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Agricultural Research

Strawberries: More Phenolics Than We Thought

Story on pages 12-13

Agricultural Research Service • Solving Problems for the Growing World

FORUM

The Never-Ending Insect Control Contest

Thousands of different insect species are serious threats to agricultural production in the United States, and hundreds of others represent potential threats that could decimate our natural, agricultural, and urban landscapes. Without the pest control measures that we have today, insects would be eating or otherwise putting to ruin about half of all the crops grown in the United States each year, causing significant damage to our livestock, and even inflicting harm to us and our capacity to live peacefully in our homes.

Luckily, scientific research has provided a large toolbox of measures and strategies to help farmers and ranchers, gardeners, homeowners, and the general public control many of these problem insects. These tools include chemical insecticides, biological control agents, lures and traps, insect-resistant crops, organic treatments and insecticides, decoy (trap) crops, area-wide control programs, physical barriers, and many others. For livestock insect pests, there are dips, repellents, and insecticidal sprays and dusts, as well as novel management strategies, natural enemies, and more.

Many of the innovations for successful control have resulted from the work of Agricultural Research Service scientists, such as the discovery in 1936 that selenium is absorbed by plant roots and carried to foliage where it kills aphids, which became the first systemic insecticide; finding genetic resistance to corn earworm in a line of flour corn in 1941; and developing the sterile male insect release technique that led to the eradication of the screwworm from the United States in 1966, a method that is being used today to keep this and other pests like the Mediterranean fruit fly out of the country.

Unfortunately, we cannot rest on previous successes, even successes like those described above, because insects continue to evolve countermeasures. They find ways to bypass or overcome virtually every control method, which then calls for scientists to find even more novel approaches. Whether insecticide resistance will evolve is not among the leading questions asked by entomologists; the accepted consensus is that it will. Instead, the questions are when will the resistance evolve, what are the mechanisms underlying it, how can we slow the evolution of resistance, and are there new mechanisms remaining to be discovered? There is a continuing "arms race" between research to find new control measures and the countermeasures of the insects.

Beyond that, the expectations and requirements of end-users and policymakers, and even social attitudes, keep changing, which generally means the bar for what is expected from insect control measures keeps being raised. Today, we want insect control methods to be not only highly effective, but also narrowly focused, that is, effective against a target pest insect, but not harmful to nontarget species; less persistent in the environment; less harmful to human applicators as well as the general public; and have less of an impact on the environment in terms of the raw ingredients from which they are made. Finally, in addition to all of the above, new control methods have to be economical to develop and use.

To meet all of these high expectations, ARS scientists are continually seeking novel ways to control insects. For example, researchers at the ARS Imported Fire Ant and Household Insects Research Unit, in Gainesville, Florida, are testing a group of unique alkaloids from Central and South American poison frogs as potential compounds to control fire ants. See the story on page 4.

In Tifton, Georgia, an ARS entomologist is trying out low-tech, low-impact insect control measures, like plastic barriers between peanut and cotton fields, to see whether they will stop native stink bugs from moving between crops. That story is on page 6.

At the other end of the technology scale, ARS researchers are turning to our growing knowledge of genomics and biochemistry to explore novel ways to develop very targeted pest control measures. For example, they are combining genome sequencing with bioinformatics and molecular biology tools, such as high-throughput proteomics, microarrays, and RNA interference, in an effort to develop a vaccine against cattle ticks using information encoded in the cattle tick genome.

ARS scientists are also breeding new, very specific insect-resistance traits right into the crop itself. One such project is under way to develop soybeans resistant to soybean aphids, which arrived here from Asia around 2000. ARS researchers are conducting fine mapping, metabolomics, transcriptomics, and proteomics studies of several lines of soybeans that make a specific biochemical change when the aphids attack, a change that could lead to natural resistance to this pest.

A challenging goal of ARS research is to stay ahead of the insects, finding new ways to keep them from doing serious harm, because the insect pests will continue to adapt and evolve, sometimes quite rapidly. Fortunately, so will science and scientists.

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LYNDON PORTER (D3331-1)

Pea plant with disease symptoms caused by *Pea enation mosaic virus*.
Story begins on page 16.

Cover: There's more to strawberries than just great taste and a pleasing color. They are also high in a class of health-promoting compounds known as "phenolics." Thanks to ARS scientists, we now know that strawberries have even higher amounts of phenolics than previously thought. Story begins on page 12. Photo by Peggy Greb. (D3073-1)

Fire Ants Are No Match for Poison Frogs

SANFORD PORTER (D3337-1)

SMITHSONIAN'S NATIONAL ZOO (D3333-1)

Above: A native of Central America, the tropical fire ant, *Solenopsis geminata,* has two types of workers—very large and small. Poison frogs are known to eat the workers and use the ant's alkaloids for their own defense. Left: Blue poison dart frog or blue poison arrow frog, *Dendrobates tinctorius "azureus,"* one of the frogs alkaloid samples were taken from. Below: Strawberry dart frog, *Oophaga pumilio.*

Since its accidental introduction into the United States from South America in the 1930s, the red imported fire ant, *Solenopsis invicta,* has spread throughout the southern United States, decimating small-animal populations, damaging crops and other plants, and inflicting painful and sometimes lethal venomous stings on humans and livestock. Scientists are constantly looking for effective methods to control this pest.

Robert Vander Meer, research leader in the Imported Fire Ant and Household Insects Unit at the Agricultural Research Service's Center for Medical, Agricultural, and Veterinary Entomology in Gainesville, Florida, partnered with other researchers to determine whether toxic alkaloids from the brilliantly colored skin of poison frogs would deter fire ants.

Toxic alkaloids—naturally occurring compounds—on the skin of these frogs were discovered in the early 1960s in Central and South America. However, Amerindian tribes had discovered the effects of the alkaloids long before then, when they learned how to secure alkaloid secretions from the skin of poison frogs of one genus to make poison darts for hunting. Poison frogs do not naturally produce the alkaloids, but instead sequester them by ingesting millipedes, ants, mites, and other arthropods that do produce the alkaloids.

Vander Meer's collaborators include engineer Cliff Hoffman at ARS's Southern Plains Agricultural Research Center in College Station, Texas; chemical ecologist Paul Weldon at the Smithsonian Conservation Biology Institute at the National Zoological Park in Front Royal, Virginia; and chemist Thomas F. Spande at the National Institutes of Health (NIH) in Bethesda, Maryland.

"After finding that the frogs had unique alkaloids, NIH scientist John Daly, now deceased, began exploring possible medical applications of the compounds," Vander Meer says. "Over the years, scientists have identified and isolated about 900 alkaloids from the skin of poison frogs."

