the red coloring when combined with tartrazine, which is a yellow food-coloring agent.

But *Lactobacilli* can be beneficial, too. During testing, the FSRU team noted that several *Lactobacilli* also modify azo dyes—dyes used in the textile industry that may be passed along to wastewater streams if untreated. This is the first report that food-related microorganisms can transform azo dyes.

Azo dyes impart vivid and warm colors such as red, orange, and yellow to fabric. Though many azo dyes are nontoxic, some have been found to be mutagenic. “Considerable effort has been made to identify microorganisms capable of degrading azo dyes in wastewater streams from the textile industry,” says Pérez-Díaz. “If food-grade *Lactobacilli* capable of degrading a range of azo dyes were identified, they might be organisms of choice for waste-treatment applications.”

This revelation spawned from research that began long ago.

Thirty years ago, ARS scientists fingered *Lactobacillus casei* as the cause of the red spoilage. Another outbreak some years later prompted further research, which identified another bacterium in the *Lactobacillus* genus—*L. paracasei*—as the culprit when FD&C yellow no. 5 (tar-



**Inset:** Microbiologist Ilenys Pérez-Díaz uses spectrophotometry to examine loss of yellow coloring in a sample of cucumber brine. **Above:** A preserved cucumber that has developed red-colored spoilage from *Lactobacilli*.

trazine) was added to the brine solution. Commercial dill pickle makers use either this dye or turmeric to impart a yellowish tint to the pickles' brine.

Pérez-Díaz and her colleagues isolated *Lactobacilli* from spoiled jars of hamburger dill pickles and used those isolates to inoculate nonspoiled jars of hamburger dill pickles. “Jars that contained brines with tartrazine developed the red hue on the pickle skins; those that had turmeric or no added coloring did not,” she says. “We also found that pH level played a role in the development of red discoloration on pickle skin. A pH between 3.9 and 3.5 supported bacterial growth, disappearance of tartrazine, formation of red-colored spoilage, and a drop in pH.”

Seven treatments were tested in an

attempt to find a preventive measure for red-colored spoilage. Pérez-Díaz found that adding sodium benzoate prevented bacterial growth, disappearance of tartrazine, and development of red spoilage. According to Pérez-Díaz, this would be a practical method to prevent development of red-colored spoilage in hamburger dill pickles.—By **Sharon Durham**, ARS.

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

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# Citrus Chemical Call to Arms Found



A *Diaprepes* weevil feeds on a citrus leaf.

When chewed on by hungry caterpillars, corn, cotton, and tobacco plants release chemical distress signals that marshal help from parasitic wasps. Similarly, lima beans attacked by spider mites attract predatory mites.

Now, an Agricultural Research Service-University of Florida team has shown that some citrus trees also resort to signaling when their roots are attacked by the grublike larvae of *Diaprepes abbreviatus*, the citrus root weevil. But instead of fast-flying wasps or nimble-legged predatory mites, the rescuers are wormlike organisms called “nematodes,” which wriggle inside the grubs and feed on them internally, killing the citrus pests in 24 to 48 hours.

“When weevil larvae feed on roots,” says ARS chemist Hans Alborn, “the roots release volatiles, including terpenes, that diffuse into the surrounding soil.” Nematodes in the soil track those chemical cues back to their source—namely, roots needing rescue from further harm.

Potentially, the finding could yield new, improved ways of using the nematodes to

biologically control *D. abbreviatus*. The species, which is native to the Caribbean region, was accidentally introduced into Florida in 1964. Today, it’s considered a major agricultural pest that causes \$70 million annually in losses not only to citrus, but also to ornamental plants and other crops.

The weevil also poses an aboveground threat to Florida’s citrus crop, valued at \$993 million during the 2008-2009

season. As an adult, the pest feeds on leaves, giving them a notched appearance. “Notching is one of the first things you see when entering an infested grove,” says Jared G. Ali, a University of Florida researcher collaborating with Alborn, who is in the ARS Chemistry Research Unit in Gainesville, Florida.

Adult weevils can be controlled by spraying the tree canopy with foliar insecticides. Also effective, though far more time-consuming and labor-intensive, is shaking the canopy and trapping the weevils that fall from trees or emerge from the soil.

Synthetic pyrethroids are used to control the grubs. Some of these chemicals are meant to serve as a barrier to larvae that fall to the ground after hatching from eggs deposited in the canopy. Even then, “many larvae still make it into the soil,” notes Ali, who works in the laboratory of Lukasz Stelinski, an assistant professor

ARS chemist Hans Alborn loads an autosampler with vials for gas chromatography and mass spectrometer analyses of root volatile collections.

## Could Improve Use of Nematode “First Responders”

PEGGY GREB (D2051-1)



Above: University of Florida collaborator Jared Ali adds insect-killing nematodes to a six-arm olfactometer to test their attraction to different citrus rootstock cultivars attacked by *Diaprepes* root weevil larvae. The olfactometer is filled with sandy soil to simulate conditions below ground.



PEGGY GREB (D2057-1)

at the University of Florida's Citrus Research and Education Center in Lake Alfred, Florida.

Once below ground, the grubs feed on the tree's fibrous roots until ready to pupate, emerging as adults anywhere from 6 to 15 months later. Severe infestations weaken the tree and reduce overall fruit yield. Feeding also increases the likelihood of infection by *Phytophthora* fungi, which cause root rots that can speed the tree's demise at great cost to growers.

### Biological Alternatives

Life below the citrus grove is not without its hazards, however. That's because the grubs are a favorite food of some species of insect-killing, or "entomopathogenic," nematodes such as *Steinernema riobrave* and *Heterorhabditis indica*. Both have been commercially formulated into biopesticide products that can be applied to grub-infested groves using existing herbicide applicator technology or other micro-sprinkler systems.

After encountering a grub, and infecting it, the nematodes release symbiotic bacteria that render the pest's tissues into a kind of slurry, which the nematodes then eat. Once the food is gone, the nematodes exit the carcass to start the cycle over again. The nematodes and their bacteria target a slew of insect hosts, but pose no danger to humans, pets, livestock, or wildlife.

A variety of factors, including soil type and temperature, can affect the nematode's performance as a biocontrol agent—with reductions in grub numbers at treated sites ranging from 0 to 90 percent. But until recently, little attention had been paid to the complex chemical tête-à-tête that occurs between citrus tree roots, grubs that feed on them, and the surrounding soil's resident nematodes.

When a plant chemically recruits a predator or parasite to dispatch of a herbivorous attacker—in this instance, citrus calling on nematodes to kill grubs—scientists call the phenomenon a "tritrophic interaction."



PEGGY GREB (D2054-1)



University of Florida assistant professor Lukasz Stelinski applies a nematode attractant to a nematode lure to be tested below ground in a citrus field.

PEGGY GREB (D2055-1)

A chamber used in an underground field test of nematode attractants. The chamber is filled with sand, a root weevil larva, and a filter paper impregnated with the attractant.

While aboveground tritrophic interactions are well documented, far less is known about underground ones.

"We're one of the first groups to look at these interactions from a citrus-chemical ecology perspective," says Ali who coauthored a paper on the work with Stelinski and Alborn in the March 2010 issue of the *Journal of Chemical Ecology*. "Our specific focus is gaining insight into what a citrus plant can do to protect itself," Ali adds.

### Catching the Action in Real-Time

To determine what signaling compounds citrus roots released when attacked by grubs, the scientists used a specialized glass

chamber called a "root-zone olfactometer."

"With this system, you can see the weevils chewing on the roots and pull the volatiles off as they're being released," says Ali. "You can also tease out which direction the nematodes are going" and extract them for analysis, he adds.

In lab and greenhouse trials with small trees derived from the commercial rootstock "Swingle citrumelo," roots damaged by captive grubs attracted up to three times more *S. diaprepesi* nematodes than roots that had been mechanically damaged or left intact. When root volatiles were collected and analyzed by gas chromatography-mass spectrometry, terpenes accounted for four of the six compounds that stood out. Interestingly, "the roots only released the volatiles when being fed on by larvae," notes Alborn.

The team's research could one day lead to new varieties developed from rootstocks shown to be adept at recruiting nematodes. So far, they have tested the signaling capacities

of 5 rootstocks and hope to screen as many as 20 more by the end of 2011.

The researchers are also studying the volatiles' effect on other denizens of the citrus root zone, including nematodes that parasitize plants.

Within the next 2 years, the team hopes to recommend rootstock-nematode combinations that growers can use as part of an integrated approach to managing the weevil.—By **Jan Suszkiw**, ARS.

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

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Technician Amanda Rinehart (foreground) and ecologist Nancy Kokalis-Burelle study eggplant roots for nematode damage and root health.

**A**s they search for soil treatments to replace methyl bromide, Agricultural Research Service scientists in Fort Pierce, Florida, are using one technique that sounds as if it were borrowed from a family recipe—taking molasses and heating it up.

Farmers have been using methyl bromide since the 1930s, but these days it is subject to strict environmental controls because it depletes Earth's ozone layer. As part of an international agreement, growers worldwide are being required to find a replacement, a tall order because methyl bromide eliminates a broad spectrum of pests, including weeds, nematodes, and plant pathogens. The mandate is particularly challenging in Florida, where sandy soils limit organic alternatives and the mild winters serve as a safe harbor for many of nature's most pernicious pests. Nutsedge is a particular weed problem in Florida and is a major reason methyl bromide is still used there.

"We don't usually see the cold winter temperatures that you have in other places, so pests aren't killed off here by Mother Nature the way they might be elsewhere," says Erin Roskopf, a microbiologist at the U.S. Horticultural Research Laboratory in Fort Pierce.

For Florida growers, the stakes are enormous. Fresh-market tomatoes and bell peppers, both raised in Florida with methyl bromide, netted growers there a combined \$889 million in 2008. Caladiums, an ornamental also produced largely with methyl bromide, are a \$15 million crop in Florida.

In their search for alternatives, ARS researchers are studying two approaches: a biologically based cropping system that builds on success overseas, and two recently developed fumigants. Similar approaches have had some success elsewhere, but the question is whether they will be effective in Florida, with its unique soils, climate, and pest pressures. Preliminary results are promising, but the researchers still need to evaluate whether the biologically based treatments can sufficiently control nutsedge.

#### **A Biological Alternative**

At the Fort Pierce lab, Roskopf, ecologist Nancy Kokalis-Burelle, and soil scientist David Butler are raising bell peppers followed by eggplant in a field to test a biological approach that uses a combination of composted broiler litter, molasses, and anaerobic soil disinfestation (ASD). Lab colleagues Gregory T. McCollum and Joseph Albano are evaluating fruit quality and soil nutrients.

In ASD, a carbon source—in this case molasses—is added to stimulate microbial activity, and the soil is covered with a clear plastic tarp. The topsoil is saturated with water and allowed to heat. The sun-drenched tarp "cooks" the weed seeds in the soil, and the carbon and water increase microbial activity and create anaerobic conditions conducive to pest control.

In this study, before heating the soil, the researchers treated plots with different

# Investigating T to Replacing M

levels of organic amendments: with and without poultry litter; with and without molasses; and with 2 inches of water per acre, 4 inches per acre, or no water at all. The poultry litter increased soil moisture and added organic matter, and the molasses provided a readily available carbon source for soil microbes. The molasses they used is a waste product of the sugarcane processing industry. The researchers are also conducting studies to evaluate mustard meal, sorghum, and cowpeas as possible alternatives to molasses.

