

Agricultural Research



CORN Boosting Quality, Productivity, and Safety

pp. 2-17

A Systems Approach to Corn

The United States is the world's largest producer and exporter of corn grain, producing more than 40 million metric tons each year. Eighty million acres of land in this country are planted to corn, with the majority being grown in the Midwest, and about 20 percent of our crop is exported. As one of America's major agricultural crops, corn brings in billions of dollars every year.

In addition to being an important food source for humans, corn and its coproducts are increasingly being used as animal feed and to develop biofuels, plastics, adhesives, and medicines. As uses and world demand for corn increase, so must our knowledge of the plant. This is why the Agricultural Research Service is taking a systems approach to corn by examining the factors that play important roles in producing this valued crop and how we can use them to our advantage.

ARS is proudly leading the way in examining the rich genetic diversity of corn, key to future advances in crop breeding and improvement. ARS scientists and university cooperators have recently developed the Nested Association Mapping (NAM) population, the largest plant genetic resource ever assembled. (See story on page 4.) NAM captures almost all of corn's genetic diversity. With this extremely useful tool, we are moving from manipulating just one gene to exploiting multiple genes for complex traits.

Working closely with other government agencies and universities, ARS is pioneering the use of new genetic resources and methods for crop improvement. For example, some of our scientists are working on projects funded by the USDA National Institute of Food and Agriculture, the U.S. Department of Energy, and the National Science Foundation to apply information gained from corn research to improve several types of plants for biofuel production. One project focuses on selecting genetic regions in corn's DNA that will improve the plant's energy value.

ARS is also working with other countries to improve corn. ARS and Mexican scientists have collaborated in maize and teosinte genome-sequencing projects, funded by the National Science Foundation and the Mexican government. Teosinte is a wild relative of corn and widely believed to be corn's ancestor. Insight into these genomes will help us learn what genes have been manipulated as corn has been domesticated and, more importantly, what genes we can manipulate to improve corn even more.

We have developed other useful tools in corn that will help all crop breeders select and utilize genes for valuable traits. ARS scientists and colleagues are using mutant corn genes as 'reporters' to identify novel genes and gene variants for traits of interest. (See story on page 7.) And the ARS-led Germplasm Enhancement of Maize (GEM) project is looking to broaden the genetic base of our country's corn crop by crossing exotic corn germplasm with currently used lines to help growers access valuable traits that may not have otherwise been available to them. (See story on page 15.)

In addition to examining the corn genome, ARS researchers are finding ways to control mycotoxin-producing *Aspergillus* fungi that can contaminate the grain. Some species of *Aspergillus* can severely affect crop yield and produce aflatoxins in corn at

levels unsafe for human or livestock consumption. ARS scientists are developing innovative ways to combat this serious problem. They are using beneficial species of *Aspergillus*—ones that don't produce aflatoxins—as natural, biological pesticides to combat the deadly *Aspergillus* species. (See story on page 8.)

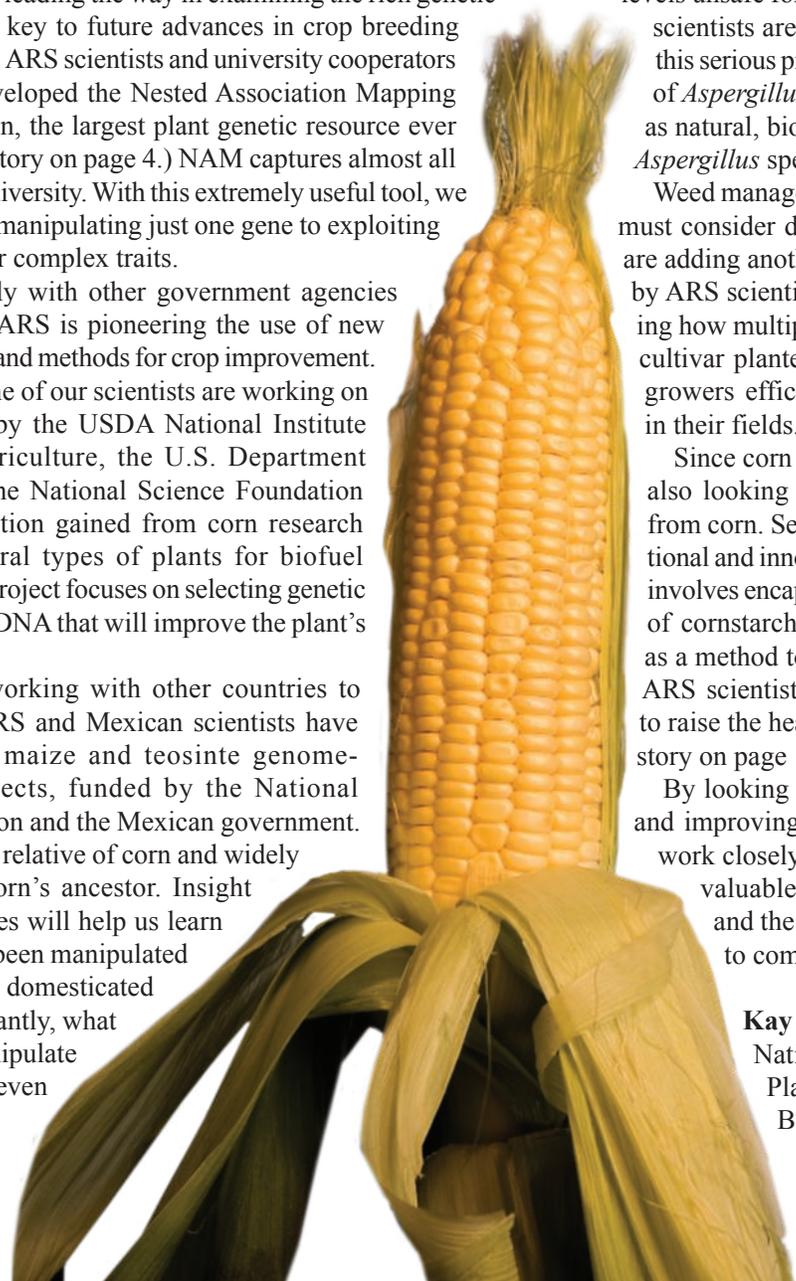
Weed management is another important area growers must consider daily. And the effects of climate change are adding another challenge to growers. New research by ARS scientists stresses the importance of considering how multiple factors such as planting date, type of cultivar planted, and the weed seed bank affect how growers efficiently and successfully manage weeds in their fields. (See story on page 14.)

Since corn is not only used as food or feed, we are also looking at ways to develop biobased products from corn. Several ARS researchers are finding functional and innovative uses for cornstarch. One such use involves encapsulating compounds inside tiny particles of cornstarch. These "nanoparticles" could be used as a method to deliver pesticides, for example. Other ARS scientists are developing corn-based modifiers to raise the heat tolerance of corn-based plastics. (See story on page 11.)

By looking at all the factors involved in sustaining and improving corn production and by continuing to work closely with collaborators, we will ensure this valuable crop remains available to consumers and the industries that rely on it for many years to come.