Traditionally, the frogs' coloration and alkaloids were thought to be used as protection against mammals and birds, not as defenses against arthropods like ants, he says. However, these frogs could be fair game for many aggressive, predacious ant species in Central and South America, such as fire ants.

Vander Meer and his team developed a way to measure the toxicity of 20 poison-frog alkaloids, most of which were purified from frog-skin extracts that Daly had collected and prepared. Not all of the alkaloids deterred fire ants at concentrations that would be found in nature, but some were highly effective.

In Central America, the tropical fire ant, *S. geminata,* occupies the same territory as the poison dart frog, *Oophaga pumilio,* Vander Meer says. The major alkaloid produced by *S. geminata* is found on the skin of *O. pumilio,* showing that this frog eats *S. geminata* ants. However, the bioassay showed that this alkaloid was not very effective against *S. invicta*.

"Interestingly, this same frog has a varied diet of ants and mites," he says. "Mite-derived alkaloids have also been reported on *O. pumilio's* skin, and these compounds were found to be highly effective at incapacitating *S. invicta*."

The varied frog diet appears to protect the frogs from ant predation, he adds. This supports the observation that poison frogs are not attacked by predatory ants in their natural habitat, but if the frogs are raised on a diet that does not contain alkaloids, they are readily attacked when exposed to ants.

"Once the frogs get these alkaloids into their systems, they may modify some of the compounds," Vander Meer says. "Without those starting materials from the arthropods, they cannot produce the alkaloids."

This study, which was published in *Naturwissenschaften* in January 2013, broadens the use of these alkaloids by poison frogs to include protection against ant predation.

In earlier work, published in *Proceedings of the National Academy of Sciences,* Weldon collaborated with scientists at ARS and NIH to test poison-frog alkaloids against the yellowfever mosquito. They found pumiliotoxin 251D to be the most potent of the ones tested. When mosquitoes landed on surfaces treated with this alkaloid, they were unable to fly, and they died. After testing chemical structures related to 251D, the researchers determined that the natural 251D structure was 10 times more potent than the others.

STEPHANIE VEGA (D3345-1)

Above: Chemist Robert Vander Meer prepares to test the effect of poison dart frog alkaloids on fire ants. Below: A fire ant is exposed to an alkaloid placed on the tip of a syringe plunger. The ant is then released for scientists to take note of its responses, such as convulsions or decreased ambulation.

The discovery that pumiliotoxin 251D and other poison-frog alkaloids act on contact with arthropods (fire ants and mosquitoes) was significant, Weldon says. "Previous studies with pumiliotoxin 251D focused on injecting it into test insects—tobacco hornworms. By injecting it, they missed the most remarkable property of this compound—its ability to act as a contact toxin, penetrating the surface of the insect."

"Disease transmission by mosquitoes is on the rise worldwide and is an extremely important problem" Vander Meer says. "Derivatives of these frog alkaloids may be useful in the future because when they are applied to surfaces, mosquitoes that land there die—preventing them from biting and transmitting disease."

The main concern is the high toxicity of pumiliotoxin 251D. However, other alkaloids identified from frogs or derivatives of the alkaloids may have greater mosquito toxicity and less mammalian toxicity, scientists suggest. Further research is needed to find compounds that maintain the desirable effects of the alkaloids while reducing their toxicity.—By **Sandra Avant,** ARS.

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

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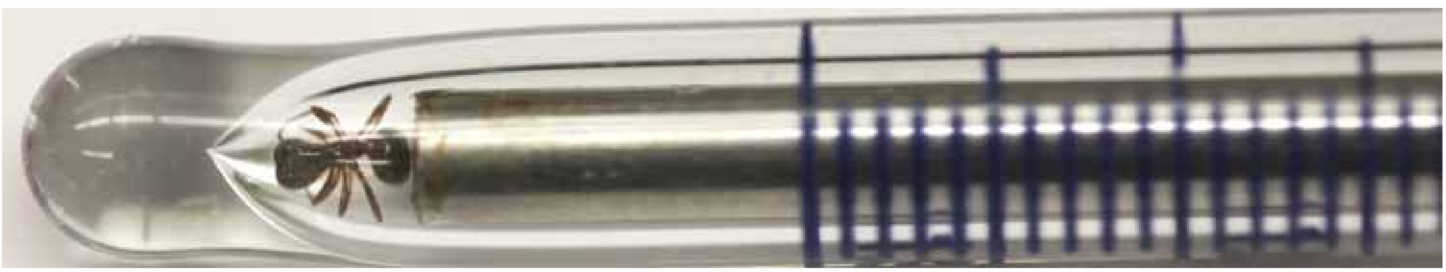

ROBERT VANDER MEER (D3335-1)

KRISTIE GRAHAM (D3339-1)

Controlling Native Stink Bugs in the Southeast

PEGGY GREB (D3308-1)

A plastic barrier wall placed between peanuts and cotton prevents stink bugs from moving from low-growing peanuts into cotton.

HERB PILCHER (D3338-1)

Two southern green stink bugs *(Nezara viridula)* mating on a sorghum seed head. This species causes damage to corn and cotton crops.

The invasive brown marmorated stink bug *(Halyomorpha halys)* has become a familiar nuisance in many areas of the United States, but three native stink bugs have been attacking crops for decades in Georgia and other parts of the Southeast. The southern green stink bug *(Nezara viridula)* and the brown stink bug *(Euschistus servus)* will damage corn, and both can develop in peanut and move into cotton to cause damage as adults. The green stink bug *(Chinavia hilaris)* will damage cotton after moving into it from noncrop sources at field edges. All three are immune to the *Bt* toxin that controls other insects in corn and cotton, so growers often spray "broad-spectrum" insecticides, which kill not only stink bugs, but also beneficial insects.

Agricultural Research Service entomologist Patricia Glynn Tillman, who is in the Crop Protection and Management Research Unit in Tifton, Georgia, has found ways to help growers reduce losses and adopt greener alternatives. Results of her studies highlight the effectiveness of several innovative strategies, including the use of nectar-producing plants to attract beneficial insects and the placement of barriers between crops to discourage migrations.

Where Do They Go?

Each spring, many farmers in Georgia plant corn first, then peanuts, and then cotton, often near each other or side-by-side. Using hand-held nets and drop cloths, Tillman and her colleagues collected stink bugs from the three crops for 6 years to study stink bug migration patterns.

In one report, they evaluated the influence of different types of plants along field edges on stink bug dispersal, whether adjacent woodlands contribute to infestations, and the effects of plant height on how far the pests penetrate into fields.