They planted peppers in the fall and eggplant in the spring for 2 years, using the type of raised-bed production system common in Florida. Before planting, the researchers introduced *Phytophthora capsici* to the fields, an oomycete that causes crown rot and root rot, so they could track control rates. They buried packets of it 6

In an anaerobic soil disinfestation trial for growing eggplant, researchers conduct soil nutrient analysis and plant pathogen population assessments.



# Two Pathways Methyl Bromide

inches deep in the soil and retrieved them after 3 weeks of ASD to evaluate the effects. They also sampled the soil for nematodes, counted the number of nematodes extracted from crop roots, assessed weed populations and soil properties throughout the trials, and measured crop yields.

They found that at depths of 6 inches, the ASD treatments heated the soil temperature to about 113°F, which was at or just below lethal levels for many soil pathogens. They also found that nematode populations were reduced when treated with molasses and poultry litter, that molasses and poultry litter controlled grass weeds just as well as methyl bromide did, and that the ASD treatments controlled *P. capsici* in the buried packets as well as methyl bromide did, regardless of how much water, poultry litter, or molasses was applied.

ant, soil scientist David Butler collects a soil sample for ment.



“The nutsedge pressure at the test location wasn’t really adequate to show treatment differences, so we are still investigating that component,” Rosskopf says.

### Evaluating New Commercial Products

Rosskopf and Kokalis-Burelle completed large-scale demonstration field trials comparing the effectiveness of two recently developed fumigants with methyl bromide at two Florida sites, one where they are raising delphiniums and the other caladiums. Both dimethyl disulfide, approved for experimental use under the trade name “Paladin,” and methyl iodide, being marketed as “Midas,” have shown promise at controlling weeds, nematodes, and plant diseases.

The researchers combined each treatment with chloropicrin, a pesticide that kills fungi and is often used in combination with methyl bromide. They applied each treatment to about a half-acre of delphiniums in a field in Hobe Sound and 2 acres of caladiums at another site in Zolfo Springs. They raised two varieties of delphiniums and four varieties of caladiums.

In the caladium trials, where the pest pressure was much higher than in the delphinium trial location, their grower cooperator also applied a soil treatment often used on caladiums known as “Telone II” (1,3-dichloropropene). They measured weed density, weeding time required, and nematode and fungal populations in the soil. They also examined roots for disease and compared the sizes and numbers of flowers produced under each treatment.

Preliminary results show the alternative fumigants were just as effective as methyl bromide at suppressing grass weeds and at controlling nematodes, but that the ef-



Bell peppers and a wide variety of other vegetables and fruits may benefit from anaerobic soil disinfestation, a potential alternative to methyl bromide fumigation for pest control.

fectiveness of Paladin was reduced when it was used year after year.

In the delphinium trial, there were no significant differences in weed density and total weeding time or in total flower yields. In the caladium trial, they found no significant differences among methyl bromide, Paladin, and Midas in terms of total weeds, “rogue” or off-variety plants produced, or in total hours of labor for weeding, but yields were dependent on the combination of fumigant and cultivar.

“This interaction indicates that growers will have harder decisions to make, because their choice of cultivar is often market-driven. They may have to choose a fumigant that provides the greatest return for that specific cultivar, making it much more difficult than when they could use methyl bromide with everything they grew,” said Rosskopf.

The fumigant field trials are attracting widespread interest. A recent field day organized to explain the project at Zolfo Springs attracted dozens of cut-flower and caladium growers.—By **Dennis O’Brien**, ARS.

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

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**Microcracks** in the shells of eggs pose a major food safety concern to consumers. Research has shown that cracked eggs are more likely to harbor bacteria, including foodborne pathogens. A new technology—called the “modified-pressure imaging system”—combines negative pressure with imaging and can accurately detect almost 100 percent of cracked eggs.

The device, developed by Agricultural Research Service scientists in Athens, Georgia, was discussed in detail in “A Better Way To Spot Eggshell Cracks,” *Agricultural Research*, February 2009. It uses a negative-pressure chamber to essentially pull the eggshell outward very gently to clearly show any microcracks present in the shell.

The next step was to see whether quality suffered after eggs were subjected to the device.

Food technologist Deana Jones, research leader Kurt Lawrence, engineer

## Egg Quality Preserved After Exposure to Egg Crack Detection Technology

Seung-Chul Yoon, and hyperspectral image specialist Gerald Heitschmidt, in the Egg Safety and Quality Research Unit, conducted a study to determine whether exposure to the modified-pressure imaging system had any effect on egg quality during storage.

“After 5 weeks of refrigerated storage, only a slight difference in the amount of water in the whole egg was noted between imaged and nonimaged eggs,” says Jones. “All other quality attributes were the same for imaged and nonimaged eggs.”

These other quality attributes include the egg’s weight, albumen (egg white) height, shell strength, vitelline (yolk) membrane strength, and Haugh unit. A

Haugh unit—considered by the egg industry as the “gold standard” of interior egg quality—is a mathematical formula that takes into account the egg’s weight and the height of the albumen.

“This information lets us know that the use of the modified-pressure imaging system to detect cracked eggs does not affect egg quality, making it an important tool for enhancing the safety of shell eggs for U.S. retail sale,” says Jones.—By **Sharon Durham**, ARS.

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## New Primer Helps Identify More DNA Markers

**DNA** markers are important tools for identifying genes that control traits of interest to plant breeders, such as disease resistance or fruit quality. DNA markers are usually generated using “primers,” short segments of DNA that are used by geneticists to look for genes that control specific traits. In general, geneticists use random primers in their search for DNA markers, but that process is often long and arbitrary, and it can result in researchers spending a lot of time and money to identify just one DNA marker.

To streamline the process, Agricultural Research Service geneticist Amnon Levi and plant pathologist Pat Wechter have developed a new method for identifying DNA markers. They used genomic data to search for small pieces of DNA, called “oligonucleotides,” that are prevalent in

watermelon genes and could be used as primers. Levi and Wechter believed that these new primers would generate a larger number of markers because they are more targeted than random primers.

Zhangjun Fei, an ARS-funded bioinformatics researcher at the Boyce Thompson Institute for Plant Research in Ithaca, New York, collaborated with Levi and Wechter and wrote a computer script to identify oligonucleotides that exist in high numbers in genes of watermelon. They named the new primers “high-frequency oligonucleotides targeting active genes,” or HFO-TAG for short.

Working from the U.S. Vegetable Laboratory in Charleston, South Carolina, the scientists and fellow ARS and university colleagues tested their theory on 12 closely related watermelon cultivars.

The researchers found that the HFO-TAG primers identified more DNA fragments than random primers did. Finding more fragments means researchers have a greater chance of finding DNA markers for genes that control desirable traits. And they don’t have to invest as much time and money to identify the markers.

Levi and Wechter are currently using the HFO-TAG primers to look for watermelon genes that control disease or pest resistance and fruit quality. The primers will also be useful in genetic studies and genetic mapping of watermelon.

According to the scientists, this simple and straightforward method can be applied to genetic studies of other plants as well as animals. A full description of this study has been published in the *Journal of the American Society for Horticultural Science*.—By **Stephanie Yao**, formerly with ARS.

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# A New Approach to an Old Problem: Devising Formulas To Enhance Growth

**T**wo researchers at the U.S. Horticultural Research Laboratory in Fort Pierce, Florida, are attracting international attention in diverse scientific fields for their approach to a common problem in science: how to predict every possible variable in an experiment where you simultaneously manipulate numerous components.

Researchers who use culture media to grow and study cells, tissues, or any biological entity typically try to optimize the media by varying one ingredient at a time, while holding all the other ingredients constant. The problem is that changing the amount of any single ingredient in a mixture changes not only the amount of that ingredient, but also its proportion to every other ingredient in the mix. The one-ingredient-at-a-time—or one-factor-at-a-time—approach fails to take into account the effects of proportions. In addition, this can be an expensive and time-consuming process, with no significant improvement in culture growth.

But geneticist Randall P. Niedz and ecologist Terence J. Evens have developed a new approach that can help researchers reformulate any type of mixture, whether it is a culture medium, fertilizer, potting soil, insect diet, or animal feed.

Niedz and Evens encountered the problem a few years ago while each was studying mineral nutrients. Niedz was trying to improve the growth of citrus tissue culture, an important area for crop improvement, but one that tends to be a challenge. Evens was studying the effects of algal growth in runoff from ornamental plants in nurseries.

A typical plant/algae culture medium contains about 16 individual mineral nutrient ions, and its effects are determined by both the amount and the proportion of each ingredient in the mix. Niedz and Evens recognized that creating a nutrient solution is a special type of mixture problem and should be defined by the composition of its individual ions. They found that by examining



**Geneticist Randall Niedz (left) and ecologist Terence Evens inspect two green algae cultures for an ongoing study of the effects of fertilizers on algal growth.**

ions independent of their parent salts, they could make sense of the specific ion effects in their solutions.

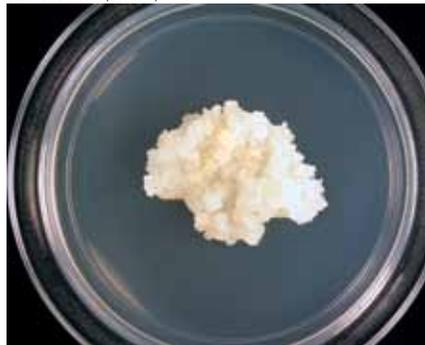
The method they developed of reconfiguring a “recipe” is complex and must be done with a computer using both an algorithm they devised and experimental designs that separate out the effects of proportionality and amount. Results are teased out in ways that reveal every possible outcome and are considerably more accurate, resource efficient, and knowledge rich than results from standard one-ingredient-at-a-time approaches, the researchers say. When applied to citrus tissue and algal culture, the results were dramatic, saving time and money and enhancing cell growth. In the citrus experiments, growth of citrus tissue cultures increased nearly 200 percent. In the algal experiments, they found unusual mixtures where algae grew vigorously and at pH levels far beyond where traditional science said they should grow.

The approach developed by Niedz and Evens is being used not only by scientists in their fields, but also by entomologists, medical researchers, and even chemists around the world to gauge the effects of varying ingredients in mixtures. They explained the approach in a letter to *Nature Methods* in June 2006 and a report in *Scholarly Research Exchange* in May 2008. They have also released a free online software package, for mineral nutrient research, that has been downloaded more than 300 times by scientists in 39 countries. The software package is available at [www.ars.usda.gov/services/software/download.htm?softwareid=148](http://www.ars.usda.gov/services/software/download.htm?softwareid=148). — By **Dennis O’Brien, ARS.**

*The research is part of Water Availability and Watershed Management, an ARS national program (#211) described at [www.nps.ars.usda.gov](http://www.nps.ars.usda.gov).*

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ELDRIDGE WYNN (D1785-1)



ELDRIDGE WYNN (D1786-1)



**Citrus cell lines growing on standard media formulation (left) and on a formulation identified after a single experiment using the new ion-based approach. The cells on the new formulation are growing almost 200 percent faster. The new approach can be used to make better formulations for many things, including potting soils, animal diets, fertilizer blends, and algae nutrient solutions.**



Hulled winter barley ready for harvest.

## Boosting Barley for Bioenergy

**A**gricultural Research Service (ARS) scientists Kevin Hicks and David Marshall want winter barley to become a prime-time player in bioenergy production.

“The 2007 Energy Independence and Security Act requires production and use of 36 billion gallons of renewable transportation fuels by 2022. Today we only make 9 billion,” says Hicks. “We see winter barley as the perfect biofeedstock for making biofuels on the East Coast.”