Kay Simmons

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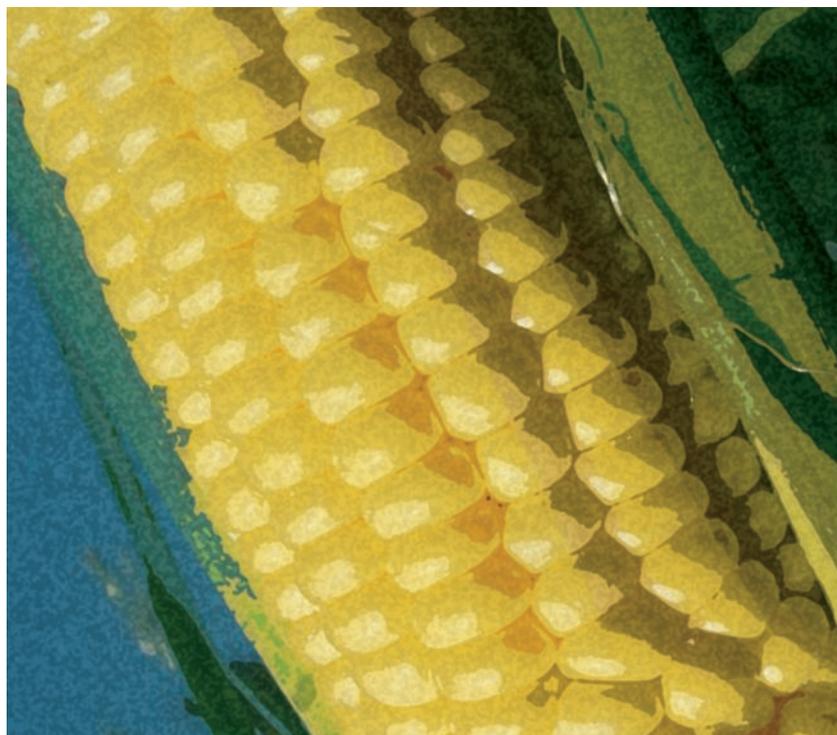
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Cover: Ecologist Marty Williams examines a sweet corn plant. He and his colleagues found weedy fields yielded more corn when planted in June or July, while less weedy fields had better crop yields when planted earlier. Story on page 14. Photo by Peggy Greb. (D1914-1)

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.

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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.

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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.

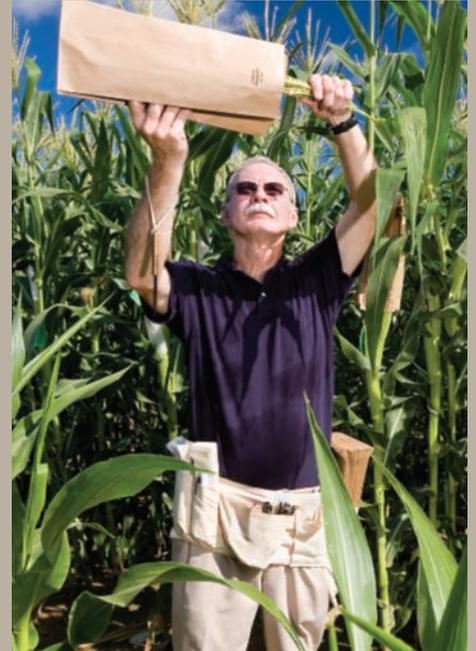
In related research, by comparing aflatoxin-susceptible with aflatoxin-

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.



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



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.

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Helping Earth-Friendly, Corn-Based Plastics Take the Heat

Those little plastic coffee-cup lids that keep your steaming hot java from sloshing all over you might someday be made from biodegradable, corn-based plastic. Making corn-derived plastics more heat-tolerant, so that they won't distort when they're not supposed to, is one of several top-priority targets of collaborative research under way since 2007 at the Agricultural Research Service's Western Regional Research Center in Albany, California, near San Francisco.

Chemist William J. Orts, who leads the center's Bioproduct Chemistry and Engineering Research Unit, works with collaborators Allison Flynn and Lennard F. Torres of Santa Barbara-based Lapol, LLC, to broaden the range of applications for which corn-based plastics would be ideal. The partnership, carried out under terms of a cooperative research and development agreement, aims at making those plastics an alluring alternative to petroleum-derived plastic goods.

Plastics made from corn are generally biodegradable, and corn is a renewable natural resource. While plastics made from petrochemicals can be biodegradable, they are derived from a finite, nonrenewable source.

What's more, manufacture of corn plastics usually causes less pollution, including fewer greenhouse gas emissions, than does the production of petro plastics.

Corn-based plastics are made by fermenting corn sugar to produce lactic acid. In turn, the lactic acid is used to form a bioplastic known as "polylactic acid," or PLA. Trouble is, PLA has a lower heat tolerance than some petroleum-based plastics. "That excludes PLA from being used for some applications," says Flynn.

In the plastics industry, the upper limit of PLA's heat tolerance, that is, the temperature at which it may begin to distort, is referred to as its "heat-deflection temperature."

To overcome this obstacle, Flynn, Torres, and Orts are developing a new product. Known as a "heat deflection temperature modifier," it can be blended with PLA to make PLA more heat tolerant. The modifier itself is more than 90 percent corn based and is fully biodegradable, according to Flynn.

Preliminary tests at the Albany lab indicate that, when blended with PLA, the modifier can raise PLA's heat-deflection temperature by at least 50°F. With further research and development, the heat modifier might make it possible for hundreds of products that currently can't typically be made with PLA to one day be manufactured with this bioplastic.

The products of the future might include not only coffee-cup lids, but perhaps food or beverage bottles or other containers that are "hot-filled," that is, filled at the food-manufacturing or beverage-bottling plant while the food or beverage is still hot from pasteurization. Examples might include, among other popular items, tomato catsup or some kinds of fruit juice. Today's PLA typically can't take the heat of hot-filling.

ARS and Lapol are currently seeking a patent for the invention.



ARS chemist William Orts (left) works with collaborators Allison Flynn and Lennard Torres of Lapol, LLC, to improve the heat tolerance of environmentally friendly plastic made from corn.

"Right now, there are no commercially available heat-deflection temperature modifiers for PLA," says Lapol chief operating officer Randall A. Smith. "It's an emerging market."

The encouraging preliminary results for the experimental heat-deflection temperature modifier suggest that new opportunities for PLA may indeed be heating up.—By **Marcia Wood, ARS.**