They found that brown stink bugs and southern green stink bugs are present in all three crops and often migrate from one crop to the next, and that green stink bugs are rare in corn and peanut, but common in cotton. They also found that stink bugs are likely to migrate into cotton from adjacent woodlands and into corn from both woodlands and nonwooded areas. The height of the crop also made a difference: The taller the crop plant, the less penetration occurred. Results were published in 2014 in the *Bulletin of Entomological Research.*

They also evaluated stink bug colonization in three cropping systems common in the Southeast: corn/cotton, corn/peanut/cotton, and peanut/cotton. Results showed that for southern green stink bugs and brown stink bugs, the risk of colonizing cotton was highest in corn/peanut/cotton cropping systems, and for green stink bugs, the risk was highest in peanut/cotton systems. Green stink bugs are more attracted to cotton than to corn, but corn is more likely than cotton or peanuts to harbor southern green stink bugs and brown stink bugs.

Stink bugs have long been more of a concern for cotton and corn growers than for peanut producers, but the results show that peanut growers also have reason for concern. While peanuts are a poor host for green stink bugs, southern green stink bugs and brown stink bugs grow and develop in peanuts, and both subsequently will move readily into cotton and cause significant damage. Results were published in 2013 in *Environmental Entomology.*

Barriers Can Help

Tillman also placed barriers between crops to see if they would prevent stink bugs from migrating from one crop to another. She also evaluated whether nectar-producing plants will attract beneficial parasitoids that control stink bugs. In one study, she placed nectar-producing buckwheat plants, sorghum Sudan grass (an annual grass that grows to about 8 feet), and plastic sheets of two different heights (about 6 feet high and about 2 feet high) between peanut and cotton fields to assess their effectiveness as barriers. The grass and plastic sheets were erected specifically to see if they would prevent stink bugs from migrating from low-growing peanut plants into cotton. She found that the plastic and the grass were effective, as long as the barriers were at least as high as the cotton (4.5 feet). She also found that the buckwheat attracted the parasitoid *Trichopoda pennipes,* which cut back on stink bugs in nearby cotton. Results were published in 2014 in the *Journal of Pest Science.*

Finally, Tillman and her colleagues placed potted milkweed plants about 4 feet from each other along the edges of cotton fields in peanut/cotton production areas and sampled the cotton for levels of stink bugs and *T. pennipes*. They found significantly higher numbers of *T. pennipes* parasitizing stink bugs in cotton near nectar-producing milkweed than in control plots. They also reported an unrelated benefit: The milkweed attracted Monarch butterflies, an eye-catching migratory species that has prompted concern among conservationists because of the loss of milkweed habitat. Results were published in 2014 in *Environmental Entomology*.

The strategies will not eradicate stink bugs, but the studies show they will help. "These measures have the potential to provide some control of stink bug populations, but they will work best if used as one part of an overall management plan," Tillman says.—By **Dennis O'Brien,** ARS.

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

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Sorghum Sudan grass grown between peanuts (on right) and cotton (on left) reduces populations of stink bugs in cotton.

KRISTIE GRAHAM (D3340-1)

SCOTT BAUER (D3334-1)

Closing In on Butanol for Biofuel

At the ARS Bioenergy Research Unit in Peoria, Illinois, chemical engineer Nasib Qureshi observes and controls a fermentor in which butanol is produced from corn stover and recovered simultaneously with a vacuum.

Butanol is the go-to industrial solvent for products such as lacquers and enamels, but it might also play a substantial role in the production of renewable fuels. Gallon for gallon, it has 30 percent more energy than ethanol and only around 4 percent less energy than a gallon of petroleum-based gasoline.

Agricultural Research Service chemical engineer Nasib Qureshi has studied a range of feedstocks for producing "biobutanol." His most recent work suggests that using barley straw and corn stover—both agricultural byproducts—could be the answer.

"To produce butanol most economically, we will need to produce it from agricultural byproducts, not from dedicated crops," says Qureshi, who works in the ARS Bioenergy Research Unit in Peoria, Illinois.

Qureshi has confirmed that both materials can be converted to butanol via "separate hydrolysis, fermentation, and recovery" (SHFR) or by "simultaneous saccharification, fermentation, and recovery" (SSFR). SHFR requires two operations in two reactors—one for hydrolysis and the other for fermentation combined with recovery. In SSFR, releasing the plant sugars, fermenting them to butanol, and recovering the butanol are combined into a single operation that is performed in a single reactor.

In one recent study, Qureshi, ARS chemist Badal Saha, and ARS microbiologist Mike Cotta evaluated the effectiveness of SHFR in fermenting barley straw plant sugars into butanol. They pretreated the barley straw to release the plant sugars, which created a broth called a "hydrolyzate" that could be fermented into butanol.

Then the researchers observed the hydrolyzate's fermentation rates. They also used a process called "gas stripping" to remove the resulting butanol simultaneously produced during fermentation. With this approach, Qureshi and partners obtained a final butanol yield that was 182 percent of the yield obtained from a control study.

Next, the scientists used the same protocols with pretreated corn stover and were able to ferment more than 99 percent of the sugars in the hydrolyzate. This resulted in butanol yields that were 212 percent greater than yields observed from the controls, and 117 percent higher than the butanol yields from the barley straw.

Qureshi and his research partners applied these results to another investigation that evaluated the effectiveness of producing butanol from corn stover in an SSFR process that used vacuum technology—not gas stripping—to simultaneously recover butanol during fermentation. The team included Saha, Cotta, ARS molecular biologist Siqing Liu, University of Illinois professor Vijay Singh, and Ohio State University professor Thaddeus Ezeji.

During the hydrolysis process, more than 97 percent of the stover sugars were released and were available for fermentation. The total butanol yield was 0.34 grams per liter per hour—higher than the glucose control yield of 0.31 grams per liter per hour. The scientists published their results in *Bioresource Technology* and *Food and Bioproducts Processing,* both in 2014.

"Several years ago when I tried to ferment butanol from barley straw, the yields were much lower than the control yields, and I was not able to obtain any yields from corn stover," Qureshi says. "So we're pleased with our findings, and we look forward to scaling up and commercializing butanol biofuel production from agricultural residues such as barley straw and corn stover."—By **Ann Perry,** ARS.

This research is part of Biorefining, an ARS national program (#213) described at www.nps.ars.usda.gov.

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Assessing the Risks of HoBi-Like Viruses in Cattle

Scientists are tracking a new species of pestivirus that could pose a threat to U.S. cattle. Tentatively called "HoBi-like viruses," they are related to bovine viral diarrhea viruses (BVDV), which cause significant economic losses to cattle producers worldwide.