So Hicks and others in the ARS Crop Conversion Science and Engineering Research Unit in Wyndmoor, Pennsylvania, are developing new sustainable technologies to convert varieties of hulled and hull-less winter “energy” barley into fuel ethanol. This initiative also includes Virginia Polytechnic Institute and State University scientists Carl Griffey, Wynse Brooks, and Mark Vaughn, who are supervising ongoing research efforts to develop improved varieties of hulled and hull-less barley.

Their combined efforts could help farmers from southern Pennsylvania to South Carolina develop a profitable 2-year rotation of winter barley, corn, and soybeans. Winter barley is grown on seasonally fallow land. It acts as a cover crop by protecting soil and nutrients and preventing migration of fertilizers from crop fields to the Chesapeake Bay—which is why the Chesapeake Bay Commission supports the development of winter barley as an energy crop. And since the field would otherwise be left fallow, producing biofuels from winter barley would not interfere with food production.

Now, too, there is a major marketing opportunity for growers of winter barley. Osage Bio Energy, headquartered in Glen Allen, Virginia, is well under way in constructing the first major barley-to-ethanol

production facility in the United States.

Meanwhile, in Raleigh, North Carolina, Marshall coordinates the regional winter barley testing nursery, which has the best experimental lines from both public and private winter barley breeding programs in the United States. He and other scientists in the ARS Plant Science Research Unit are just a few years into making crosses between hull-less barley and barley with resistance to Ug99, a stem rust that can inflict crop losses of up to 100 percent.

Once the researchers have developed robust lines that contain both traits, they’ll begin breeding for traits to enhance ethanol production, such as starch content. “In several years, we hope to release barley varieties with traits for enhanced agronomic performance, good grain-to-ethanol qualities, and good resistance to stem rust,” Marshall says.—By **Ann Perry**, ARS.

*This research is part of Plant Genetic Resources, Genomics, and Genetic Improvement (#301), Bioenergy and Energy Alternatives (#307), and Quality and Utilization of Agricultural Products (#306), three ARS national programs described at [www.nps.ars.usda.gov](http://www.nps.ars.usda.gov).*

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# Wheat

## ARS Wheat Rust Resistance Roundup

**T**he stem rust fungus *Puccinia graminis* has long plagued the world's wheat, barley, and other grain crops. But none of the fungus's main types have matched the devastation wrought by Ug99. It's a virulent new race that even stem rust-resistant varieties cannot withstand.

So pressing is the threat posed by Ug99, known scientifically as "*Puccinia graminis* f. sp. *tritici*," that the Agricultural Research Service has committed multiple labs to the global fight against the pathogen. ARS's efforts also support the Borlaug Global Rust Initiative, an international effort to rush newly developed rust-resistant cultivars to affected wheat growers, whether they be in regions where Ug99 already occurs—namely, parts of North Africa and the Middle East—or areas it could spread to next.

The labs, followed by summaries of their Ug99 research, include:

### Cereal Disease Laboratory, St. Paul, Minnesota—

Characterizes and monitors Ug99 and other emerging rust strains; tests breeding germplasm of U.S. wheat and barley for resistance to Ug99 in the greenhouse; identifies new sources of resistance in cultivated and wild relatives of wheat; develops molecular markers; and creates genetic maps of Ug99 and other important rust strains. (See "World Wheat Supply Threatened!" *Agricultural Research*, November/December 2007, pp. 4-6.)

### Plant Science Research Unit, Raleigh, North Carolina—

Coordinates Ug99 screening of U.S. wheat and barley submissions at the Njoro Research Center of the Kenya Agricultural Research Institute; developed wheat lines with multiple genes for resistance to Ug99 and other stem rusts; and identified resistance sources in several barley lines being tested in Kenya. (See "International Wheat and Barley Screening Collaboration Helps Uncover Stem Rust-Resistant Material," *Agricultural Research*, February 2010, pp. 8-9.)

### Small Grains and Potato Germplasm Research Unit, Aberdeen, Idaho—

Coordinates acquisition and shipping of seed from wheat breeders across the United States for testing in Kenya; prescreens landrace wheats from the National Small Grains Collection against local stem rust races; and crosses resistant landraces tested in Kenya to study inheritance in offspring as part of the effort to discover new resistance genes. (See "International Wheat and Barley Screening Collaboration Helps Uncover Stem Rust-Resistant Material," *Agricultural Research*, February 2010, pp. 8-9.)

### Cereal Crops Research Unit, Fargo, North Dakota—

Identified novel sources of stem rust and Ug99 resistance in rye, goat grasses, perennial wheat grasses, and other wild species; and combined classical cytogenetics with molecular marker techniques to develop bread and durum wheat lines carrying resistance genes derived from wild relatives of wheat, including

*Sr37*, *Sr39*, *Sr43*, and *Sr47*, that are free of "linkage drag"—unwanted segments of chromosome that can be inherited from wild species along with rust resistance genes, hindering commercial breeding efforts.

### Hard Winter Wheat Genetics Research Unit, Manhattan, Kansas—

Identified four new resistance genes from wild relatives of wheat to combat Ug99 and developed genetic stocks for their use in wheat breeding; developed genetic markers and improved germplasm for several Ug99-effective resistance genes, including *Sr22*, *Sr35*, and *Sr40*; used chromosome-engineering techniques to shorten introduced chromosome segments from wild relatives of wheat and thus reduce undesirable linked characters; and use marker-assisted selection to move useful resistance genes into elite hard winter wheat varieties for Central and Southern Plains breeders.—By **Jan Suszkiw**, ARS.

*This research is part of Plant Genetic Resources, Genomics, and Genetic Improvement (#301) and Plant Diseases (#303), two ARS national programs described at [www.nps.ars.usda.gov](http://www.nps.ars.usda.gov).*

*To reach scientists involved in these projects, contact Jan Suszkiw, USDA-ARS Information Staff, 5601 Sunnyside Ave., Beltsville, MD 20705-5129; (301) 504-1630, [jan.suszkiw@ars.usda.gov](mailto:jan.suszkiw@ars.usda.gov). \**

YUE JIN (D1797-1)



Ug99-infected wheat from a nursery in Njoro, Kenya.

# Throwing a CITRUS PEST Off Its Scent

**A citrus leafminer larva creates a gallery, or mine, within a citrus leaf, thereby gaining protection from externally applied pesticides and leaving behind a fecal trail. The wound created by the larva also makes the plant more susceptible to citrus canker disease.**

Agricultural Research Service scientists are working on controlling a pest that poses an increasing threat to Florida citrus groves by exploiting the insect's own reproductive habits.

The leafminer moth, *Phyllocnistis citrella*, forms channels as it feeds inside citrus leaves and, as a result, often makes the plant more susceptible to canker disease. Further exacerbating the leafminer problem is the spraying of more insecticide to combat another pest—the Asian citrus psyllid. The insecticide is killing off the leafminers' natural enemies, allowing the pest to increase in numbers.

Commercial traps can sometimes help, but Stephen Lapointe, an ARS entomologist with the U.S. Horticultural Research Laboratory in Fort Pierce, is exploring a control strategy that has proved effective with other pests: mating disruption.

Many insects release pheromones to attract mates. In some cases, scientists have synthesized those pheromones and

developed treatments that throw males off the scent of fertile females in orchards and fields. The technique has been used to control gypsy moths, codling moths, and several other pests that attack fruit and vegetable crops. These treatments are considered environmentally friendly because they reduce the need for insecticides and are designed to target specific pests. "In all of them, we are basically just interfering with the male's ability to receive the signal from the female," Lapointe says.

Lapointe is working with Lukasz Ste-linksiki of the University of Florida's Citrus Research and Education Center to see whether they can disrupt leafminer mating by manipulating either of two compounds—a triene and a diene—used by female leafminers to attract mates.

The researchers set out to examine whether the formulation of three parts triene to one part diene would be optimal for attracting males in a trap. They also wanted to see what formulations would be most effective at confusing males and disrupting their ability to pick up the female scent.

The researchers created synthetic female-scented traps, placed them in a citrus grove, and counted the number of males caught. They confirmed that the three-to-one ratio of triene to diene worked better than triene or diene alone as an attractant. In 2.5 months, more than 48,000 males were captured in traps baited with the 3:1 blend compared with around 3,500 males



A 1-gram dollop of SPLAT containing the sex pheromone to disrupt citrus leafminer mating.

captured in traps baited with the triene and only 70 in traps baited with the diene.

In a second phase of the project, the researchers surrounded the female-scented traps with 17 different combinations of the two synthetic compounds—as well as each compound on its own—to see which would be most effective at preventing males from finding the traps. They placed the experimental treatments around female-scented traps at different points inside a 59-acre grove of grapefruit trees. If the experimental treatments prevented the males from reaching the female-scented traps, the males theoretically would be unable to find females in natural settings, the researchers say. They used a waxy substance known as "SPLAT" that slowly released the experimental treatments over time, and they checked once a week over several months to see which treatments were most effective at keeping leafminers out of the female-scented traps.

Results showed that either compound used alone was as effective as the 3:1 blend at keeping male leafminers away from the female-scented traps. The diene-only treatment resulted in an 89-percent reduction in moth catches, and the triene-only treatment resulted in an 83-percent reduction. Greater amounts of diene are required to disrupt the moths, but diene is also much cheaper to synthesize, Lapointe says.

The work was recently published in the *Journal of Chemical Ecology*. Lapointe is also working with ISCA Technologies, Inc., of Riverside, California, a manufacturer of the SPLAT technology, to use these results to develop a marketable leafminer mating-disruption technology.—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](http://www.nps.ars.usda.gov).*

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ARS research leader David Hall (left) observes as entomologist Stephen Lapointe applies a formulation of SPLAT containing the sex pheromone of the citrus leafminer.

# Newcastle Disease Virus Vaccine on the Horizon

Newcastle disease virus (NDV) is an important pathogen that causes disease and death not only in domestic and commercial poultry, but also in wild bird populations around the world. Current Newcastle disease (ND) vaccines are used widely in commercial poultry and protect the vaccinated birds from disease but do not prevent them from becoming infected and carrying the virulent virus or shedding it in their feces. Therefore, the current vaccines do not eliminate virulent virus transmission from infected to healthy birds.

A vaccine that reduces virulent virus shed and transmission is sorely needed by the poultry industry.

Using reverse genetics technology, researchers in the Endemic Poultry Viral Disease Research Unit and the Exotic and Emerging Viral Diseases Research Unit of the Southeast Poultry Research Laboratory in Athens, Georgia, have developed a new vaccine from parts of a virus that is similar to the wild-type NDV circulating in the environment today. This new vaccine not only reduces mortality and severity of ND symptoms in poultry, but it also decreases the amount of virulent virus shed from vaccinated birds.

“Currently, most vaccines used in the United States are formulated with NDV

isolated in the 1940s, which is similar to the virulent NDV circulating at that time,” says poultry unit microbiologist Qingzhong Yu. “Unfortunately, with time, new NDV strains have emerged that are genetically very different from commonly used vaccine strains.

“Reverse genetics technology enabled us to generate a new vaccine by exchanging a gene from the original vaccine with a similar gene of the current circulating virus. We found that when the new vaccine, containing gene sequences similar to the wild-type virus, was used in vaccination studies, the vaccinated birds were protected from disease and shed less of the wild-type virus after challenge,” says Yu.

Yu, Daniel King (retired ARS researcher), David Suarez, Patti Miller, and former ARS researcher Carlos Estevez (now with Texas A&M) submitted a patent application for the vaccine in 2009. Licensing by the USDA Animal and Plant Health Inspection Service’s Center for Veterinary Biologics would have to follow before the vaccine could be used.