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

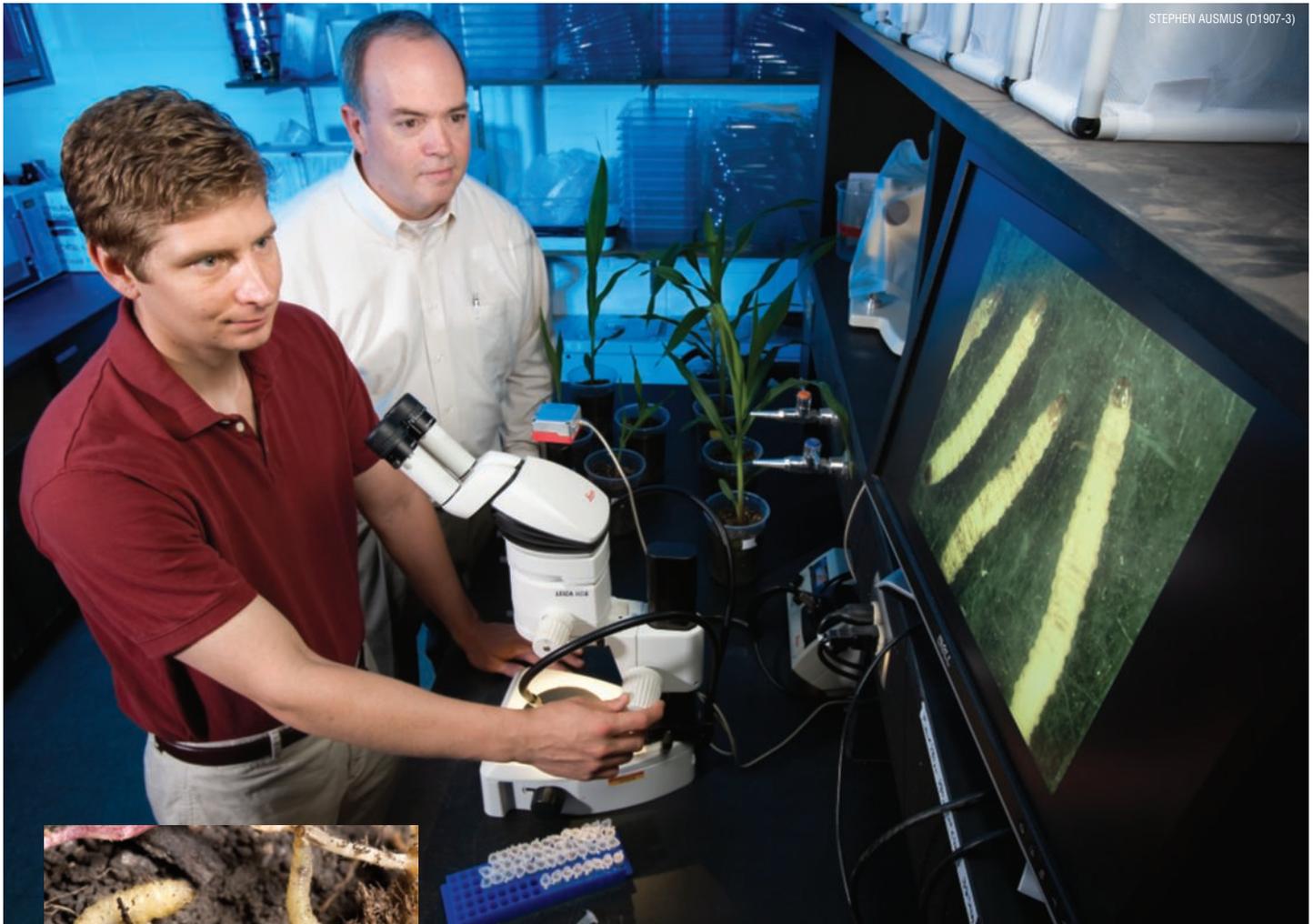
*William J. Orts is with the USDA-ARS Western Regional Research Center, Bioproduct Chemistry and Engineering Research Unit, 800 Buchanan St., Albany, CA 94710; (510) 559-5730, bill.orts@ars.usda.gov. **

A Cornucopia of Corn-Based Products

Agricultural Research Service scientists are busily inventing new, ecologically sound products from corn for home and industry. Several articles published in previous issues of Agricultural Research ([archived at \[ars.usda.gov/ar\]\(http://archived.ars.usda.gov/ar\)](http://archived.ars.usda.gov/ar)) will tell you more about their work.

- "From Lubricants to Packaging Materials: ARS Scientists Just Say 'No' to Petroleum," September 2009
- "Corn: The Latest Glue Ingredient?" September 2009
- "Zein—A Corn Compound With Diverse Valuable Uses," November/December 2008
- "Corn-Based Absorbent Dries Soggy Books," May 2007
- "Corn: A New Ingredient for Detergents?" April 2007
- "Electroactive Bioplastics Flex Their Industrial Muscle," December 2005

Rooting Out Rootworm Resistance



Above: Entomologists Aaron Gassmann (left), of Iowa State University, and Tom Sappington, with ARS, observe magnified western corn rootworm larvae to measure the size of their head capsules. Larvae exposed to *Bt* corn roots may grow more slowly than unexposed larvae, and head capsule width is an accurate indicator of larval growth stage. **Inset:** Larvae of western corn rootworm, *Diabrotica virgifera virgifera*, feeding on corn roots.

The western corn rootworm (WCR) is a highly destructive pest that has required more acres of conventional insecticide use per year than any other pest since it invaded the Midwest in the 1960s. Today, WCR continues to be the most important pest of corn production across the Midwest, in part because it has developed resistance to conventional crop rotation, which was once a highly effective management tactic.

Certain types of *Bt* corn, which have been genetically engineered to produce a particular insect-specific toxin protein

derived from the bacterium *Bacillus thuringiensis*, are effective in the control of WCR. These products have partially replaced the use of conventional insecticides for WCR control. But laboratory tests strongly suggest that if *Bt* technology is not managed effectively in the field, WCR will acquire resistance to *Bt* over time.

Entomologists Bruce Hibbard, in the Plant Genetics Research Unit (PGRU) in Columbia, Missouri, and Tom Sappington, in the Corn Insects and Crop Genetics Research Unit (CICGRU) in Ames, Iowa, together with Blair Siegfried, at the Uni-

versity of Nebraska-Lincoln, and Aaron Gassmann, at Iowa State University, are analyzing the evolution of *Bt* resistance in WCR to help safeguard this technology for the future.

Some *Bt* corn lines targeting WCR produce a protein toxin known as “Cry3Bb1.” Hibbard and collaborators recently documented that WCR exposed to these corn lines, at low to moderate doses under greenhouse conditions, developed *Bt* resistance after just three generations. This result was later validated in a controlled field test.



Several colonies of WCR that are resistant to Cry3Bb1 have now been established and are being used by Sappington's group to identify genetic markers associated with the resistance. Similar studies are being conducted with *Bt*-corn lines that produce mCry3A and other Cry proteins.

"Development of colonies resistant to distinct *Bt* products will allow us to evaluate the potential for cross-resistance in WCR," says Hibbard.

Rapid Resistance

As a strategy to control the evolution of *Bt* resistance in WCR populations, Hibbard and collaborators are currently testing the concept of using non-*Bt* plants as insect "refuges." This method promotes the mating of *Bt*-resistant insects with *Bt*-susceptible insects that emerge from refuge plants, which do not produce *Bt*. In this way, *Bt* susceptibility is maintained in the WCR population.

In 2006, Hibbard, along with CICGRU and University of Missouri collaborators, documented that without a refuge, WCR populations quickly develop *Bt* resistance under laboratory conditions. At the university's Bradford Research and Extension Center, the team evaluated *Bt*-resistant and *Bt*-susceptible (control) WCR colonies. *Bt* corn and non-*Bt* corn plants were infested with 500 viable rootworm eggs from the resistant or control colonies. The number of *Bt*-resistant insects recovered from *Bt* corn was 10 times greater from eggs taken from the *Bt*-resistant colony than from eggs taken from the control colony. Each colony did equally well on the non-*Bt* corn.

"This emphasizes the importance of farmers planting refuges, because resistance developed in the absence of a refuge," says Hibbard. Current work specifically tests the effectiveness of different refuge types.

Gathering Genetic Intelligence To Combat Western Corn Rootworm

At the Ames lab, DNA marker technology is being used to glean valuable

genetic intelligence on the rootworms. DNA marker sequences are short stretches of DNA along the pest's genome that uniquely identify a region of that chromosome. A DNA marker that is located near a gene that confers a trait of interest can often be detected more easily than the gene itself.

Sappington's team is also using DNA markers to genetically locate the gene or genes responsible for "rotation resistance," an egg-laying behavior that enables some rootworm populations to survive rotations of corn with soybean, a nonhost crop on which the pest's caterpillars cannot feed.

Farmers in the Midwest have long used corn-soybean rotations, which allows them to forgo using soil insecticides on their corn. But starting in 1987, reports emerged from eastern Illinois that female rootworms were flying out of corn fields to deposit their eggs in nearby soybean crops—in effect, synchronizing their larval offsprings' emergence to the next season's rotational corn crop.