Originally found in South America, HoBi-like viruses have since been associated with disease outbreaks in Southeast Asia and more recently in Europe. Signs of infection include slowed growth, reduced milk production, higher rates of reproductive and respiratory diseases, and a higher incidence of death among young animals. No vaccines are available to specifically prevent HoBi infection in cattle.

At the Agricultural Research Service's National Animal Disease Center (NADC) in Ames, Iowa, microbiologist Julia Ridpath and her colleagues are characterizing HoBi-like viruses and determining whether available BVDV diagnostics and vaccines are effective in detecting and preventing them.

"We've found that cattle vaccinated against BVDV have little or no protection against HoBi infections," Ridpath says. "Our current BVDV tests and commercial vaccines are not adequate to detect HoBi-like viruses and protect cattle against them."

These findings, published in *Veterinary Microbiology* and the *Journal of Veterinary Diagnostic Investigation,* indicate a need for new diagnostics and vaccines specific to these viruses.

Ridpath, who is in NADC's Ruminant Diseases and Immunology Unit, also demonstrated that acute HoBi infections in cattle result in immunosuppression. Like BVDV, HoBi has the ability to cross the placenta, infecting the fetus and establishing a lifelong infection in an animal. Such animals are referred to as "persistently infected."

"A calf born persistently infected with BVDV is like a 'Typhoid Mary,' shedding virus into the environment through its tears, blood, urine, feces, and skin," Ridpath says. "All of its tissues carry the virus. We have found that the same is true of calves persistently infected with HoBi-like viruses, and they can transmit the virus to other calves, sheep, goats, and pigs."

Ridpath and her colleagues at NADC are collaborating with researchers in Brazil and Italy to investigate the prevalence of HoBi. In a study published in the *Journal of Veterinary Diagnostic Investigation* in 2014, the team conducted a survey of fetal bovine serum collected from animals in the United States. No HoBi-like viruses were detected. This suggests that the viruses have not invaded the country.

The first published report of HoBi in European cattle was in a 2010 respiratory disease outbreak. However, ARS scientists and their colleagues from Italy demonstrated that HoBi had been in circulation in Europe earlier than previously reported. They detected the virus in archived samples sent to a diagnostic laboratory in Italy in 2007.

Future efforts will continue to involve conducting immunology surveys to ensure that HoBi-like viruses have not entered the United States, Ridpath says. Additionally, scientists are working to develop tests to screen imported animals and animal products to make sure they are free of these viruses.—By **Sandra Avant,** ARS.

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

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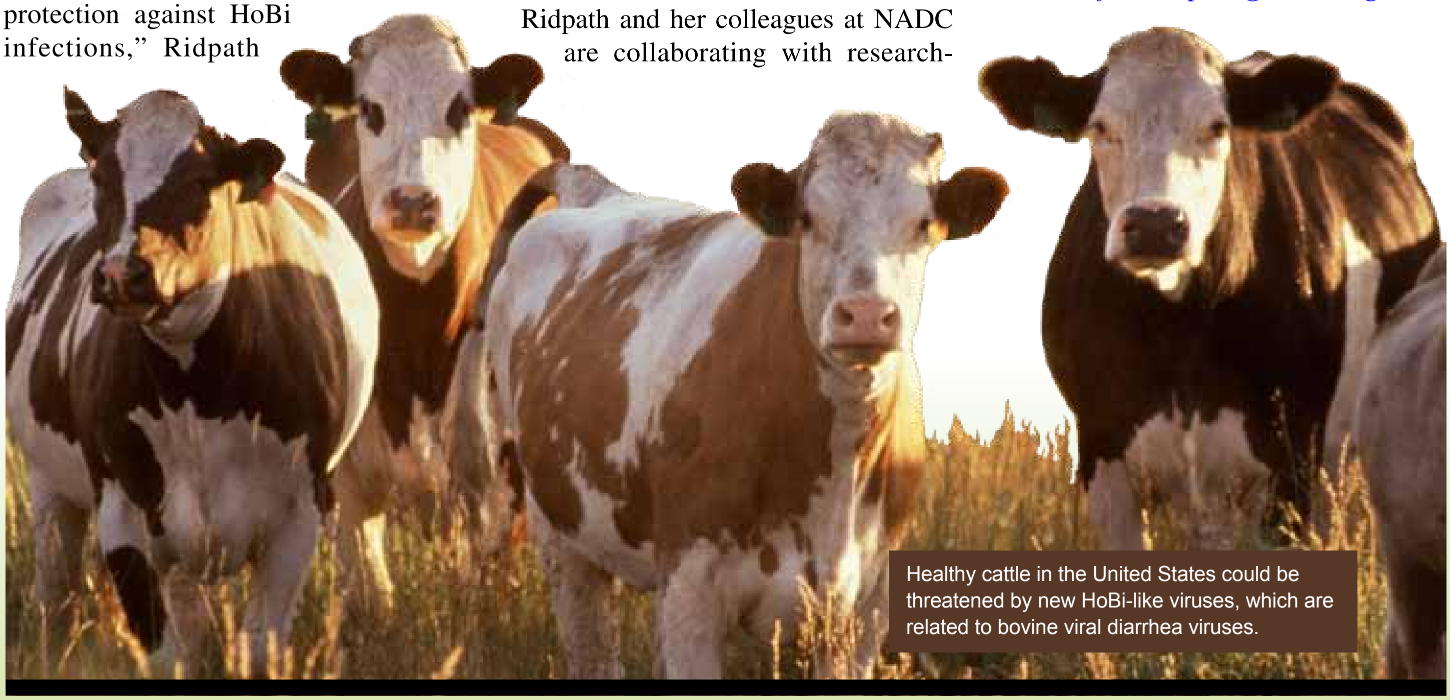

Healthy cattle in the United States could be threatened by new HoBi-like viruses, which are related to bovine viral diarrhea viruses.

KEITH WELLER (K4328-8)

STEPHEN AUSMUS (D3329-2)

Making Peach Trees Fit Better on the Field

ARS plant molecular biologist Chris Dardick (left) and postdoctoral geneticist Courtney Hollender evaluate branches of 1-year-old transgenic stone fruit trees engineered to have altered branch angles. Dardick's tree has wide branch angles, while Hollender's tree has vertical branches.

When you think of the traits a perfect fruit tree should have, you may think of fragrant blossoms or juicy fruit, but the shape of the tree might not occur to you. In fact, tree shape is very important in agriculture. A tree used for agricultural production—whether for fruit, nuts, or lumber—requires extensive land space, mostly because of its large size and spreading growth habit.