NDV causes disease in more than 250 species of birds and typically affects the respiratory, gastrointestinal, and/or nervous system. Symptoms may include gasping, coughing, lack of appetite, drooping wings, and diarrhea. ND is clinically similar to avian influenza, and the two diseases may be confused, which impairs the rapid diagnosis of a disease outbreak.

The most severe form of ND can result in disease and mortality rates exceeding 90 percent in susceptible chickens. The most recent U.S. outbreak—which occurred in 2002-2003 in California, Nevada, Arizona, and Texas—illustrates the devastation and financial cost that can result: More than 3.4 million birds were destroyed, and the cost of controlling the outbreak in California alone was more than \$160 million.

“Newcastle disease continues to be a danger to the commercial poultry industry because it can spread rapidly and can exact a heavy toll,” says Yu. “Vaccines for ND have been used for more than 50 years to control the disease and are successful in reducing mortality and the severity of symptoms. Our goal is to create a vaccine to decrease virus spread as well.”—By

**Sharon Durham, ARS.**

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

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Microbiologist Qingzhong Yu examines recombinant Newcastle disease virus vaccine candidates in infected cells.



PEGGY GREE (02048-1)

# Archiving Parasites and Protozoa for Research, Diagnoses, and More

In the United States, parasitic infections contribute to losses of more than \$2 billion annually in livestock alone. In turn, some parasites, like tapeworm, can infect humans who eat uncooked or undercooked beef or pork.

Fortunately, ARS maintains archival collections of parasites for research, identification, and diagnostic purposes, the vast majority of which are held at the U.S. National Parasite Collection (USNPC), which is curated by ARS zoologist Eric Hoberg.

Hoberg is with the Animal Parasitic Diseases Laboratory, which is part of the Henry A. Wallace Beltsville Agricultural Research Center in Beltsville, Maryland. The collection was established in 1892 and is among the largest parasite collections in the world.

Current holdings include more than 20 million catalogued specimens representing nematodes, tapeworms, flukes, spiny-headed worms, and some parasitic arthropods, such as fleas, ticks, and lice. Detailed biodiversity information about hosts and geographic distribution is maintained in an on-line database.

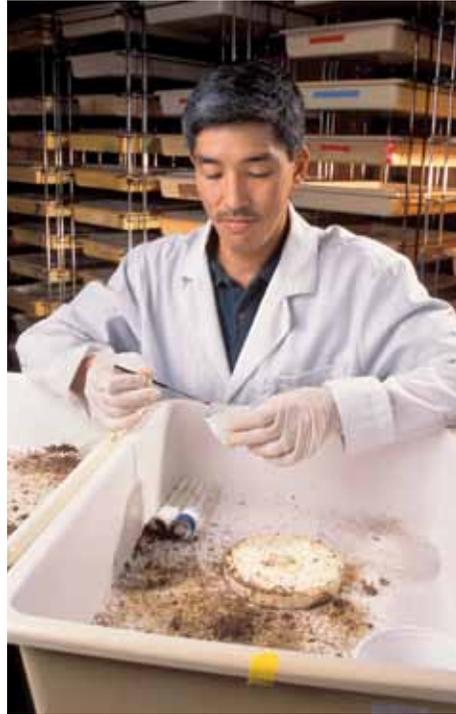
The USNPC contributes significantly to explorations of diversity, evolution, and distribution, furthering our understanding of the socioeconomic and ecological significance of parasites and pathogens. Archival collections serve as critical baselines to understand ecological disturbance. Archives provide a foundation to identify shifting geographic and host ranges for parasites and diseases that may emerge with accelerated global change.

## Newly Reclassified Protozoa at Work

ARS researchers have also assembled and maintain invertebrate protist collections at three research locations for the purpose of in-house and joint projects.

At the Center for Medical, Agricultural, and Veterinary Entomology (CMAVE), in Gainesville, Florida, researchers are using a collection of microsporidia—formerly classified as protozoa and now considered fungi—to act as soldiers of biological

PEGGY GREB (K10225-1)



Entomologist David Oi collects infected fire ants from a colony decimated by the fire ant pathogen *Kneallhazia solenopsae*.

warfare at the tiniest level. Species of these spore-producing parasites, such as *Kneallhazia solenopsae*, are being used to bring about declines in red imported fire ant (*Solenopsis invicta*) populations. In Argentina, these infectious soldiers are associated with localized declines

PEGGY GREB (D1578-1)



Eric Hoberg, chief curator of the U.S. National Parasite Collection, examines roundworm specimens from raccoons, which pose a potential threat for human infection.

of 53 percent to 100 percent in fire ant populations, according to CMAVE entomologist David Oi.

In addition, Oi and CMAVE colleagues Sanford Porter and Steven Valles were able to get *K. solenopsae* to infect phorid flies without harming them. That's important because the flies may serve as vectors to infect red imported fire ants with the microsporidia—perhaps facilitating the spread of infection to other colonies.

*K. solenopsae* not only reduces fire ant colony size, it also reduces the number of reproducing ants, decreases the survival of queens, and increases the mortality rate of colonies, says Oi. The collection enables scientists to research the potential for using these and other microbes as biocontrol agents.

In Sidney, Montana, ARS scientists at the Northern Plains Agricultural Research Laboratory used the U.S. Rangeland Grasshopper Collection's microsporidia holdings to develop the first microbial control agent registered for use in the United States against grasshoppers. Large-scale outbreaks of grasshoppers occur about every 8 to 10 years. In 2001, such an outbreak caused an estimated \$25 million of damage to crops in Utah alone.

And researchers at the Grain Marketing and Production Research Center in Manhattan, Kansas, maintain protozoa in a collection that is used to develop biocontrol agents against beetles and other bugs that burrow into stored grain products.—By **Rosalie Marion Bliss** and **Sharon Durham**, ARS.

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

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## Troublesome Microbes That Resist Antimicrobials The Antimicrobial Resistance Collection

In an effort to monitor pathogenic microbes' resistance to antimicrobials, the National Antimicrobial Resistance Monitoring System (NARMS) was established in 1996 by the U.S. Food and Drug Administration's Center for Veterinary Medicine in collaboration with the Centers for Disease Control and Prevention and the U.S. Department of Agriculture.

The animal component of NARMS focuses on zoonotic pathogens (those that can be transferred from livestock or wildlife to humans) and is housed within ARS's Bacterial Epidemiology and Antimicrobial Resistance Research Unit. Led by microbiologist Paula Cray, the unit is part of the ARS Richard B. Russell Research Center in Athens, Georgia.

The NARMS animal component tests samples, or isolates, obtained from healthy on-farm animals, animals being diagnosed for illness, and food animals at slaughter. The specimens are stored in the **Antimicrobial Resistance Collection**, maintained by Cray's team.

Resistance of *Salmonella*, *Campylobacter*, *Enterococcus*, and *Escherichia coli* to antimicrobials such as streptomycin and tetracycline is tested, monitored, and tracked in an attempt to better understand foodborne pathogens' resistance trends.

"Nontyphoid *Salmonella* was chosen as a sentinel organism of NARMS's animal component, which was launched in 1997," says Cray. A sentinel organism is one used to track changes in resistance over time. "Testing of *Campylobacter* isolates began in 1998, while *E. coli* was included in 2000. *Enterococcus* testing has also been added to the data set.

"It is vital that we track these trends of resistance and disseminate the information to animal producers, veterinarians, and consumers."—By **Sharon Durham**, ARS.

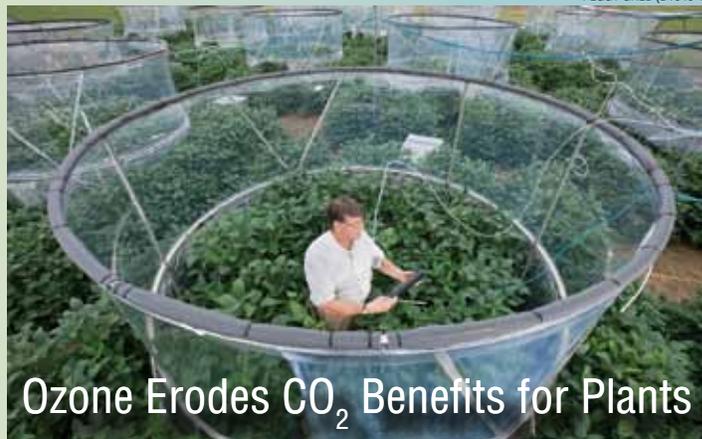
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PEGGY GREB (D602-1)



Research leader Paula Cray (left) and technician Lori Ayers perform antimicrobial susceptibility testing on a bacterial culture.

PEGGY GREB (D1548-1)



## Ozone Erodes CO<sub>2</sub> Benefits for Plants

Plant physiologist Fitzgerald Booker prepares to place an optical scanner into a tube positioned in the soil to photograph soybean roots. The photos will show changes in root growth caused by elevated carbon dioxide and ozone, pumped into the open-top field chambers.

Experiments by Agricultural Research Service scientists at Raleigh, North Carolina, and Urbana, Illinois—and their university colleagues—have shown that future higher levels of ozone may cancel out some of the benefits to crops expected from higher carbon dioxide (CO<sub>2</sub>) levels.

For one thing, crops wouldn't achieve the 30-percent increase in photosynthesis—and resulting higher yields—expected as CO<sub>2</sub> levels continue to rise.

The scientists have also found that ozone can thwart expected high CO<sub>2</sub> benefits by lowering crop quality. It can also help invasive weeds outcompete forage and other crops.

"We are beginning to look at interactions between ozone and diseases such as stripe rust and stem rust of wheat," says David Marshall, research leader of the Plant Science Research Unit at Raleigh, and an expert on wheat rusts.—By **Don Comis**, ARS.





# Breeding Plants for a High-Ozone World

Soybean breeder and geneticist Tommy Carter inspects research plots during extreme drought stress at the North Carolina State Sandhills Research Station. Carter evaluates approximately 25 acres (5,000 plots) of soybean for drought/stress resistance annually.

**Geneticist Thomas Carter** has bred soybeans for the Agricultural Research Service for 30 years, but for the last couple of years, he has been trying to solve a mystery: Why do the world's most stress-resistant soybean types hail from a little village in far-northern Sweden?

Stress resistance is a rare commodity in soybean, but these Swedish varieties put up with a host of problems—drought, iron deficiency, toxic soil aluminum and salts, and even high levels of ozone.

Although Sweden is not known for its soybean production, Swedish breeders made a major breakthrough—but one that went unnoticed for decades by the soybean community and even by the breeders.

“We now know that they did something very right. The mystery is how,” Carter says.

Carter, who is in the ARS Soybean and Nitrogen Research Unit at Raleigh, North Carolina, has long been interested in breeding for stress resistance. Ozone resistance has become his most recent interest, in large part because of his collaboration with ozone

expert Kent Burkey, a plant physiologist in the ARS Plant Science Research Unit at Raleigh.

Their stress studies led the scientists to the treasure trove of genetic resistance in Swedish soybeans, which appear to have an even more pronounced resistance to ozone than to the other stresses. Understanding the ozone effect may be a key to unraveling the secrets of the broad stress resistance of the Swedish soybeans.

## Tracing the American Soybean's Family Tree

Carter uncovered the resistance mystery after analyzing thousands of soybean types to generate the family tree of North American soybeans. From this, he found 30 ancestors, which together account for 92 percent of the genetic material in North American soybeans.

Carter screened these ancestors first for salt tolerance. He found two lines of vegetable soybeans—Fiskeby 840-7-3 and Fiskeby III, both from the Swedish village of Fiskeby—that were the most salt

tolerant. Then he screened for aluminum tolerance, and again the Fiskeby plants stood out.