The trait has since spread to parts of Indiana, Ohio, Michigan, Missouri, Iowa, and Wisconsin and to Ontario, Canada. A DNA marker associated with rotation resistance would help alert farmers to when the trait has, or has not, spread to their region.

DNA markers are also being developed for examining genetic variation and gene flow within and between WCR populations.

"Though difficult to achieve, information on the population ecology and genetics of WCR are crucial for designing effective resistance-management strategies to maintain the viability of current control technologies, including *Bt* corn," says Craig Abel, CICGRU research leader. "Molecular markers are important tools for obtaining this information."

Sappington, David Grant, Nicholas Miller, and others at Ames are collaborating with university colleagues and the Institut National de Recherche Agronomique in France to conduct more sensitive analyses

of genetic variation in populations. The scientists are taking specimens from WCR's native range in the Great Plains and trying to get better population-structure data than that obtained previously from the Corn Belt, where the pest appeared more than 40 years ago—not long when it comes to random genetic change in huge populations, says Sappington.

"By analyzing these long-established populations, we hope to estimate how far rootworm adults move per generation," he says. "Such estimates are important for accurately predicting the development and spread of *Bt*-resistance and rotation-resistance traits over long distances—almost impossible to do using conventional methods such as mark-release-recapture experiments."—By **Alfredo Flores**, formerly with ARS, and **Jan Suszkiw**, ARS.

This research is part of Integrated Agricultural Systems (#207) and Crop Protection and Quarantine (#304), two ARS national programs described at www.nps.ars.usda.gov.

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

STEPHEN AUSMUS (D1910-33)



Adult female western corn rootworm, *Diabrotica virgifera virgifera*, on a corn leaf.

Sweet Corn Weed-Management Systems for the Changing Climate

Can you imagine a world without sweet corn—the variety of corn we humans enjoy eating the most? Summer barbecues wouldn't be the same if ears of fresh, sweet corn on the cob weren't on the menu. And let's not forget the many recipes that use canned or frozen sweet corn. Served with other ingredients or on its own, sweet corn is one of America's favorite vegetables.

Thanks to ecologist Marty Williams, we won't have to worry about forgoing a helping of this tasty treat anytime soon. Williams is working hard to help improve sweet corn production by examining the ecological factors farmers face in growing this popular food crop. He works in the ARS Global Change and Photosynthesis Research Unit in Urbana, Illinois—right in the heart of our country's Corn Belt.

In a recent study published in *Field Crops Research*, Williams and colleagues examined how the changing climate is affecting weed growth in corn fields. As the climate in the Midwest gets warmer, weeds commonly seen in the South are now moving north. Changing environmental conditions, such as warmer temperatures and periods of both drought and flooding, are bound to affect corn's growth and productivity, too.

The researchers examined 175 commercial sweet corn fields throughout the north central United States, a major sweet corn processing region. They sought to identify the important weed species commonly found in these fields and learn more about which management practices are and aren't working well.

The study produced some interesting results. "The highest yielding fields were those that received tillage between crop rows," says Williams. "Fields located in the northern part of the region and fields that were planted to a sweet corn hybrid that matured in less than 84 days also produced higher yields."

The researchers also identified several characteristics of more sustainable weed-management systems. Adequate rainfall or irrigation improved the effectiveness of herbicides, and fields in the northern part of the region experienced lower weed interference and produced fewer weed seeds. While corn yields were related to the amount of weed pressure, the impact of weed pressure on yields depended on planting date. For example, weedy fields yielded more corn when planted in June or July, while less weedy fields had better crop yields when planted earlier.

Surprisingly, the research team found that fields receiving the most expensive weed-control programs and highest rates of herbicide also had the highest weed interference and weed seed production. This shows that even the most rigorous herbicide program doesn't routinely provide complete control, underscoring the importance of taking a long-term approach to managing the weed seed bank in the soil.

"The fact that latitude showed up repeatedly as an important descriptor of successful production systems and that environmental conditions in the region are changing suggests that best management practices are a moving target," says Williams. "Our findings show the areas where growers may



Sweet corn after silk emergence. ARS scientists are examining how the changing climate is affecting weed development and how that affects crop growth and productivity.

want to consider changes to their production systems."—By **Stephanie Yao, ARS.**

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

*Marty Williams is in the USDA-ARS [Global Change and Photosynthesis Research Unit](http://www.ars.usda.gov), 1102 S. Goodwin Ave., Room N-325, Urbana, IL 61801; (217) 244-5476, martin.williams@ars.usda.gov. **

Genetic-Diversification Program Is a "GEM" of a Resource for Corn Growers

Since 1994, the ARS-led Germplasm Enhancement of Maize (GEM) project has sought to help broaden the genetic base of America's corn crop by identifying promising exotic germplasm and crossing it with domestic lines.

New hybrids derived from such crosses have provided corn researchers and the producers they serve with access to many valuable traits that might not otherwise have been readily available to them. These may include improved or alternative native sources of resistance to insect pests such as corn rootworms and diseases like northern leaf blight, better stress tolerance, superior silage, increased oil content, and enhanced bioenergy potential.

GEM's aim is to provide sources of useful genetic maize diversity to help producers reduce risks from new or evolving insect and disease threats or changes in the environment or respond to new marketing opportunities and demands, says Mike Blanco, a plant geneticist who oversees GEM at the North Central Regional Plant Introduction Station, Ames, Iowa.

Blanco coordinates the evaluation and development of germplasm geared to midwestern corn production systems. Plant geneticist Matt Krakowsky, with ARS's Plant Science Research Unit in Raleigh, North Carolina, coordinates breeding and evaluation activities for the southeastern United States. Collaborating with them are more than 60 GEM cooperators from other ARS labs, state and foreign universities, private industry, international organizations, and nongovernmental organizations.

During the 2009 growing season, the Ames and Raleigh

locations managed or coordinated evaluations on 17,200 nursery plots as well as 14,000 yield trial plots in Ames and 12,000 in Raleigh. A new "allelic diversity" study is devoted to exploring and capturing the genetic variation represented by over 300 exotic corn races. In 2010, 420 nursery rows at Raleigh and 369 rows in Ames are devoted to the objective of the allelic diversity project, which is to develop and release adapted germplasm lines representing this diversity.

GEM researchers use newer technologies to achieve their objectives and, when necessary, devise new ones. For example, using the "doubled haploid" method reduces the time it takes to produce homozygous plants derived from tropical or semitropical corn from the 8-10 growing seasons required by conventional breeding to just 2 growing seasons. Use of photoperiod-control "shade houses" created in Ames enables tropical corn lines to flower in midwestern long day-length periods.

"This allows us to make crosses we'd normally have to go to a short day-length nursery in the Tropics to make," says Blanco. More crosses can be made, affording greater quality control over research.

Since 2001, GEM has released 221 new corn lines to cooperators for further development into elite commercial hybrids.—By **Jan Suszkiw, ARS.**

*Mike Blanco is with the USDA-ARS Plant Introduction Research Unit, Iowa State University, G212 Agronomy Hall, Ames, IA 50011; (515) 294-3758, michael.blanco@ars.usda.gov. **

Corn Genomics: At the Touch of a Keystroke

The Maize Genetics and Genomics Database (MaizeGDB) is a web-accessible resource (www.maizegdb.org) that furnishes geneticists, breeders, and others with all things corn—from molecular markers and sequence data to genetic maps and bioinformatics tools for mining them.