Changing the tree's shape, either by pruning or through genetics, will affect how that tree fits among other trees in the field and the number of trees that can be grown in an area. Pruning is expensive and labor intensive. A better option is to genetically alter trees to be more compact so they can be planted closer together to increase productivity.

Agricultural Research Service scientists in Kearneysville, West Virginia, along with colleagues at several universities, have identified a gene that controls tree shape in peach trees, specifically the angle at which their branches grow. The work was done using a new gene-mapping method that the team developed.

"To identify the genes responsible for the trait that causes trees to grow in a desirable columnar shape, we devised a strategy we call 'pnomes' (pronounced *pea-nomes*)," says ARS plant molecular biologist Chris Dardick. "The pnomes strategy is based on sequencing the complete set of genes, called the 'genome,' of plants that have a trait of interest, like a particular tree shape, and then looking for a common gene or set of genes responsible for that trait."

Using this technique, the group identified a gene called *"PpeTAC1,"* which promotes horizontal growth of branches in peach trees. "When this gene is disrupted, the tree branches grow more vertically, producing a pillar or columnar shape to the tree," says Dardick.

This work was recently published in *The Plant Journal* and is currently the subject of a 5-year project funded by the USDA National Institute of Food and Agriculture. The researchers filed a patent application on using the gene to manipulate tree architecture.

The team also showed that *PpeTAC1* has the same effect in the model plant *Arabidopsis thaliana*. "Surprisingly, this same gene was found to perform a very similar function in the distantly related rice and corn," says Dardick. "The knowledge gained from this work should provide technologies for optimizing plant architecture."

The research team also includes Ann Callahan, Courtney Hollender, Karina Ruiz, and Ralph Scorza, all with ARS's Appalachian Fruit Research Laboratory; and scientists from the University of Rostock (Germany), and Clemson University.—By **Sharon Durham,** 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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Finding Rice Traits That Tackle Climate-Change Challenges

Above: Cultivated rice competing with weedy red rice (the taller plants among the rows) near Stuttgart, Arkansas. ARS scientists are examining weedy red rice for characteristics that could be used to adapt cultivated rice to climate change. Below: In Beltsville, Maryland, plant physiologist Lewis Ziska examines the response of different rice cultivars to changes in carbon dioxide and temperature.

People around the world depend on food crops adapted to an array of temperature and precipitation regimes, but those conditions are in flux because of global climate change. So scientists want to identify plant traits that could be used to develop food-crop cultivars that thrive despite—or perhaps because of—shifts in carbon dioxide (CO_2) levels, water availability, and air temperature.

As part of this effort, Agricultural Research Service plant physiologists Lewis Ziska, Martha Tomecek, and David Gealy conducted a study of several rice cultivars to determine whether changes of temperature and CO_2 levels affected seed yields. They also looked for visible traits that could signal whether a plant cultivar has the genetic potential for adapting successfully to elevated CO_2 levels.

For their study, the scientists included weedy red rice, which infests cultivated rice cropland. Despite the plant's downside, previous assessments indicated that weedy rice growing under elevated CO_2 levels had higher seed yields than cultivated rice growing under the same conditions.

The scientists used environmental growth chambers to study genetically diverse rice cultivars at current and future projections of atmospheric CO_2 and a range of day/night air temperatures. They observed that on average, all the rice cultivars put out more aboveground biomass at elevated CO_2 levels, although this response diminished as air temperatures rose.

For seed yield, only weedy rice and the rice cultivar Rondo responded to elevated CO_2 levels when grown at optimal day/night air temperatures of 84 ˚F and 70 ˚F. The researchers were also intrigued by an additional observation: Only weedy rice gained significant increases of aboveground biomass and seed yield under elevated CO_2 levels at the higher temperatures expected for rice-growing regions by the middle of the century.

When Ziska and colleagues analyzed the study data for the weedy rice, they observed that seed-yield increases under elevated CO_2 resulted from an increase in panicle (seed head) and tiller production. Tillers are stalks put out by a growing rice plant, and as the plant matures, the seed heads—where rice grain is produced—develop at the end of the tillers.

Since rice tiller production is determined in part by a plant's genetic makeup, crop breeders might someday be able to use this weedy rice trait to develop commercial rice cultivars that can convert rising CO_2 levels into higher seed yields. To the researchers, these findings also suggest that the weedy, feral cousins of cultivated cereals could have other traits that would be useful in adapting to the environmental challenges that may come with climate change.

"We know that atmospheric CO_2 and air temperatures will increase together," says Ziska. "Ideally, we can develop plants that respond well to elevated CO_2 levels and incorporate traits that favor plant survival despite temperature changes."

Ziska and Tomecek are with the ARS Crop Systems and Global Change Laboratory in Beltsville, Maryland, and David Gealy is with the ARS Dale Bumpers National Rice Research Center in Stuttgart, Arkansas. The researchers published their findings in 2013 in *Functional Plant Biology*.—By **Ann Perry,** ARS.

PEGGY GREB (D1555-1)

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

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PEGGY GREB (D910-10)

New Tests Count Total Phenolics in Fruits and Veggies

ARS scientists have developed more accurate tests to analyze phenolics (health-promoting compounds) in spinach (shown here) and strawberries (opposite).

Agricultural Research Service investigators have a long history of designing and developing reliable analytical methods for measuring nutrients and other compounds in foods. ARS scientists have now devised new analytical methods for detecting and measuring concentrations of phytochemicals called "polyphenols" in plant materials.

The class of health-promoting compounds is found in certain foods and beverages and is also referred to as "phenolics." At the ARS Eastern Regional Research Center (ERRC) in Wyndmoor, Pennsylvania, scientists first reported on the new test and used it on a variety of samples of beverages, such as teas and juices; grains, such as rice and quinoa; and flaxseed.

In the laboratory, the scientists used the new method to measure the amount of phenolics in the various food samples by mixing them with Fast Blue BB diazonium salt. Under alkaline conditions, diazonium salt specifically couples with phenolics to form stable complexes that can be directly measured.

When compared to results using a traditional method to assess phenolic concentrations, the new Fast Blue BB method assessed higher amounts of total phenolics in most of the beverage and grain samples tested, and lower amounts in flaxseed and some juice blends tested. The results suggested the traditional method does not assess or pick up all phenolics present in samples tested and inadvertently measures other compounds besides phenolics.

The Fast Blue BB diazonium salt approach is relatively simple, inexpensive, and fast. A study describing the new method was published in the *Journal of Agricultural and Food Chemistry* in 2011.