“Then, my colleagues and I saw the same thing with drought tolerance and ozone” and realized a pattern had emerged, Carter says.

ARS scientists in Raleigh and in Urbana, Illinois, study how increases in atmospheric greenhouse gases, particularly carbon dioxide and ozone, will affect crops, especially soybeans and wheat.

To screen the ancestors for ozone tolerance in a 2003 to 2005 study, Carter turned to Burkey for help. The two scientists combined the results of this study with breeder pedigree records and found that only a few U.S. cultivars trace their ancestry to the Fiskeby stress-tolerant types. “This indicates that there is great potential to increase tolerance to ozone and other stresses in North American soybeans by adding genes from Fiskeby,” Burkey says.

He and Carter and Jim Orf, a soybean breeder/geneticist at the University of Min-

nesota at St. Paul, have crossed Fiskeby III with ozone-susceptible Mandarin Ottawa and developed 240 breeding lines from the offspring. With the help of funding from the United Soybean Board, in Chesterfield, Missouri, the team is mapping the genes in these lines to see which genes are connected to resistance to ozone and the other stresses.

“This approach can be applied to other soybean-producing regions such as China, Brazil, and Europe,” says Burkey. “Their breeding programs, in turn, may offer us other important germplasm to explore for ozone tolerance genes.”

#### Cell Wall the First Line of Ozone Defense

Ozone enters leaf stomata as a gas and then dissolves in the liquid layer, called the “apoplast,” that surrounds plant cells. The apoplast is considered the first line of defense against pollutants, where ozone can be detoxified before it reaches the leaf cell surface and causes the damage that interferes with photosynthesis and reduces yields.

In the search for ways to improve crop ozone tolerance, Burkey and Cosima Wiese—associate professor of biology at Misericordia University in Dallas, Pennsylvania—recently began studying Fiskeby to see whether leaf apoplast antioxidants might be at least part of

Fiskeby’s defense against ozone. Burkey and others have found that, in certain other species, vitamin C plays a role in keeping ozone from penetrating through the cell’s first line of defense. But soybean contains little vitamin C in the leaf apoplast, so they are looking for other antioxidants that may play a role.

#### Breeding Ozone-Tolerant Crops

ARS scientists in Urbana are taking a slightly different, but complementary, approach to the work of the Raleigh scientists. They are using SoyFACE (Soybean Free Air Concentration Enrichment) to screen soybean varieties for ozone tolerance and sensitivity. SoyFACE involves testing plants in open-air field conditions, under atmospheric conditions predicted for the year 2050. At that time, ozone concentrations are expected to be 25-50 percent higher than today’s concentrations.

The work is being done by molecular biologist Lisa Ainsworth, Amy Betzelberger, a graduate research assistant in the Department of Plant Biology at the University of Illinois at Urbana-Champaign, and geneticist Randall Nelson and other ARS colleagues at Urbana.

During 2007 and 2008, the team tested 10 soybean varieties that had been released between 1952 and 2003. These were selected from initial tests of 22 cultivars and experimental lines that had been evaluated for 4 years. The scientists found that, on average, exposure to

82 parts per



AMY BURTON (D2252-1)

## Ozone: Plants Don't Like It

Ozone is a greenhouse gas found in smog. It is formed mostly when sunlight “cooks” automotive and industrial pollutants that originate from combustion of carbon-based fuels.

Ozone is the most damaging air pollutant to plants.

Fitz Booker, an Agricultural Research Service plant physiologist at the Plant Science Research Unit at Raleigh, North Carolina, says, “Ozone has long been known to affect a wide range of plants, including grasses, field crops, horticultural crops, and forests. Our research and that of other scientists has shown that many crops and forages are damaged by high ozone levels, including soybeans, wheat, cotton, oats, potatoes, rice, peanuts, tomatoes, grapes, alfalfa, clover, and barley.”

In fact, during the 1950s, Howard Heggstad (deceased) discovered that what were thought to be symptoms of a plant disease on tobacco leaves in the smoggy Connecticut River Valley was actually damage from ozone. In the Washington, D.C., area, he found similar damage to plants from ozone in smog. At the time, Heggstad was an ARS plant pathologist.

Globally, yield losses from ozone have been estimated at \$14 to \$26 billion for rice, soybean, corn, and wheat combined.—**Don Comis**, ARS.

At the ARS Soybean and Nitrogen Research unit in Raleigh, North Carolina, Tommy Carter (right) and plant physiologist Kent Burkey examine a Fiskeby soybean ancestor being placed in greenhouse exposure chambers to assess its ozone injury response.





To assess oxidative stress in soybeans grown at SoyFACE under elevated ozone concentrations, ARS molecular biologist Lisa Ainsworth (back) and graduate student Kelly Gillespie use a liquid-handling robot to perform a high-throughput assay.

countries like India and China, as growing populations are able to afford more cars and build more power plants. Another concern is that ozone levels will rise in developing countries, where people can least withstand losses of staple crops such as rice and wheat.

“All this research promises to give breeders the tools they need to help farmers by providing them with more ozone-hardy crop varieties. This is great science with a solid application, the goal of all ARS research,” Carter says.—By **Don Comis**, ARS.

*This research supports the USDA priority of responding to climate change and is part of Climate Change, Soils, and Emissions (#212) and Plant Genetic Resources, Genomics, and Genetic Improvement (#301), two ARS national programs described at [www.nps.ars.usda.gov](http://www.nps.ars.usda.gov).*

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billion (ppb) ozone reduced soybean yields by 23 percent in 2007, and exposure to 61 ppb reduced yields by 12 percent in 2008. They found significant differences in ozone tolerance among the 10 varieties. “But we didn’t see any significant improvement in ozone tolerance in soybean varieties released after the 1980s,” Ainsworth says. This again shows the potential and need for breeding more ozone-tolerant varieties.

#### Ozone Damage Seen From Above

In a 2002 to 2006 study led by the National Aeronautics and Space Administration, NASA scientists, Ainsworth, ARS plant physiologist Fitz Booker, and university scientists investigated widespread ozone damage to soybeans in Iowa, Illinois, and Indiana using ozone surface monitors, satellite instruments, and historical yield data. Ainsworth says that satellite information is useful for investigating ozone’s impacts on crop yields in areas without ground-monitoring networks, such as rural regions. “Satellite observations of farmland in other countries could provide important insight into the global

extent of this problem,” she adds.

In the study, the scientists found that ozone levels above 50 ppb could reduce yields by about 10 percent in the Midwest Corn Belt, costing more than \$1 billion in lost crop production.

Their findings are consistent with those from their SoyFACE studies and studies in outdoor open-top chambers.

Ozone levels in most urban areas of the United States have declined with improvements in emission controls. But ozone levels are expected to rise in



ARS scientists Carl Bernacchi (left), Don Ort (center), and Lisa Ainsworth in a plot of soybeans treated with elevated carbon dioxide at the SoyFACE Global Change Research Facility at the University of Illinois Research Farm in Urbana.

PHOTO BY INSTITUTE FOR GENOMIC BIOLOGY/UNIVERSITY OF ILLINOIS (D1546-2)

# Helping Growers Adapt to Changing Rules on Fumigants

**For decades, methyl bromide has been an extremely important tool**

for vegetable, strawberry, deciduous fruit, nursery, and ornamental growers in their efforts to combat soil-dwelling nematodes, diseases, and weeds. But the fumigant is being phased out because of its harmful effects on the Earth's protective stratospheric ozone layer, and alternative fumigants are presenting new challenges for growers and regulatory officials who want to keep the air clean.

The Agricultural Research Service has been conducting research to find the best alternatives to the fumigant since the mid-1990s, and because of the issue's critical importance, the agency initiated a special areawide pest-management project 5 years ago that made several additional research efforts possible. As part of that 5-year effort, ARS researchers in Florida and California are helping to minimize release of the alternative fumigants into the atmosphere with studies focused on fumigant emission rates and the effectiveness of tarps used as barriers to cover fumigated soil. The work also is designed to assist the U.S. Environmental Protection Agency



DAN CHELLEMI (D2246-1)

In a fumigated, tarped field in Tifton, Georgia, (left to right) horticulturist Jerry Johnson, plant pathologist Randy Driggers, and technicians Taylor Ivy and Nick Rotindo collect soil and air samples for analysis of chemical fumigants. Samples were collected to evaluate the impact of good agricultural practices on the soil fate and atmospheric emission of chemical fumigants.

(EPA) and other regulators charged with developing new fumigant requirements to better protect people who use them or live near treated fields.

Under requirements being imposed by EPA, growers who use fumigants will need to establish buffer areas around treated fields to protect neighbors from excessive exposure and develop detailed management plans that include either fumigant monitoring or notifying neighbors of fumigant applications. Experience in California suggests that many growers

and pesticide applicators have been able to adapt to these types of fumigant requirements within about a year. But many smaller operations, particularly those near suburbs, may be unable to meet the proposed standards because of their proximity to homes, institutions, and public rights-of-way. These include some California strawberry growers and south Florida growers of tomatoes, peppers, and cucumbers, says Dan Chellemi, an ARS plant pathologist at the U.S. Horticultural Research Laboratory in Fort Pierce, Florida.

The financial implications could be significant. In Florida, for instance, tomatoes were a \$622 million crop in 2008, and bell peppers were valued at \$267 million.

Field studies conducted by Chellemi, Husein A. Ajwa, a former ARS scientist now with the University of California-Davis, and colleagues, showed that implementation of recently developed application equipment and methods reduced emissions to levels far below those found in previous studies. "We found that the differences were quite significant," Chellemi says.

Chellemi and colleagues applied several alternative fumigants under commercial application conditions at three sites near Duette, Florida, and three sites near Tifton, Georgia. The fumigants included chloropicrin, metam sodium, metam potassium,

The vertical air-monitoring station located in the center of a fumigated field at a Duette, Florida, site is designed to continuously collect air samples at several heights. Fumigant-collection tubes were exchanged and air-sampling pumps calibrated every 6-12 hours for 10 consecutive days to ascertain fumigant flux rates from soil.



DAN CHELLEMI (D2247-1)

dimethyldisulfide, and 1,3-dichloropropene (sold as Telone). The fumigants were injected into the soil using shanks and low-disturbance coulters mounted on tractors, application methods that are becoming standard practice. The soil was then immediately covered with plastic tarps designed to prevent the fumigant from escaping.

The researchers used different types of plastic tarps, selected sites that included different soil types, and recorded the temperatures and moisture levels of the soil at times when the fumigants were applied. They also set up weather stations to monitor wind speeds and air sampling stations to track emission levels.

They found that emission rates could be drastically affected by the quality of the soil and the type of covering used. Coverings include tarps made with polyethylene or metal and VIF's (virtually impermeable films), which have layers of nylon or other materials imbedded in them. The researchers found that in dry soils with low organic matter content, VIFs worked best at keeping emissions low, while in areas with moisture above field capacity, a more permeable metalized film was equally as effective at reducing emissions. Their studies confirmed that good agricultural practices are critical factors in determining how much fumigant is released into the atmosphere. The EPA has used the results, published in the journal *Atmospheric Environment*, along with results from other recent research, to develop the fumigant standards currently being considered.

#### Testing Film Quality

ARS researcher Sharon Papiernik and her colleagues used specially designed chambers to test the permeability of dozens of films used in field trials to come up with a "resistance factor" that measures each film's ability to serve as a fumigant barrier. Papiernik sandwiched each film between two chambers, injected fumigants into one chamber, and measured both the fumigant that passed through the film into the second (receiving) chamber and fumigant that

remained in the source chamber. Because each fumigant had a different chemistry, each behaved differently with each tarp.