"MaizeGDB puts data together in a way that would have taken researchers a long time to chase down and compile on their own," says Carolyn Lawrence, a plant geneticist and MaizeGDB director at ARS's Corn Insects and Crop Genetics Research Unit (CICGRU) in Ames, Iowa.

Launched in 1991 as "MaizeDB," MaizeGDB is the product of many collaborators from CICGRU, ARS's Plant Genetics Research Unit in Columbia, Missouri, ARS's Plant Gene Expression Center in Albany, California, and cooperators worldwide. In addition to its support from ARS, MaizeGDB is also funded by the National Science Foundation and other organizations.

For all the powerful science behind it, MaizeGDB is surprisingly easy to use and offers straightforward explanations of key terminology—including how database information can be used.

For example, under a subhead titled "data centers," clicking on the term *QTL* links the user to the following explanation: "A *QTL* (or quantitative trait locus) refers to a particular region of

the genome that is associated with a particular trait. This association is made through statistical methods based on the counting and measurement of easily observed traits, such as the weight of 1,000 kernels, the height of the second leaf at a particular stage of development, and so forth."

Conduct a *QTL* search for *plant height* and you get a list of experiments that evaluated plant height as well as links to the trait itself and 60 references describing it.

"At an early stage, the MaizeGDB team partnered with potential users to develop the database," says CICGRU research leader Craig Abel. "The resulting feedback and the creativity of the MaizeGDB team to develop solutions have produced an exceptional genomic database."

A recent noteworthy addition is a new genome browser that graphically displays sequence data for the inbred line B73. Another is the "Locus Lookup Tool." According to Lawrence, this tool can help researchers locate specific genes that will help them develop a better corn plant. Ultimately, growers will benefit from this technology as healthier and higher yielding corn plants are developed.—By **Jan Suszkiw, ARS.**

*Carolyn Lawrence is in the USDA-ARS Corn Insects and Crop Genetics Research Unit, 1034 Crop Genome Informatics Laboratory, Iowa State University, Ames, IA 50011; (515) 294-4294, carolyn.lawrence@ars.usda.gov. **

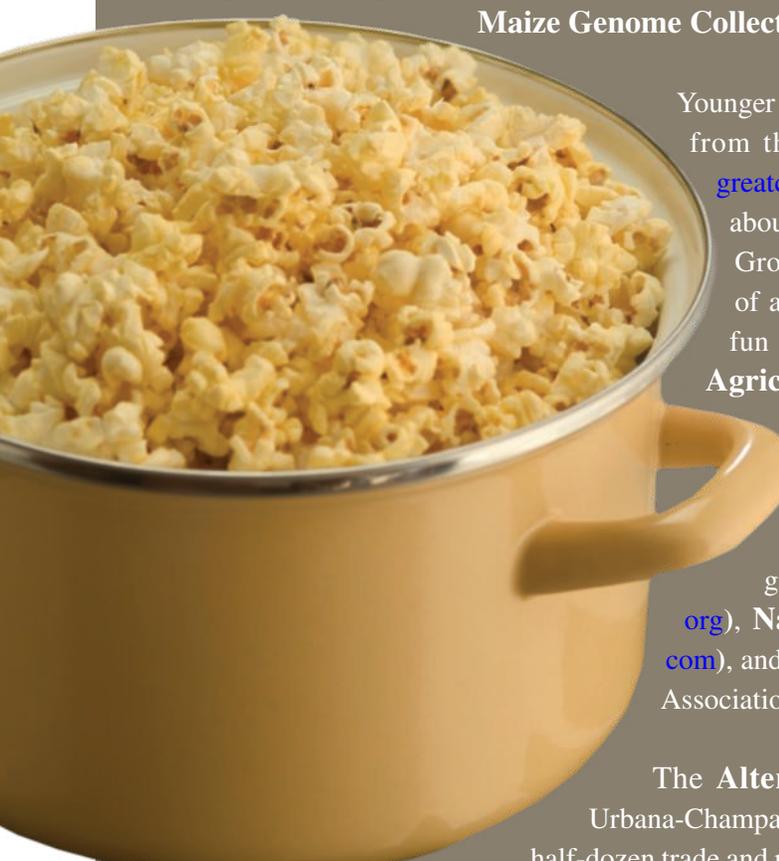
CORN!

See These Sources To Learn More

Reference librarian Felecia Tyler at the ARS National Agricultural Library in Beltsville, Maryland, and Washington, D.C., recommends these sources as starting points for those who want to learn more about corn.

More than 600 links to articles, images, and more about corn are offered at the National Science Foundation-funded **National Science Digital Library** (tinyurl.com/NSDL-corn).

Need help understanding corn genetics? See the science tutorial at the **Plant and Soil Sciences eLibrary** (tinyurl.com/UNL-corn) for self-directed lessons. You'll put what you learned to good use when you step up to the compilation of scientific articles in the Public Library of Science's **PLoS Genetics: 2009 Maize Genome Collection** at www.plos.org/cms/node/498.



Younger viewers may delight in **The Great Corn Adventure**, from the University of Illinois Extension (tinyurl.com/greatcorn). Teachers of 4th or 5th graders will want to know about **Corn in the Classroom** from the Kentucky Corn Growers' Association (tinyurl.com/KYcorn). And kids of all ages will enjoy the National Agricultural Library's fun and informal **Popcorn: Ingrained in America's Agricultural History** (tinyurl.com/NALpopcorn), which takes a nostalgic look at one of the nation's all-time favorite snacks.

Browse these corn industry sites for a quick background: **Corn Refiners Association** (www.corn.org), **National Corn Growers Association** (www.nega.com), and the "Choose Ethanol" site of the Renewable Fuels Association (ethanolrfa.org/index.php).

The **Alternative Uses** page of the University of Illinois at Urbana-Champaign's Corn Information venue identifies more than a half-dozen trade and research organizations that are developing new uses for corn (tinyurl.com/altcorn).

Explore two more sites to see what online corn resources the National Agricultural Library has chosen for its ever-expanding **AGRICOLA** (AGRICultural On-Line Access) database. Choose from more than 500 online books, reports, and the like at go.usa.gov/i75, or select from more than 5,000 articles from scientific journals and related publications at go.usa.gov/i7n.—By **Marcia Wood, ARS**. *

THE POPCORN BOARD

ARS National Research Program on Corn

The Agricultural Research Service (ARS) has an extraordinarily strong research program in corn, with leading scientists at multiple locations cooperating to make crucial advances in one of the country's and the world's major crops. Those advances are not only benefitting corn, but are also serving as the basis for new discoveries in all plant sciences.

Most of the ARS corn research program centers on the critical need to broaden genetic diversity in corn. Corn is a highly bred plant, which has resulted in a very narrow genetic base. Researchers are looking outside the limited gene pool now in common use to find sources for essential new traits that growers need for the future.

The ARS corn research program concentrates on new insect and disease resistance, resilience to climate change, nutritional improvement for health benefits, quality improvements for both food and biobased products, and enhanced food safety with aflatoxin resistance—

objectives that all reflect the priorities of U.S. Department of Agriculture Secretary Tom Vilsack.