Removing Interference

A later ARS study conducted at the Henry A. Wallace Beltsville [Maryland] Agricultural Research Center (BARC) further explored assessing total phenolics using Fast Blue BB. This study was performed on strawberries and was headed by plant physiologist Gene Lester with ARS colleagues at BARC and ERRC. They demonstrated that the Fast Blue BB assay provides a higher and more accurate estimate of total phenolics than the traditional assay, called Folin-Ciocalteu, or FC, that has been used for decades. FC uses a reducing reagent that detects phenolics indirectly and lacks the ability to be specific, or screen, for measuring phenolics alone, according to the scientists.

In the study, the researchers used the FC and Fast Blue BB tests to analyze total phenolic concentrations in five different genotypes of strawberry fruit that are commonly grown in the United States. Strawberries are an important source of phytochemicals, in particular phenolics. The team then compared the results of both tests.

The team found that the traditional FC test interacted with vitamin C (ascorbic acid) and sugars that are abundant in strawberry, which alters the accuracy of the assessment of phenolics. That means the FC method picks up other compounds in the plant itself and also in the media used. These additionally measured compounds are referred to as "interfering substances," and they include fructose, glucose, and sucrose and other organic compounds naturally found in the extraction media. There are upwards of 50 interfering substances that impede an accurate measure of the types and amounts of phenolics when using the FC assay method to test for total phenolics.

During the study, the researchers also measured each known FC assay-interfering substance, including vitamin C and the fruit sugars, within the five genotypes of strawberries. They wanted to determine the effect of these interfering substances on the accuracy of the two assays for measuring total phenolics.

The FC method had a significant correlation with vitamin C, meaning it assessed vitamin C as phenolics, and was found to underreport or fail to assess total plant phenolics by nearly threefold. The FC method does not include a step to correct for picking up the interfering substances that are counted among total phenolics, according to the scientists. The researchers concluded that previous studies measuring the phenolics in strawberry fruit by use of the FC assay have greatly underestimated the amount of the fruit's total phenolic concentrations.

Significantly, the new Fast Blue BB method had no interaction with vitamin C or with the other interfering substances. "Because Fast Blue BB is a direct assay, it only targets phenolic compounds, whereas Folin-Ciocalteu is an indirect assay—you can get false positives, primarily by picking up ascorbic acid and other substances," says Lester.

The study was published in the *Journal of Food Composition and Analysis* in 2012.

Fast Blue BB Goes Green

While the Fast Blue BB method as originally developed at the ERRC was aimed to measure phenolics only in plant tissue that does not contain chlorophyll, the BARC researchers reasoned that the test should also work with green vegetables if modified. Lester led a study to gauge how summer and winter weather conditions affect levels of specific nutrients and compounds, including phenolics, in spinach. For the study, he and colleagues modified the Fast Blue BB assay so it could be used to test green plant material.

"Chlorophyll absorbs at the same wavelength as the Fast Blue BB," says Lester. "We developed a simple, rapid, chlorophyll-removal step that eliminates this confounding factor in the original assay so that the method could be used to assess green plant tissues."

The scientists used the modified method to gauge the amount of total phenolics in different spinach cultivars grown under different production conditions. "We showed the Fast Blue BB can now be used universally to accurately assess total phenolics for all fruit and vegetable plant tissues," says Lester. The study was published in the *Journal of Agricultural and Food Chemistry* in 2013.—By **Rosalie Marion Bliss**, ARS.

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

Gene E. Lester is with the USDA-ARS Office of National Programs, 5601 Sunnyside Ave., Beltsville, MD 20705-5138; (301) 504-4616, gene.lester@ars.usda.gov. *

PEGGY GREB (D3075-1)

Using a new test, ARS scientists have determined that strawberries contain higher levels of healthful compounds known as "phenolics" than previously thought.

PEGGY GREB (D3074-2)

Delicious, Nutritious, and a Colorful Dish for the Holidays!

America's favorite vegetable is almost fat free and a good source of potassium, iron, and vitamin C. It has 3 grams each of protein and fiber, low sodium, and no cholesterol, and costs a quarter per serving at the produce aisle.

If you haven't guessed which vegetable yet, it's only going to get harder. Agricultural Research Service scientists have bred and released colorful new varieties. Some have relatively high levels of beneficial red-to-purple pigments called "anthocyanins," and others have high levels of yellow-pigmented carotenoids.

We're talking about the popular potato. ARS researchers have developed three new varieties of potatoes with red and purple flesh and skin, which are now available to consumers. If you can get the new varieties soon, you'll have colorful potatoes gracing your holiday dinner table. See the box on page 15 for information on availability.

STEPHEN AUSMUS (D3327-3)

High-nutrient, great-tasting Purple Fiesta potato variety, a fingerling midseason specialty potato with smooth purple skin and dark purple flesh.

What's In a Color?

All potatoes contain an assortment of nutrients and other health-promoting compounds. The colored-flesh potatoes have anthocyanins and carotenoids. The amount and type depend on the variety of the potato. Breeding efforts by ARS researchers in Beltsville, Maryland, led to the release of yellow-pigmented potatoes with up to three times more carotenoids than a yellow-fleshed imported variety.

As the most-eaten U.S. vegetable, phytonutrient-rich potatoes can have a strong impact on health, according to plant geneticist Charles Brown, who is with ARS in Prosser, Washington. In a study, Brown and his colleagues analyzed and compared concentrations of phytochemicals in yellow- and purple-pigmented potatoes and in white potatoes. The team reported that yellow potatoes had a 45-fold greater concentration of carotenoids than white potatoes, and purple potatoes had a 20-fold greater concentration of anthocyanins than yellow potatoes. No detectable amounts of anthocyanins were found in white potatoes.

During the same study, the team also compared sensory evaluations of pigmented potatoes to those of white potatoes. When yellow, purple, and white potatoes were ranked by a consumer panel, no significant differences in flavor or overall acceptance were observed. The study was published in *Food and Nutrition Sciences* in 2013.

Coming to the Table

Brown carefully bred the three unique red- and purple-pigmented potatoes at ARS's Vegetable and Forage Crops Production Research Unit in Prosser.

"Getting them to consumers has taken decades," says Brown. "The cost in labor alone to bring a new variety to market is considerable." The steps to market included selections, crosses, evaluations, and extensive field-testing around the nation. Growers, buyers, and industry representatives make further postharvest evaluations based on consumer-acceptability features prior to release.

The three great-tasting potato varieties with colored flesh that are now available for consumption are TerraRosa, AmaRosa, and Purple Fiesta (also known as Purple Pelisse). They perform well across a variety of preparation methods such as baking, roasting, microwaving, steaming, and mashing.