The researchers tested 200 film-chemical combinations, including those used in large-scale field trials from the areawide pest management project, and came up with a resistance factor that can be used to determine emission rates for each film and fumigant under a wide range of growing conditions and weather patterns. Papiernik is research leader of the North Central Agricultural Research Laboratory in Brookings, South Dakota.

The results, reported online last year in the *Journal of Environmental Quality*, showed that the VIFs were in fact significantly better barriers to fumigant diffusion than the polyethylene films, but

At an experimental site near Bakersfield, California, a tractor injects fumigants 46 centimeters deep into the soil while technician Qiaoping Zhang walks along the field and places boundary markers to identify the area of treated soil. Gas samples will be taken to determine fumigant emission rates in the field.



their effectiveness varied depending on the fumigant tested. Some VIFs were less effective under higher humidity levels.

The EPA is developing this approach as the standard testing method for evaluating agricultural plastics used in soil fumigation. The results, along with those from other studies, have provided basic standards for film manufacturers and guidance for growers on which films offer the best options for reducing fumigant emissions.

#### Math Makes It Simple

A major goal in many fumigant studies is determining the amount of gas released from the soil during the fumigation period. But measuring and calculating emissions is no easy task. It means trying to estimate how much of a fumigant is released in an outdoor environment, where variables

range from the chemistry of the fumigant to the temperature and the amount of water vapor in the air.

Such constantly shifting variables make it difficult to determine not only the amount of fumigant being released, but also its effectiveness at killing pests.

Researchers also need to determine how emissions rates are affected by a complicated list of crop-management decisions, such as the permeability of the film being used, the amount of time the film covers the fumigated soil, and the depth of the shank used to inject the treatment into the soil.

Scott Yates, research leader of the Contaminant Fate and Transport Unit at the U.S. Salinity Lab in Riverside, California, took a mathematical approach to the problem and developed a model focused on determining

fumigant volatilization rates, the amount of fumigant retained in the soil, the amount released into the air, and the relationship between soil-chemical properties and emissions.

In work published in the *Journal of Environmental Quality*, Yates used the model to calculate fumigant emission rates that compared reasonably well to actual methyl bromide emissions observed in field trials where a polyethylene film was used to cover an 8-acre field. The model can be used to determine how a

fumigant will be distributed throughout a field and offers a consistent method for determining emission rates under a wide variety of crop-management scenarios.—  
By **Dennis O'Brien**, ARS.

*This research supports the USDA priority of responding to climate change and is part of Methyl Bromide Alternatives (#308) and Air Quality (#203), two ARS national programs described at [www.nps.ars.usda.gov](http://www.nps.ars.usda.gov).*

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Sound Science  
Sound Air

# Cattle Pastures May Improve Soil Quality



At an experimental field site in Oconee County, Georgia, technician Steven Knapp cuts a test plot to determine hay yield.

**Decades of plowing** throughout the Piedmont region of the United States have degraded the soil, allowing much of it to be washed away and robbing what is left of nutrients and organic matter. Sorghum, cotton, soybean, and wheat are still widely grown in the region, which stretches all the way from Alabama to New Jersey. But because the soil is so degraded, growers have allowed much of the land to revert to forests and pastures.

“Growers need guidance on whether keeping the land unused is the best way to restore degraded soils or whether allowing cattle to graze on it is a viable option,” says Alan Franzluebbbers, an Agricultural Research Service ecologist at the J. Phil Campbell, Sr., Natural Resource Conservation Center in Watkinsville, Georgia. The center was started in 1937 to look for ways of improving soil quality for farmers in the southeastern United States.

Franzluebbbers led a project where researchers planted grasses on 37 acres of rolling, eroded land in northeastern Georgia and allowed beef cattle to graze there to assess the effects on soil quality. Coastal bermudagrass was planted initially, and after 5 years, tall fescue was drilled into it, when the bermudagrass was in a dormant winter stage, to extend the grazing season from 5 months to 10 months of the year.

The research team, which included retired ARS scientists John Stuedemann and Stan Wilkinson, varied the number of cattle per acre, and over 12 years they assessed how the soils would respond to four different scenarios: moderate grazing (average of 23 steers for every 10 acres), intensive or heavy grazing (35 steers per 10 acres), no grazing and letting the grass grow, and no grazing but cutting the grass for hay. Under each scenario they looked

at the amount of soil compaction that occurred, the amounts of soil organic carbon and nitrogen found in the soils, and the amounts of surface plant residues, which help prevent erosion. Soil compaction makes it harder to grow crops. They also looked at the effects on the soil of three different fertilizer treatments (inorganic fertilizer alone, organic broiler litter alone, and a mix of inorganic fertilizer and organic broiler litter).

The team found that fertilizer type made little difference, but different grazing scenarios produced dramatically different effects. Land that was grazed produced more grass than ungrazed land, and grazing led to the most carbon and nitrogen being sequestered in soil. Sequestering carbon and nitrogen in the soil has become a major goal for agriculture because it reduces greenhouse gas emissions. Whether grass was grazed moderately or intensely made little difference on sequestration rates.

Cutting grass for hay reduced the amount of surface residue and increased soil compaction but didn’t change the amounts of organic carbon and nitrogen in the soil. Land left unused had the highest surface residue and least soil compaction and was better at sequestering carbon in the soil than haying.

From an environmental standpoint, grazing has traditionally been viewed as less desirable than leaving the land unused. But the results, published in the *Soil Science Society of America Journal*, demonstrate that if growers manage cattle so that pastures are grazed moderately, they’re restoring soil quality and cutting greenhouse gases by keeping carbon in the soil as organic matter rather than releasing it into the atmosphere as carbon dioxide.—  
By **Dennis O’Brien**, ARS.

*This research is part of Climate Change, Soils, and Emissions, an ARS national program (#212) described at [www.nps.ars.usda.gov](http://www.nps.ars.usda.gov).*

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# New Red Imported Fire Ant Enemies in Place for Fight



Red imported fire ants first arrived in the United States in the early 1930s and have been expanding along the southern portion of the country ever since. These ants inhabit more than 350 million acres in 12 southern states and Puerto Rico, and they have recently become established in isolated sites in California and New Mexico. For more than a decade, ARS scientists have gone on the offensive against red imported fire ants by using natural enemies against them.

Entomologist Sanford Porter has worked to collect, breed, and release phorid flies that are now used to control fire ant populations in the southern regions of the United States. Porter is at the Center for Medical, Agricultural, and Veterinary Entomology's (CMAVE) Imported Fire Ant and Household Insects Research Unit, in Gainesville, Florida.

The fire ants' large numbers and potent sting have resulted in medical, agricultural, and environmental economic impacts that cost the U.S. public billions of dollars each year. Scientists at CMAVE and cooperators in several states conducted a program to suppress fire ants in large areas. The area-wide project involved cooperators in five states and was successful in establishing self-sustaining and spreading populations of phorid flies from South America.

The fire ant biocontrol program, which began in 1995, has released five species of phorid flies to parasitize the various sizes of fire ants—from the large to the very small. "The relationship between phorid fly and fire ant is very specific: The introduced phorid fly species only attack imported fire ant species," says Porter.

After several years of testing, *Pseudacteon cultellatus* was recently approved for field release. This new species of phorid fly is currently being released at several sites in Florida to control tiny fire ant workers that belong to multiple-queen colonies. "These colonies are particularly problematic, because they usually house two to three times the number of worker ants," says Porter. "Target release sites typically have a large number of fire ants, diverse plant life, and water nearby.

"Of the four phorid fly species previously released, only one has failed to establish itself and widely spread out. *P. litoralis*, released in 2004 and 2005, was able to establish only in Alabama," says Porter. "The others—*P. tricuspis*, *P. curvatus*, and *P. obtusus*—have expanded well beyond their release sites and are attacking fire ants in large regions. *P. tricuspis* and *P. curvatus* each cover about half of the U.S. fire ant range and are expected to increase to well over two-thirds of the range by the end of 2011."—By **Sharon Durham**, ARS.

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

*Sanford Porter is in the USDA-ARS Imported Fire Ant and Household Insects*

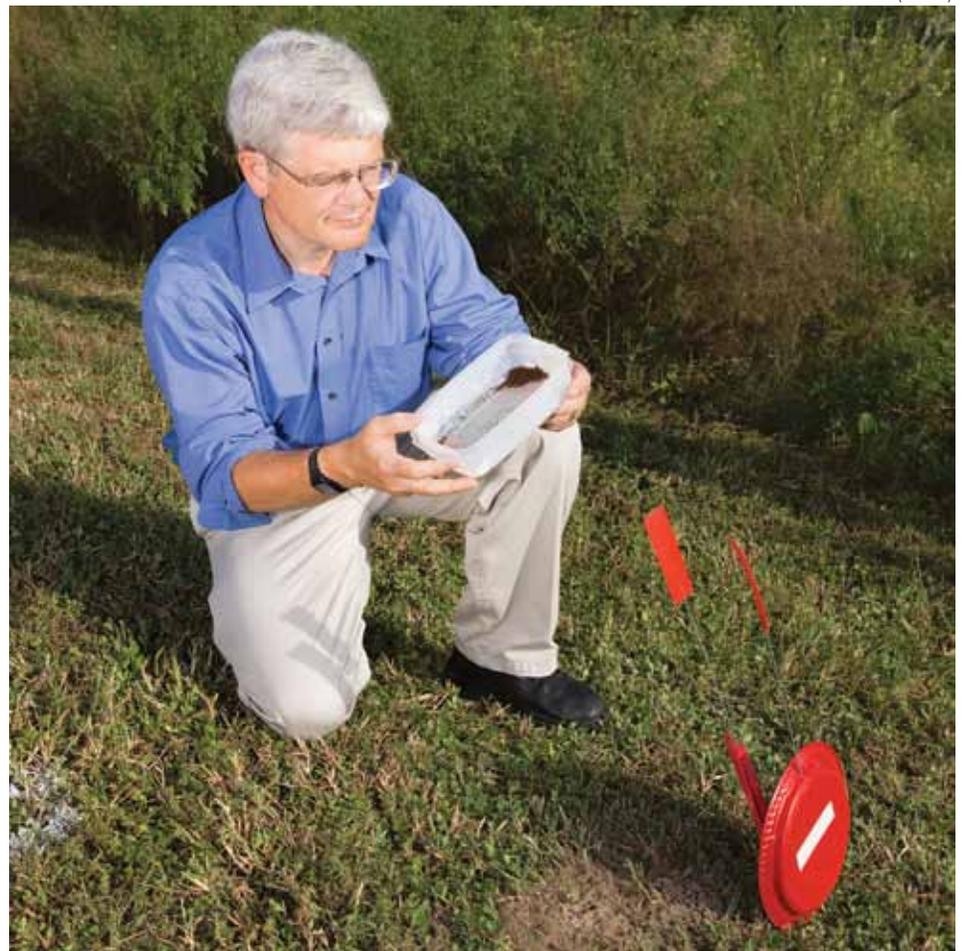
*Research Unit, Center for Medical, Agricultural, and Veterinary Entomology, 1600-1700 S.W. 23<sup>rd</sup> Dr., Gainesville, FL 32608; (352) 374-5914, [sanford.porter@ars.usda.gov](mailto:sanford.porter@ars.usda.gov). \**

Below: A phorid fly (left), *Pseudacteon cultellatus*, about 1 mm long, and a red imported fire ant (right), *Solenopsis invicta*, about 3 mm long. The fly can lay eggs inside the ant's head. Fly maggots that hatch will then slowly decapitate the ant.