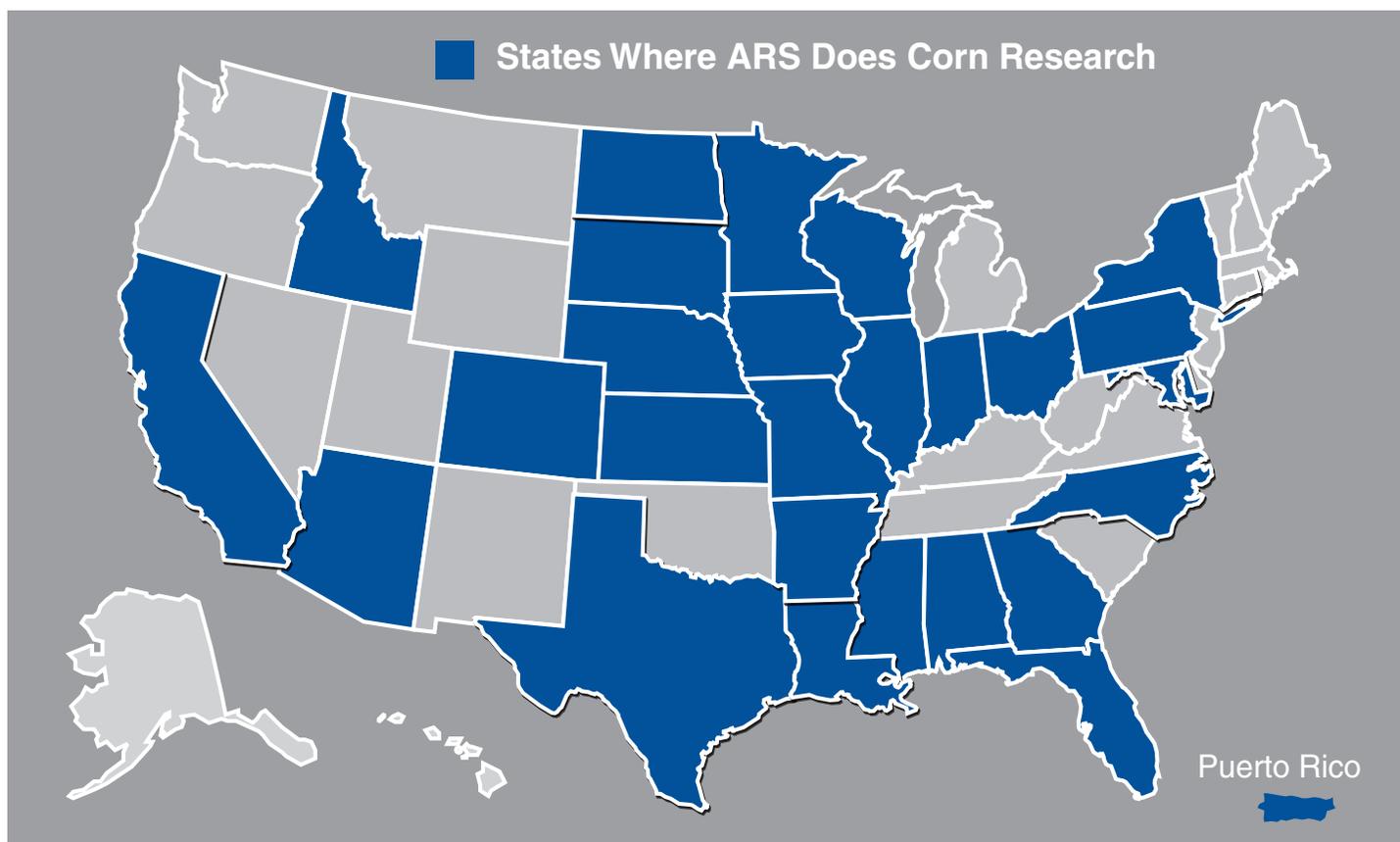
As part of the effort to broaden corn's genetic base, ARS researchers are leading the Germplasm Enhancement of Maize (GEM) project, a long-term, wide-scale collaboration of international, federal, state, and private partners to bring tropical and other exotic maize genes into the breeding pool. ARS scientists in GEM are coordinating testing and evaluation of thousands of new, more diverse corn lines under a range of environmental conditions.

ARS has also been a partner in the sequencing of the corn genome, which is revealing its functional regions. From the sequencing data, ARS researchers have assembled a haplotype genetic map (mapping of alleles located closely together on the same chromosome that tend to be inherited together) that lays out portions of the genome shared by 27 diverse inbred lines of corn.

To better understand natural diversity in corn, ARS scientists and collaborators have developed a massive genetic resource, which required assessing more than 1 million corn plants. These projects have received major support from the National Science Foundation, along with the USDA National Institute of Food and Agriculture and the U.S. Department of Energy.

Corn researchers in ARS are also developing new scientific tools and techniques to speed up the efficiency of crop breeding and developing more effective and environmentally sensitive production methods.

ARS scientists are also using corn as a model for grasses that can be used to produce biofuels. By extrapolating from the wealth of genomic and genetic information available for corn, scientists are making swifter progress in developing new bioenergy feedstocks from sorghum and perennial grasses. ✱



Hawaii Hosts **Wasp-on-Wasp** Battle

HAWAII DEPARTMENT OF AGRICULTURE (D1923-1)

A black, 2-millimeter-long wasp from East Africa is helping wage war on one of its own kind—the *Erythrina* gall wasp (*Quadrastichus erythrinae*), an invasive species that has decimated Hawaii’s wiliwili and coral bean trees. Hawaii Department of Agriculture (HDOA) officials made the



Light infestation of galls on coral tree leaves.

first release of the beneficial African wasp—500 specimens mass-reared in captivity—on November 28, 2008, at a gall-infested wiliwili stand in the Liliuokalani Botanical Gardens, Honolulu.

Michael Gates and Gérard Delvare’s scientific description and naming of the beneficial species (*Eurytoma erythrinae*) earlier that year helped HDOA obtain the necessary federal approvals to make the release. Gates and Delvare, both entomologists, are with the Agricultural Research Service and the Commonwealth Scientific and Industrial Research Organization, respectively.

“The gall wasp has been traveling around the globe for years,” says Gates, who works in ARS’s Systematic Entomology Laboratory at the Smithsonian’s National Museum of Natural History in Washington, D.C. “It is native to East Africa and has spread via the Indian and Pacific Ocean basins. It was detected in

Oahu in April 2005 and in Florida in the fall of 2006.”

How the gall wasp arrived is unknown, but the pest quickly found suitable hosts on which to feed and reproduce, first on Oahu, then on other Hawaiian islands. HDOA officials hope their biologically based approach to controlling the invasive wasp will spare further harm to Hawaii’s thinning ranks of indigenous wiliwili trees and introduced tropical coral bean trees, which are commonly used for landscaping, soil and water conservation, and as windbreaks.

The gall wasp’s larval stage is to blame for the harm that’s been caused. After hatching from eggs deposited in leaf and stem tissue, the larvae induce irregular plant growths called “galls” in which to feed. They pupate and emerge as adults 14 days later to feed, mate, and start the cycle of destruction over again. Heavily galled leaves become curled and misshapen.

HAWAII DEPARTMENT OF AGRICULTURE (D1924-1)



Heavy infestation of galls on coral tree leaves.

Severe infestations can defoliate and eventually kill afflicted trees.

The galls, coupled with the pest’s establishment throughout the Hawaiian islands, have made topically applied insecticides ineffectual and systemic ones too costly.

Royale

But now, these selfsame galls could become the pest's tombs, thanks to the ability of female *E. erythrinae* wasps to find the chambers and deposit their own eggs inside. Upon hatching, the brood feed on gall wasp larvae, then pupate and emerge 2 weeks later as adults. Importantly, *E. erythrinae* wasps pose no apparent danger to nontarget insects, such as native wasps.