AmaRosa is a "fingerling" (long and thin) specialty potato with smooth, vibrant, red skin and bright-red flesh. TerraRosa, described by growers as sweet and creamy, is a full-sized, oblong variety with red skin and pinkish flesh that sometimes has white marbling inside.

Purple Fiesta is a small, smooth fingerling with purple skin and dark-purple flesh. "Purple Fiesta has ranked better in taste, color, and nutrition than any other blue or purple potato I've explored," says Dan Chin, who holds the exclusive license to grow and sublicense seed. "These varieties were carefully bred to enhance all the unique qualities found in a colorful potato, including uniform size, striking color, rich vitamin and mineral content, sweet flavor, and versatility."

Brown worked on developing and evaluating the varieties as a contributing partner with the Northwest (Tri State) Potato Variety Development Program—a team that includes university researchers in Washington, Idaho, and Oregon; commissioners of the three states; and the potato industry. The Tri State Program created the Potato Variety Management Institute, a nonprofit based in Bend, Oregon, that administers licensing agreements for new varieties.

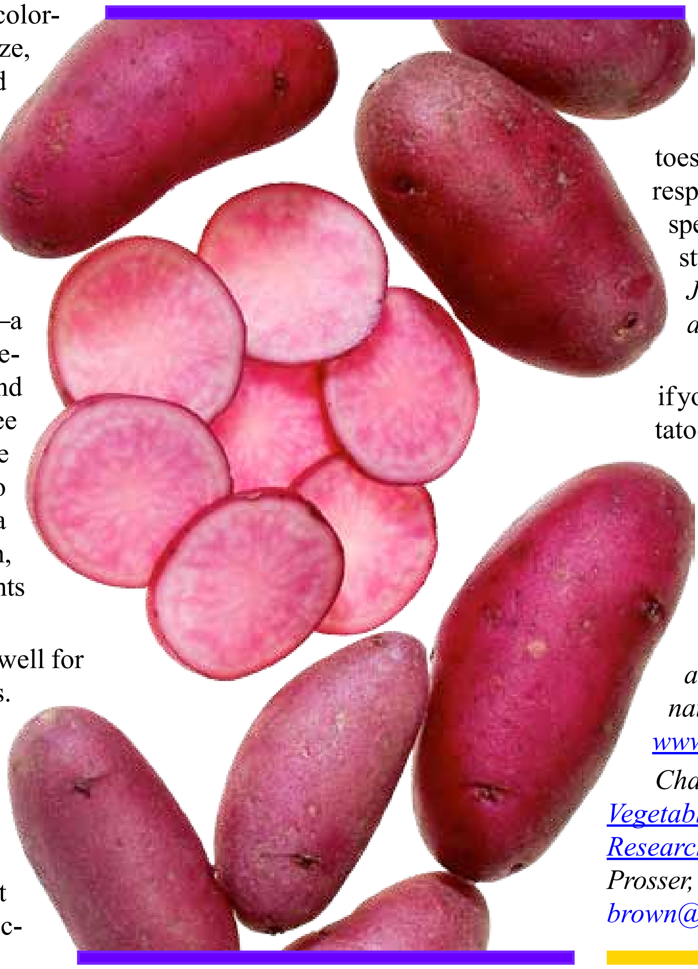
All three potatoes have ranked well for use in making potato chips and fries. "One of the sensory evaluations ranked AmaRosa highest among 10 contenders when prepared as fried chips," says Brown. "The chips retained their rosy red color and resisted fading, showing great potential for the chipping snack sector as well."

Articles describing the attributes of AmaRosa and Purple Fiesta were published in the *American Journal of Potato Research* in 2012.

The Power of Pigments

While the new potato varieties are festively colored and taste good, their value is far from skin deep. Foods rich in antioxidative bioactive compounds have been linked to reduced incidence of chronic diseases. So Brown and geneticist Duroy (Roy) Navarre, who is also at the ARS Prosser laboratory, worked with lead author Boon Chew and colleagues at Washington State University on a human nutrition study. They tested whether the carotenoids and anthocyanins from pigmented potatoes decreased oxidative stress, inflammation, and improved immune status. Their study was based on USDA-ARS colored-potato breeding lines developed at Prosser.

For the study, 36 healthy male volunteers were randomly assigned to consume white-, yellow-, or purple-fleshed potatoes for 6 weeks. Every day, the three groups (12 volunteers each) consumed two-thirds of a cup (150 grams) of the type of cooked potatoes assigned to them. All the potatoes were prepared and served at the research site.



STEPHEN AUSMUS (D3326-11)

High-nutrient, great-tasting AmaRosa potato variety, a fingerling midseason specialty potato with smooth, vibrant red skin and bright red flesh.

Fasting blood samples were collected from the participants at the start and end of the 6-week intervention. Their blood was tested for cytokines, including interleukin-6, which is secreted by lymphocytes (immune system white blood cells), while initiating an inflammatory response. Blood samples were also tested for lymphocyte proliferation, C-reactive protein (another biomarker of inflammation), and phenotype.

The researchers found that the participants who were fed the carotenoid-rich yellow potatoes and anthocyanin-rich purple potatoes had lower plasma IL-6 than those fed white potatoes. Volunteers consuming the purple potatoes showed a heart-healthy decrease in C-reactive protein from the study's start. And those who ate the yellow-pigmented potatoes showed an enhanced immune response, as indicated by increases in specific lymphocyte populations. The study was published in the *American Journal of Advanced Food Science and Technology* in 2013.

With this many positive features, if you decide to serve these colorful potatoes this holiday season, you might want to be sure to have enough to send home with guests and for leftovers the next day.—By **Rosalie Marion Bliss,** 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.

Charles R. Brown is in the USDA-ARS Vegetable and Forage Crops Production Research Unit, 24106 North Bunn Rd., Prosser, WA 99350; (509) 786-9252, chuck.brown@ars.usda.gov.

Where To Find AmaRosa, Purple Fiesta, and TerraRosa

Red- and purple-fleshed potatoes are available between September and May. A list of growers and information on retailers and shipping can be found at the link below. Please scroll down to "Specialty Varieties" after clicking:

www.pvmi.org/varieties/varieties.htm

VIRGINIA COFFMAN (D3330-1)

Markers Highlight the Way to Disease Resistance in Peas

In an ARS greenhouse in Prosser, Washington, plant pathologist Lyndon Porter inspects pea plants for disease symptoms of *Pea enation mosaic virus.*

Seed companies now have a leg up in breeding new pea varieties with resistance to one of the legume crop's most costly viral scourges, the *Pea enation mosaic virus* (PEMV). Agricultural Research Service and university scientists have identified regions of the crop's DNA that can mark the location of a gene conferring resistance to the virus.