SANFORD PORTER (D2023-1)



PEGGY GREB (D2021-1)



Entomologist Sanford Porter prepares to release several hundred fire ant workers parasitized by a new phorid fly species (*Pseudacteon cultellatus*) that prefers to attack the smallest fire ant workers.

## Sweetpotatoes Pack Potent Protection

Sweetpotatoes earned U.S. producers about \$395 million in 2008—and they're packed with carotenoids, vitamin C, potassium, and fiber. Now Agricultural Research Service (ARS) scientists have found that they also contain high levels of protective phenolic compounds called "caffeoylquinic acids." These compounds, which are known for their antioxidant activity, are found in many plants, though the levels vary widely.

ARS agronomist Howard Harrison teamed up with plant pathologist Pat Wechter and plant physiologist Joseph Peterson (now retired) to measure the levels of caffeoylquinic acids in sweetpotatoes. All three scientists work at the U.S. Vegetable Laboratory in Charleston, South Carolina. Other ARS collaborators included ARS chemists Maurice Snook and Trevor Mitchell, who work at the Richard B. Russell Research Center Toxicology and Mycotoxin Research Unit in Athens, Georgia.

The scientists assessed the levels of four types of caffeoylquinic acids in 16 sweetpotato varieties from the United States, Brazil, and Africa. They also studied caffeoylquinic acids in bigroot morningglory, a sweetpotato relative with roots that can weigh more than 60 pounds.

The research team found measurable amounts of all four types of caffeoylquinic acids in the sweetpotatoes they tested. On average, the highest levels of the compounds were found in the layer of tissue just under the skin. Intermediate levels were found in the stele—the interior of the sweetpotato—and the lowest levels were found in the skin. Bigroot morningglory roots also had high levels of all four of the defensive compounds.

The scientists found that three of the compounds they tested provided some protection against *Rhizopus* soft rot, a fungus that infects sweetpotatoes after harvest by invading through breaks in the skin. All of the compounds inhibited growth of plant-infecting bacteria, and one inhibited another infectious plant fungus, *Fusarium solani*.

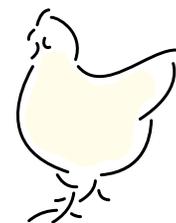
The team noted that the compound levels varied significantly among the sweetpotato varieties they evaluated, despite the study's small sample size. "If we screened a larger sweetpotato germplasm collection, we'd probably find varieties with even higher compound levels," Harrison says. He thinks that plant breeders may be able to use the compounds as biochemical markers to develop sweetpotato varieties with enhanced pest resistance and other beneficial traits.—By **Ann Perry**, ARS.

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## "Seeing" Poultry Carcass Contamination

Agricultural Research Service scientists Kurt Lawrence, Bosoon Park, Bob Windham, and Seung-Chul Yoon—all in the Quality and Safety Assessment Research Unit in Athens, Georgia—have made two improvements to a hyperspectral imaging system used to scan the surface of poultry carcasses for contaminants. They have refined the system so that it can detect even tiny amounts of fecal contamination, which can vary significantly depending on where in the digestive tract it originated. They have also developed and implemented a new image-processing method to identify and remove false-positive readings.



To facilitate the transfer of their system, a prototype on-line multispectral imaging system was installed and tested in a commercial poultry plant to detect fecal-contaminated carcasses. The system was developed through a research agreement with Stork Gamco, a manufacturer of poultry-processing equipment based in Gainesville, Georgia. Carcasses were imaged after evisceration but before washing, at a rate of 150 birds per minute.

"The system ran for several days with no hardware or software problems, and it demonstrated the feasibility of accurately detecting fecal-contaminated carcasses," says Lawrence.

The Athens team is collaborating with agricultural engineer Kevin Chao and biophysical scientist Moon Kim at the Beltsville Agricultural Research Center's Environmental Microbial and Food Safety Laboratory, who developed an on-line imaging system to differentiate systemically diseased poultry carcasses from wholesome ones.

The ARS groups and their industry partner are now merging the fecal-detection and diseased-carcass-detection systems onto a common platform that includes a line-scan hyperspectral imaging camera, lighting, and operating and detection software. Merging the two systems will aid in commercialization by creating one interchangeable imaging system that can be installed in different locations of the processing line to solve two separate and significant problems in the poultry processing industry. This will allow processors to more easily integrate such a system into their operations.

"We are currently modifying our system to work on the camera system used by the Beltsville group," says Lawrence. "Our goal is to have the new prototype tested by the end of 2009."—By **Sharon Durham**, ARS.

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## Helping the Peanut Industry

JACK DYKINGA (K4297-14)



A new technology that grades peanuts faster and more accurately is being researched by Agricultural Research Service scientists at the ARS National Peanut Research Laboratory in Dawson, Georgia.

“The U.S. peanut industry is in a period of rapid economic and technical change, and this technology will help the industry maintain a competitive edge,” says Marshall Lamb, research leader at the lab.

Lamb was approached by peanut-industry representatives for help in solving labor-shortage issues by automating peanut grading. Engineer Hank Sheppard tested x-ray technology to determine whether such a system could handle the job. When compared to official peanut-grading methods, the technology delivered a 98- to 99-percent accuracy rate, and it was faster—7 minutes versus 20 minutes per peanut grade sample.

“Official grading is labor intensive, requiring three to six people to hand-shell, pick, sort, and grade each nut,” says Sheppard. “The industry is having difficulty finding enough people to fill the worker requirements and who are willing to set aside 3 months of the year to do this difficult job.”

Accordingly, the industry recently launched an initiative to improve current procedures or develop new technologies that would make peanut grading more efficient while ensuring—or even improving—accuracy and quality.

Another processing problem being addressed by ARS research is peanut moisture. Nuts must have a moisture content of 10 percent or less to be suitable for further processing and shelling. The ability to determine moisture before grading begins would allow processors to divert high-moisture nuts for further drying instead of discarding them. Currently, the nuts are shelled, and then the moisture content is determined.

Chari Kandala, an agricultural engineer at the lab, has developed an automated in-shell moisture-detection system that could work in tandem with the x-ray grading unit to provide peanut processors a more efficient operation.

“We strive to help the peanut industry tackle important technical and quality issues. Applying these technologies may play a role in solving some of their problems,” says Lamb.—By **Sharon Durham, ARS.**

*Marshall Lamb, Hank Sheppard, and Chari Kandala are with the USDA-ARS National Peanut Research Laboratory, 1011 Forrester Dr., S.E., Dawson, GA 39842; phone (229) 995-7400, fax (229) 995-7416, e-mail marshall.lamb@ars.usda.gov, hank.sheppard@ars.usda.gov, chari.kandala@ars.usda.gov. ★*

## Biofilms Have a New Foil

*Listeria monocytogenes*—a foodborne pathogen—has been found in some ready-to-eat meats. It causes serious illness in about 2,500 people each year, resulting in 500 deaths.

Though *L. monocytogenes* is killed by cooking or pasteurization, it can survive many chemicals used in in-plant sanitation programs. Thus, food can be contaminated during or after processing. The pathogen’s ability to grow at low temperatures may allow its growth in or on raw or improperly processed ready-to-eat foods even when they are refrigerated.

One source of contamination is work surfaces of processing plants where meat products are made. ARS microbiologist Judy Arnold at the Poultry Microbiological Safety Research Unit in Athens, Georgia, has been looking for improved methods to control biofilms containing *L. monocytogenes*. Any method needs to be acceptable to the U.S. Environmental Protection Agency.

Biofilms are protective layers of proteins and polysaccharides that surround bacteria and stick to equipment surfaces. “These protective shields trap spoilage bacteria and other pathogens that contaminate food during processing, and they resist cleaning and sanitizing,” says Arnold. “Today’s longer production runs provide more opportunity for biofilms to establish themselves, and today’s longer shelf life adds to the risk of biological contamination.”

In collaboration with Sterilex Corporation of Owings Mills, Maryland, Arnold has tested a proprietary formulation—based on alkaline peroxide and phase-transfer chemistry—that appears to be a cost-effective disinfectant for use in environments for poultry and meat production and processing. The formulation uses multiple chemical and physical actions to penetrate a biofilm, kill the microorganisms, and remove the biofilm from surfaces. It was tested against multiple disinfectants for killing and removal of *L. monocytogenes* biofilm.

“Results showed that the formulation was 100 percent effective, providing total kill and more than 90 percent biofilm removal,” says Arnold. “This disinfectant is more effective than currently used disinfectants in reducing *L. monocytogenes* biofilm growth, thus minimizing the risk of pathogenic contamination. Test evaluations also resulted in instructions for use that will meet USDA ‘zero tolerance’ regulations for *L. monocytogenes*.”—By **Sharon Durham, ARS.**

*Judy Arnold is in the USDA-ARS Poultry Microbiological Safety Research Unit, Richard B. Russell Research Center, 950 College Station Rd., Athens, GA 30605; phone (706) 546-3515, fax (706) 546-3068, e-mail judy.arnold@ars.usda.gov. ★*



# Science Update

## Fungi May Hold Key to Reducing Grapefruit Juice Interactions with Medications

Grapefruit juice can interfere with the effectiveness of some medications because it contains furanocoumarins, which are one of many types of phytochemicals commonly found in plants. Furanocoumarins inhibit the enzymatic activities responsible for metabolizing certain medications and facilitating their release into the bloodstream.

Researchers have found that the fungus *Aspergillus niger*



either binds with grapefruit furanocoumarins or enzymatically breaks them down. Studies are continuing to identify the enzymes in *A. niger* that prompt the breakdown of furanocoumarins to see if these enzymes could be used to eliminate the compounds from grapefruit juice.

In another study, when edible mushrooms that are related to *A. niger*—including morels and oyster and button mushrooms—were dried, pulverized, and added to grapefruit juice, they also removed furanocoumarins. These findings provide additional evidence that proteins from *A. niger* and other fungi might someday lead to new methods for removing furanocoumarins from grapefruit juice. *Jan Narciso, USDA-ARS Citrus and Subtropical Products Laboratory, Winter Haven, Florida; (863) 293-4133, ext. 119, jan.narciso@ars.usda.gov.*

## Food "Tattoos": An Alternative to Labels for Identifying Fruit

Technology called "laser etching," developed in part by ARS researchers, uses lasers to "tattoo" produce and could someday replace current labeling practices. A carbon dioxide laser beam is used to etch information into the first few outer cells of the peel on grapefruit, creating a mark that can't be peeled off, washed off, or altered in any way.

The tiny holes etched into the grapefruit peel are effectively sealed by the carbon dioxide, which prevents decay and entry by pathogens, but a coating of wax can further protect against water loss and pathogen entry. The original testing was conducted on grapefruit and has now been extended to include other citrus fruits, tomatoes, and avocados. The U.S. Food and Drug Administration is currently reviewing its application for commercial use. *Jan Narciso, Citrus and Subtropical Products Research Laboratory, Winter Haven, FL 33881; (863) 293-4133 ext. 119, jan.narciso@ars.usda.gov.*

## It Pays To Furrow Dike

Farmers who use furrow diking in their fields plow soils into ridge-like barriers that run alongside row crops. The ridges hold water and allow it to soak into the soil instead of washing away. Researchers compared the effects on runoff and erosion in southeast U.S. cotton fields with and without furrow diking. They found that furrow diking during a moderate drought saved farmers 1 inch of irrigation water per acre, reduced runoff by 28 percent, and curbed soil erosion.