The Hawaiians found their "gall wasp gladiator" after dispatching two entomological teams to the pest's native Africa in search of natural enemies, starting in spring 2006. Quarantine studies of parasite specimens the teams returned with showed the now-named *E. erythrinae* (discovered in Tanzania by HDOA entomologist Mohsen Ramadan) to be the most host-specific and dominant of the bunch. In January 2007, Gates and Delvare were asked to identify Ramadan's candidate wasp based on their taxonomic expertise in the genus *Eurytoma*.

Their examination of 27 female and 17 male specimens included dissecting several to observe their internal and external structures and compare them with existing scientific illustrations and descriptions of other related wasp species, many of which are preserved in collections at the Museum of Natural History in Paris.

"We also used a scanning electron microscope to obtain images, and we used an Entovision system, which combines multiple images into a single, in-focus image. This essentially eliminates depth-of-field and exposure limitations encountered with traditional macrophotography," says Gates. Based on their analysis, he and Delvare concluded that the parasite was a new *Eurytoma* species. They published their findings in the April 2008 issue of the journal *Zootaxa*.

After a slow start resulting from a cool Hawaiian winter, the 2009 *E. erythrinae* population appears to have gained solid footing. As of May 2009, specimens had been recovered from 90 percent of the original release sites and, in some cases, beyond, say Neil Reimer and Juliana Yalem, both with HDOA's Division of



PEGGY GREB (D1926-1)

Entomologist Michael Gates examines specimens of the beneficial wasp *Eurytoma erythrinae*.

Plant Industry.

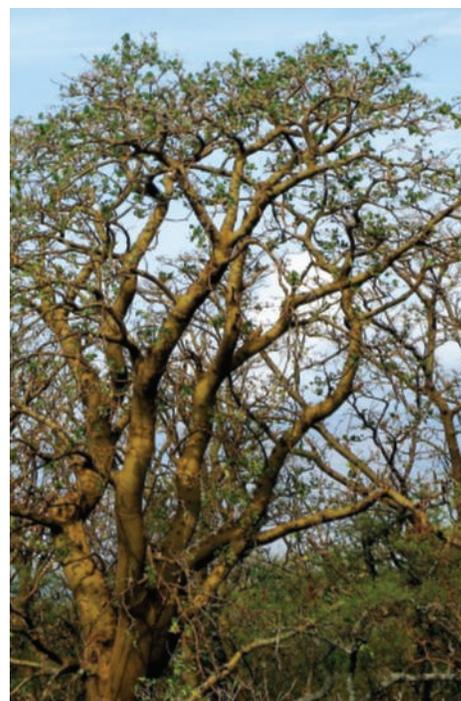
Gall-wasp parasitism has been as high as 70 percent at some sites. But continued data collection will be necessary to correlate *E. erythrinae*'s rise to declines in tree damage, especially wiliwili, which grows in dryland forests on the leeward slopes of Hawaii's islands.

"It's a bit early to tell what impact the *E. erythrinae* are having on the gall wasps, but the coral trees are starting to look pretty good," Reimer says.—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.

*Michael Gates is at the USDA-ARS Systematic Entomology Laboratory, MRC-168, National Museum of Natural History, P.O. Box 37012, Washington, DC 20013; (202) 382-8982, michael.gates@ars.usda.gov. **

HAWAII DEPARTMENT OF AGRICULTURE (D1925-1)



Gall-infested native wiliwili trees in Maui.

Designing Technology To Minimize Environmental



An aircraft fitted with Bete NF70 nozzles, which were shown in the lab to produce the optimum droplet size for oil dispersants. Field tests were conducted to simulate an actual dispersant treatment and verify the size and density of droplets deposited on the ground. **Right:** In-flight testing of water being released from the Bete nozzles.

When an oil spill occurs, it's the slick on the water that poses the main threat to the environment. Agricultural Research Service researchers are diving into the problem. They are helping fine-tune an important technology: aerial spray equipment designed to deal with the threat. Engineers at the ARS Areawide Pest Management Research Unit (AMPRU) in College Station, Texas, are working to ensure that compounds sprayed from airplanes will hit targeted oil slicks and break up oil spills.

Oil dispersants are a class of chemicals that break down oil in a waterway and disperse it into the water, reducing the

environmental damage. Aircraft generally drop them on oil spills from altitudes of about 75 feet. "You want to have a gel that breaks up the film of oil on the water surface so that it dissipates into the water column, mitigating the effects of having it wash onshore and damage the shoreline," says W. Clint Hoffmann, lead scientist in AMPRU's aerial application technology section.

There are a number of oil dispersants and scientific reports that detail what happens when they interact with an oil slick. But thankfully there are very few major spills, so scientists don't get many chances to assess oil-spill technology.

Hoffmann says the goal is to safeguard the environment by finding optimal technology for spraying three oil dispersants: Corexit 9500, Corexit 9527, and a new high-viscosity gel developed specifically to dilute oil spills.

Tunnel Vision

Engineers in the unit's aerial application technology section have spent decades studying the technology, methods, and effects of using crop dusters to spray crops such as corn and cotton with insecticides and other treatments. The unit's high-speed wind tunnel is one of a few wind tunnels nationwide capable of assessing

Damage

how aerial sprays are “atomized,” or broken into tiny droplets when released at high speeds.

The project began with researchers spraying the three dispersants separately into the wind tunnel, using different nozzles and measuring the size of the droplets produced. Droplet size plays a major role in determining where and how fast a compound will fall from an airplane, Hoffmann says. Generally, the larger the droplet, the more likely it will fall as desired—directly under the airplane.

“They want to be able to get the best results with these compounds and with their new high-viscosity gel. So the question is, ‘What droplet size would be most effective at cleaning up oil spills?’” Hoffmann says.

To measure droplet sizes, the researchers placed a double-pulse laser downstream



from the nozzles and used a high-speed camera to capture images of the droplets in flight as they went through the tunnel. Known as the “LaVision SprayMaster,” the system allowed researchers to analyze up to 70,000 droplets as they passed through the laser beams. The camera’s shutter and flash also enabled them to take between 8 and 16 images per second at a very high shutter speed, showing droplets in flight just after they were released from the nozzles. The nozzles released sprays at different pressure levels, ranging from 20 to 50 pounds per square inch (psi).

Simulating Flight

The three compounds were sent through the tunnel at about 140 miles per hour

(mph), the speed normally traveled by planes used by cleanup crews during oil-spill recovery operations. Tarps on the ground captured the material as it came out of the tunnel so that it could be safely transported to an ARS biodegradation facility in College Station.

The droplet sizes observed in the wind tunnel tests were then applied to a spray-dispersion computer model to predict how well the compounds would hit targeted swaths during an oil-spill cleanup operation. The computer model, known as “AGDISP,” is an industry standard and is used by federal regulatory agencies when they conduct environmental assessments of pesticides and other agricultural sprays.

The wind tunnel results show that the droplets produced by Corexit 9500 and Corexit 9527 were similar in size at the simulated 140-mph flight speed and that the droplets generally start to break up about 3 feet from a nozzle.

The results also show that a particular size nozzle, the Bete NF70 (Bete Fog Nozzle, Inc.), when set at 40 psi, would provide the best droplet sizes for accurate delivery. The computer modeling showed that 90 percent of either of the two compounds would land within the targeted swath, with 9.5 gallons of the spray hitting each targeted acre.

As for the gel, droplets formed from it were much larger than droplets from either of the other compounds, making it easier to ensure greater accuracy, even if dropped from higher altitudes, the researchers say. But the gel was so thick that the airborne delivery system will have to be redesigned to keep the gel from clogging the storage tank, pump, and spray booms on each aircraft.

The results were published in *Proceedings of the Arctic Marine Oil Spill Program Technical Seminar*, a book-length publication summarizing work reported at a June 2009 conference in Vancouver, British Columbia.

The work is continuing. Oil industry scientists plan on working with ARS researchers at the College Station facility



Clint Hoffmann, agricultural engineer, positions a spray nozzle in a 140-mph airstream to simulate conditions for aerial application of an oil dispersant. The size of the dispersant droplets is measured with high-speed cameras and image-processing equipment.

to test their newly designed application systems and to confirm the computer modeling results with actual flight tests.—By **Dennis O’Brien, ARS.**

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

*W. Clint Hoffmann is in the USDA-ARS Areawide Pest Management Research Unit, 2772 F&B Road, College Station, TX 77854; (979) 260-9521, clint.hoffmann@ars.usda.gov. **

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.

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Fungus-on-Fungus Fight Could Benefit Chickpeas

The fungus *Ascochyta rabiei* threatens chickpea crops the world over. During the winter, the pathogen survives on chickpea leaves and stems left behind in the field after harvest and forms sexual spores called “ascospores.” The ascospores, in turn, can infect springtime chickpea plantings and sometimes wipe out entire crops. But researchers identified and assessed 28 fungi that compete with *A. rabiei* and concluded that one of them—*Aureobasidium pullulans*—held the most promise as a biocontrol for *A. rabiei*. *Au. pullulans* has several attributes that enhance its potential, including its abundant natural populations, ready growth in culture, and safety to humans and other animals. Most important, *Au. pullulans* inhibits *A. rabiei*’s ability to form or release ascospores in winter field stubble, which curbs the pathogen’s subsequent infection of chickpea seedlings in the spring. In small-scale field trials, inoculating stubble with *Au. pullulans* spores reduced *Ascochyta* blight by 38 percent, a level that might be improved with the use of adjuvants and other standard ingredients often used in biocontrol formulations. *Frank Dugan, USDA-ARS Plant Germplasm Introduction and Testing Research Unit, Pullman, Washington; (509) 335-1783, frank.dugan@ars.usda.gov.*

Tryptophan-enriched Diet Reduces Pig Aggression

When pigs are subjected to the aggressive behavior of other pigs, they become chronically stressed, which interferes with their ability to fight off disease and maintain typical growth levels. Now scientists have found that feeding the amino acid tryptophan to young female pigs as part of their regular diet makes them less aggressive and easier to manage. Tryptophan, which is only acquired through diet, is the precursor for serotonin, which is a cerebral neurotransmitter that helps to control emotion and aggression. In the study, a diet with 2.5 times the normal amount of tryptophan was fed for 1 week to 3-month-old grower pigs and 6-month-old finisher pigs. Another group of pigs received a normal diet. Behavioral activity and aggressiveness were measured before and after the 7 days of diet supplementation. The supplemented diet raised blood concentrations of tryptophan in 3-month-old females by 180 percent and by 85 percent in 6-month-old females and reduced aggression and overall behavioral activity among the younger female pigs. *Jeremy Marchant-Forde and Heng-Wei Cheng, USDA-ARS Livestock Behavior Research Unit, West Lafayette, Indiana; (765) 494-6358 [Marchant-Forde], (765) 494-8022 [Cheng], jeremy.marchant-forde@ars.usda.gov, heng-wei.cheng@ars.usda.gov.*

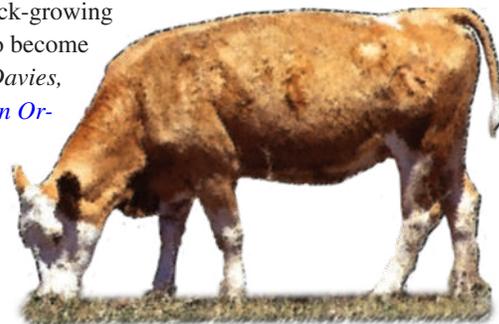


ARS Survey Helps Growers Track Two Key Cotton Pests

A comprehensive survey in Texas has resulted in identification of several new host plants for two significant cotton pests. Researchers visited more than 80 sites in Texas along roadsides and cotton fields at least twice a week for 3 years and found 13 previously unreported host plants for tarnished plant bugs and 8 for cotton fleahoppers. Tarnished plant bugs infested 4.8 million acres of U.S. cotton in 2008, making it the single most damaging insect for domestic cotton. The adults infest developing cotton buds and damage bolls. They may also transmit cotton diseases. Cotton fleahoppers, which infested 2.3 million acres in 2008, emerge from eggs in the spring and feed on weeds before moving to cotton in May and early June. In the past, spraying for boll weevils also helped control tarnished plant bugs and cotton fleahoppers, but successful boll weevil eradication efforts have reduced the need for spraying. Cotton producers can use these findings to better monitor conditions that contribute to infestations and crop losses. *Jesus Esquivel, USDA-ARS Area-wide Pest Management Research Unit, College Station, Texas; (979) 260-9519, jesus.esquivel@ars.usda.gov.*

Livestock Can Help Rangelands Recover from Fires

Results from a 14-year study suggest that rangelands that have been grazed by cattle recover from fires more effectively than rangelands that have been protected from livestock. At the start of the study, the ungrazed sites, where livestock had been excluded since 1936, had almost twice as much plant litter as the grazed sites, because much of the potential litter in the grazed areas had been removed by cattle. A controlled burn was conducted on all the sites in 1993, and vegetation cover, vegetation density, and biomass production were measured in 2005, 2006, and 2007. Researchers found that cheatgrass, an invasive annual, had infested a large portion of the ungrazed sites, which left these areas even more vulnerable to future fires. But cheatgrass did not become problematic on the sites that had been grazed, where native bunchgrass cover was almost twice as dense as bunchgrass cover on the ungrazed sites. The researchers concluded that the litter in the ungrazed sites fueled hotter fires that killed off much of the perennial vegetation, which allowed quick-growing invasive annuals to become established. *Kirk Davies, USDA-ARS Eastern Oregon Agricultural Research Center, Burns, Oregon; (541) 573-4074, kirk.davies@ars.usda.gov.*





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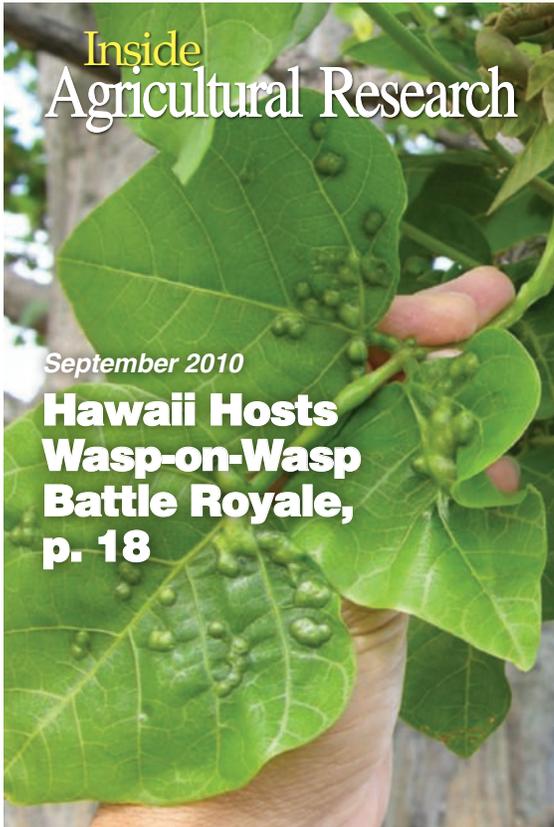
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