The following year, when drought conditions were more severe, it saved 5 inches of irrigation water per acre. The scientists also compared crop yields, water needs, and the effects of different irrigation rates between tracts of furrow-diked cotton and traditionally tilled cotton. Results indicated that in 1 of 3 years, cotton yield and net return were higher with furrow diking over four irrigation regimes, including dryland. *Russell C. Nuti, National Peanut Research Laboratory, Dawson, GA 39842; (229) 995-7449, russell.nuti@ars.usda.gov. Clinton C. Truman, Southeast Watershed Research Unit, Tifton, GA 31793; (229) 386-7174, clint.truman@ars.usda.gov.*

## First Hard Winter Wheat Varieties for Eastern U.S. Production

The first hard winter wheat varieties bred for production in the eastern United States have been developed by the Agricultural Research Service.

Hard wheat, which is used for baking bread, has been a production challenge for farmers in the eastern United States because the area's humidity increases the incidence of disease in the field, which affects yield and grain quality. But NuEast, a new variety of hard red winter wheat, has good grain yield and moderate resistance to powdery mildew and stem rust, including Ug99 races. It also has good resistance to leaf rust.

Another new wheat variety on deck is a hard white winter wheat called "Appalachian White." This variety was tested for 3 years at six locations and consistently produced good yields and better grain quality than other hard white winter wheats tested. Appalachian White also showed a higher level of resistance to powdery mildew, stripe rust, leaf rust, and Hessian fly than other hard white wheats. *David Marshall, USDA-ARS Plant Science Research Unit, Raleigh, NC; (919) 515-6819, david.marshall@ars.usda.gov.*

## Roasting Does More Than Enhance Flavor in Peanuts

Researchers have shown that increasing roast color intensity steadily ramps up the antioxidant capacities of peanuts, peanut flour, and peanut skins.

Peanuts were incrementally roasted at 362°F from zero to 77 minutes and then sampled for water- and oil-soluble antioxidant activity levels. Dark-roasting consistently increased water- and oil-soluble antioxidant capacities for both commercially available peanut flours and blanched peanuts. Peanut skins, currently considered a waste product of industrial peanut processing, had remarkably high antioxidant capacities across all roast conditions. Antioxidant increases upon roasting in blanched seed peanut were attributed to greater concentrations of phenolic compounds and/or "browning" reaction products.

The researchers also found that vitamin E degradation was most rapid in oil obtained from lightly roasted peanuts and that oil from darker roasted peanuts had better vitamin E retention than oil from lightly roasted or even raw peanuts. These findings could be a result of increased concentrations of oil-soluble Maillard reaction products, which seem to protect vitamin E from oxidation. *Jack Davis, USDA-ARS Market Quality and Handling Research Unit, Raleigh, NC; (919) 515-6312, jack.davis@ars.usda.gov.*

JACK DYKINGA (K4297-14)



**ARS studies show that the antioxidant content of peanuts and their oil is affected by roasting length.**

## Water Hardness Plays a Role in Removing Bacteria from Chicken Skin

Water used in commercial poultry-processing facilities can play a major role in the quality of the meat produced. Researchers have found that soft water is more effective than hard water in removing bacteria from broiler chicken skin. Hard water has higher concentrations of dissolved minerals like calcium and magnesium, and water is softened by removing these minerals.

Scientists compared how well very hard, moderately hard, and soft water rinsed away different bacteria—including *Campylobacter*, *Staphylococcus*, and *Pseudomonas*—from the skin of broiler chicken carcasses. They found that soft water removed up to 37 percent more bacteria than the other two water types. The effectiveness of sanitization procedures to remove microorganisms from carcasses during processing is affected by several water-quality variables, including pH, ammonia concentration, microbial contamination levels, and hardness.

These findings suggest that poultry processors might want to more closely monitor water hardness and its impact on carcass processing. *Arthur Hinton and Ronald Holser, USDA-ARS Richard B. Russell Agricultural Research Center, Athens GA 30605; (706) 546-3621 [Hinton], (706) 546-3361 [Holser], arthur.hinton@ars.usda.gov, ronald.holser@ars.usda.gov.*

## 2009 South Atlantic Area—ARS Award Winners



### **Area Senior Research Scientist**

**Bruce W. Wood**  
Byron, Georgia

For excellence in pecan production/protection research and advancing mineral nutrition management of crops.



### **Area Early Career Research Scientist**

**Mary J. Pantin-Jackwood**  
Athens, Georgia

For demonstrating the critical role of domestic ducks in the control of avian influenza viruses and for characterizing and improving diagnostics of novel enteric viruses of poultry.

## **Special Administrator's Award**

### **ARS H1N1 Influenza Virus Research Team**

**Erica Spackman**

**David Suarez**

Athens, Georgia

For outstanding, rapid research support and technology development to assist USDA, cooperating agencies, and the U.S. pork industry in responding to the 2009 H1N1 pandemic flu threat.

### **Rift Valley Fever Outbreak Early-Warning Team**

**Kenneth J. Linthicum**

**Seth C. Britch**

Gainesville, Florida

For outstanding effort and creativity in the development and transfer of Rift Valley fever outbreak early-warning system to protect global agriculture and public health.

## **Technology Transfer Awards**

### *Outstanding Efforts*

#### **Second Generation Treatment System for the Green and Profitable Management of Livestock Manure**

**Matias B. Vanotti**

**Ariel A. Szogi**

**Patrick G. Hunt**

Florence, South Carolina

For development of a green and profitable manure treatment technology.

#### **NCSU-ARS-IMS Microwave Technology Team**

**Van-Den Truong**

Raleigh, NC

For development and transfer of a novel continuous-flow microwave heating process for producing large containers of aseptic shelf-stable vegetable and fruit purées.

## 2010 South Atlantic Area—ARS Award Winners



### **Area Senior Research Scientist**

**Thomas E. Carter, Jr.**  
Raleigh, NC

For pioneering research on genetic diversity in soybean breeding and for developing the first high-yielding drought-tolerant soybean germplasm.



### **Area Early Career Research Scientist**

**Russell C. Nuti**  
Dawson, GA

For conceiving and conducting research and transferring it to applicable stakeholders in the peanut industry.

## **Technology Transfer Awards**

### *Superior Efforts*

#### **C. Corley Holbrook**

Tifton, GA

For development and transfer of Tifguard, the first high-yielding peanut cultivar with resistance to both the peanut root-knot nematode and the Tomato Spotted Wilt Virus.

#### **H1N1 Pandemic Influenza Veterinary Diagnostic Test Development Team**

##### **David Suarez**

##### **Erica Spackman**

For rapid response to the H1N1 pandemic influenza by timely development and transfer of diagnostic tests for veterinary specimens.

## 2011 South Atlantic Area—ARS Award Winners



### **Area Senior Research Scientist**

**Bosoon Park**  
Athens, GA

For pioneering research in hyperspectral and real-time multispectral imaging, and nanotechnology for food safety.



### **Area Early Career Research Scientist**

**B. Todd Campbell**  
Florence, SC

For innovative crop science research advances combining genomics and plant breeding.

## **Technology Transfer Awards**

### *Superior Efforts*

#### **Wheat Improvement Team**

**David Marshall**  
**Myron Fountain**  
**Lynda Whitcher**  
**Charlie Glover**  
Raleigh, NC

**Bill Brown**  
North Carolina State University  
Raleigh, NC

For the development, release, and adoption of specialty wheat varieties for farmers, millers, and bakers, and for organic local foods in North Carolina.

## **Sustained Effort Technology Transfer Awards**

### *Outstanding Efforts*

#### **Canal Point Sugarcane Variety Selection Team**

**Jack Comstock**  
**Serge Edmé**  
**Barry Glaz**  
**Neil C. Glynn**  
**Duli Zhao**  
**Jimmy D. Miller** (retired)  
**Peter Y.P. Tai** (deceased)  
Canal Point, FL

For the sustained transfer of high-yielding CP sugarcane varieties to Florida, and internationally, and the transfer of variety selection procedures to Central America.

## **Administrator's Outreach, Diversity, and Equal Opportunity Awards**

### *Supervisory/Managerial Category*

#### **Bacterial Epidemiology and Antimicrobial Resistance Research Team**

**Mark Berrang**  
**Paula Fedorka-Cray**  
**Charlene Jackson**  
**Jonathan Frye**  
**Richard Meinersmann**  
**Mark Englen**  
Athens, GA

In recognition of excellence in promoting diversity in ARS, mentoring students, and participating in student career and temporary employee programs.

## **AFM Support Award for Excellence**

### **Bronze Award for Excellence**

**Nancy L. Sparks**  
Raleigh, NC

For leading users of the new financial system (FMFI) through the various processes, resulting in a better understanding of the system.

## **2009 South Atlantic Area—FLC Awards**

### **FLC Award for Excellence in Technology Transfer**

**Kurt Lawrence**  
**Bosoon Park**  
**William Windham**  
Athens, GA

**Kuanglin Chao**  
**Moon Kim**  
**Alan Lefcourt**  
Beltsville Area  
Beltsville, MD

**Renfu Lu**  
Midwest Area  
East Lansing, MI

For hyperspectral imaging for food quality and safety inspection.

### **FLC Laboratory Director of the Year**

**Patrick Hunt**  
Florence, SC

## **2010 South Atlantic Area—FLC Awards**

### **FLC Laboratory Director of the Year**

**Kenneth Linthicum**  
Gainesville, FL

## **FLC Southeast Region Excellence in Technology Transfer—Honorable Mention**

**Corley Holbrook**  
Tifton, GA

For Tifguard, a peanut with resistance to both nematodes and viruses.

## **2011 South Atlantic Area—FLC Awards**

### **FLC Laboratory Director of the Year**

**Patrick Hunt**  
Florence, SC

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**USDA, Agricultural Research Service (ARS) Website**  
<http://www.ars.usda.gov>

**South Atlantic Area (SAA) Website**  
<http://www.ars.usda.gov/main/docs.htm?docid=6490>  
(on the website, click on the SAA location desired)

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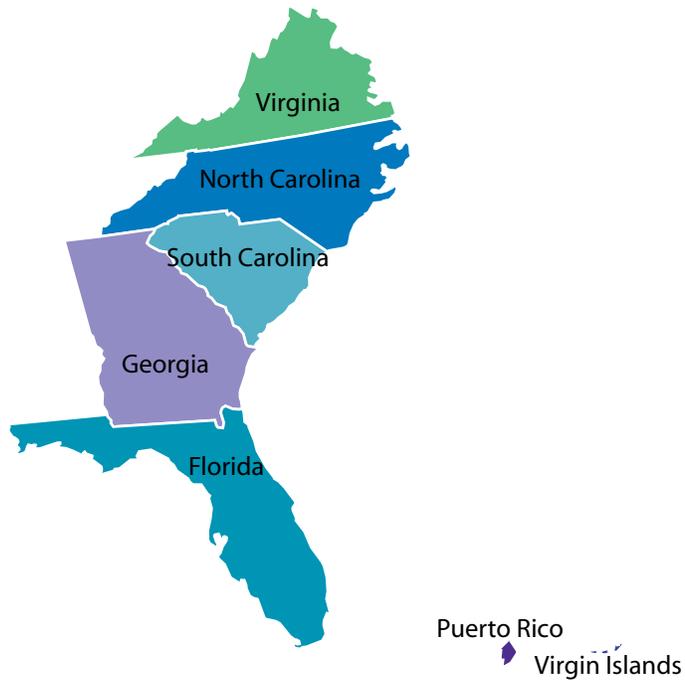
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