Using molecular procedures to detect the markers, plant breeders can rapidly identify which plant varieties have inherited the gene for resistance. Conventional methods for determining which plants have resistance genes involve growing the plants to maturity in a greenhouse or field plot, exposing them to the virus, and monitoring them for a month or more for disease symptoms.

With the marker-assisted selection method, plant breeders can determine whether individual plants have resistance in just a few hours, using samples of DNA from the mature plants, seedlings, or test-tube-grown material known as "callus." Other benefits of marker-assisted selection include savings on operational costs, resources, and labor to develop new varieties from selected plants.

PEMV attacks pea crops worldwide, with disease symptoms including stunted growth, translucent "windows" on leaves, blisterlike lesions, deformed pods, and reduced yield. In the United States, the disease is especially costly to pea growers in eastern Washington and northern Idaho, with epidemics occurring there about every 8 years and inflicting losses of up to 100 percent.

Conventional controls include spraying pea crops with insecticides to kill or repel aphids, which transmit PEMV as they feed on the plants. However, "genetic resistance to PEMV is the most cost-effective and environmentally safe means of managing this virus," says plant pathologist Lyndon Porter, in ARS's Vegetable and Forage Crops Production Research Unit in Prosser, Washington. Currently, "there are no known dry pea cultivars grown commercially in the United States that are resistant to PEMV," he adds.

To fill that order, Porter teamed with several experts in pulse crop breeding and legume genetics—namely Kevin McPhee and Shalu Jain at North Dakota State University-Fargo, Norman Weeden at Montana State University-Bozeman, and Sanford Eigenbrode at the University of Idaho-Moscow.

From field and greenhouse evaluations of 393 plants derived from crosses of susceptible and resistant pea cultivars, the team mapped PEMV resistance to a single gene, previously dubbed *En,* nestled between two molecular markers, CNGC and tRNAMet2, on the third of pea's seven chromosome pairs.

Tests to validate the markers showed they are 99.4 percent accurate in identifying pea plants with resistance to PEMV. Details were published in the November-December 2013 issue of *Crop Science.*

Ag-Biotech Inc., in San Juan Bautista, California, has converted and commercialized the markers into a breeder-friendly SNP-based marker for high-throughput marker-assisted selection. A New Zealand firm and its U.S. affiliate are now using the SNP-based marker in their pea-improvement programs.

"We have already made crosses and screened second-generation populations for resistance using these markers," reports Adrian Russell, managing director of Plant Research (NZ) Limited. "The markers will have interest wherever this virus is a problem, so the benefits will not just be seen in the United States," he predicts.—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.

*Lyndon Porter is in the USDA-ARS Vegetable and Forage Crops Production Research Unit, 24106 North Bunn Rd., Prosser, WA 99350; (509) 786-9237, lyndon.porter@ars.usda.gov.**

Your Brain and Comfort Foods

Neuroimages Capture Effects

You probably know someone who's a stress eater—a person who loads up on high-calorie goodies like vanilla ice cream or chocolate cake as a source of comfort when life gets tough. In some instances, stress-induced eating can lead to overweight and obesity, which can, in turn, increase risk of heart attack, stroke, and chronic disease such as type 2 diabetes.

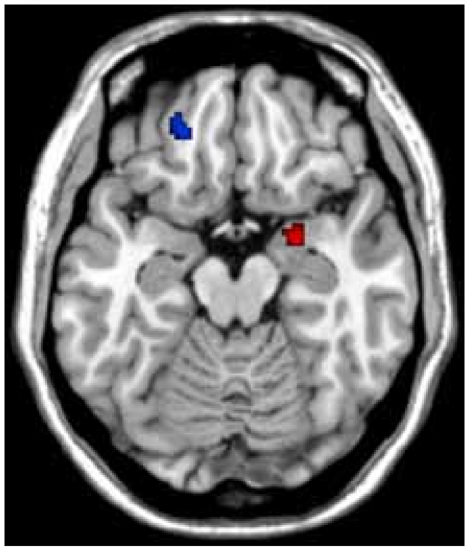
That's why Agricultural Research Service nutritionist Kevin Laugero and colleagues are taking a close look at pathways inside the brain that link stress to unhealthy eating habits, or, technically speaking, the neurophysiology of stress-eating.

The research is part of ongoing studies at the ARS Western Human Nutrition Research Center in Davis, California, to develop new, effective ways to combat America's obesity epidemic.

In studies begun in 2012, Laugero and coinvestigators have employed a leading-edge technology, fMRI (functional magnetic resonance imaging), to document brain activity associated with stress-eating. The research is designed to answer basic questions of interest to medical and nutrition researchers alike, such as: Why do some people respond to stress by overeating foods high in sugar and fat, while others don't? At what age does stress-eating become a habit? What situations are the most likely to trigger it?

For one of Laugero's most recent studies, 30 healthy female volunteers, aged 20 to 53, were asked to fill out the Wheaton Chronic Stress Survey to evaluate the amount of chronic stress caused by their work, finances, or relationships. Then, brain scans were taken while each volunteer viewed, in random order, an assortment of 200 photos of high-calorie foods, such as apple pie, lemon cake, or candy bars; 200 images of healthier foods, including a garden salad, fresh asparagus, or black bean soup; and 100 images of everyday objects such as coins, a cell phone, or a baseball.

MATTHEW TRYON (D3341-1)



When viewing pictures of high-calorie foods, study volunteers who reported high chronic stress showed enhanced activity in the amygdala region (red) of the brain, which is associated with emotions, and diminished activity in the prefrontal cortex (blue), which regulates self-control.

The scientists found that some patterns of brain activity in the "high-chronic-stress" volunteers differed markedly from those of low-chronic-stress participants. For example, activity in the prefrontal cortex, a region of the brain that regulates self-control and strategic decisionmaking, was essentially "turned off" when high-stress volunteers viewed calorie-rich foods. At the same time, the scans showed an increase in activity in the amygdala (pronounced *uh-MIG-duh-luh*), a brain region associated with emotions.

These responses were in contrast to patterns detected in brain scans of the low-stress volunteers who were shown the same food photos.

Obesity researchers have, for more than a decade, used sophisticated neuroimaging technology to detect and map the way our brains respond to food. But these findings from Laugero's research are new. They not only add to our knowledge of the neurophysiology of stress-eating, but also may help pave the way to new, science-based strategies that can help tomorrow's stress-eaters lose the habit—and unwanted pounds, as well.

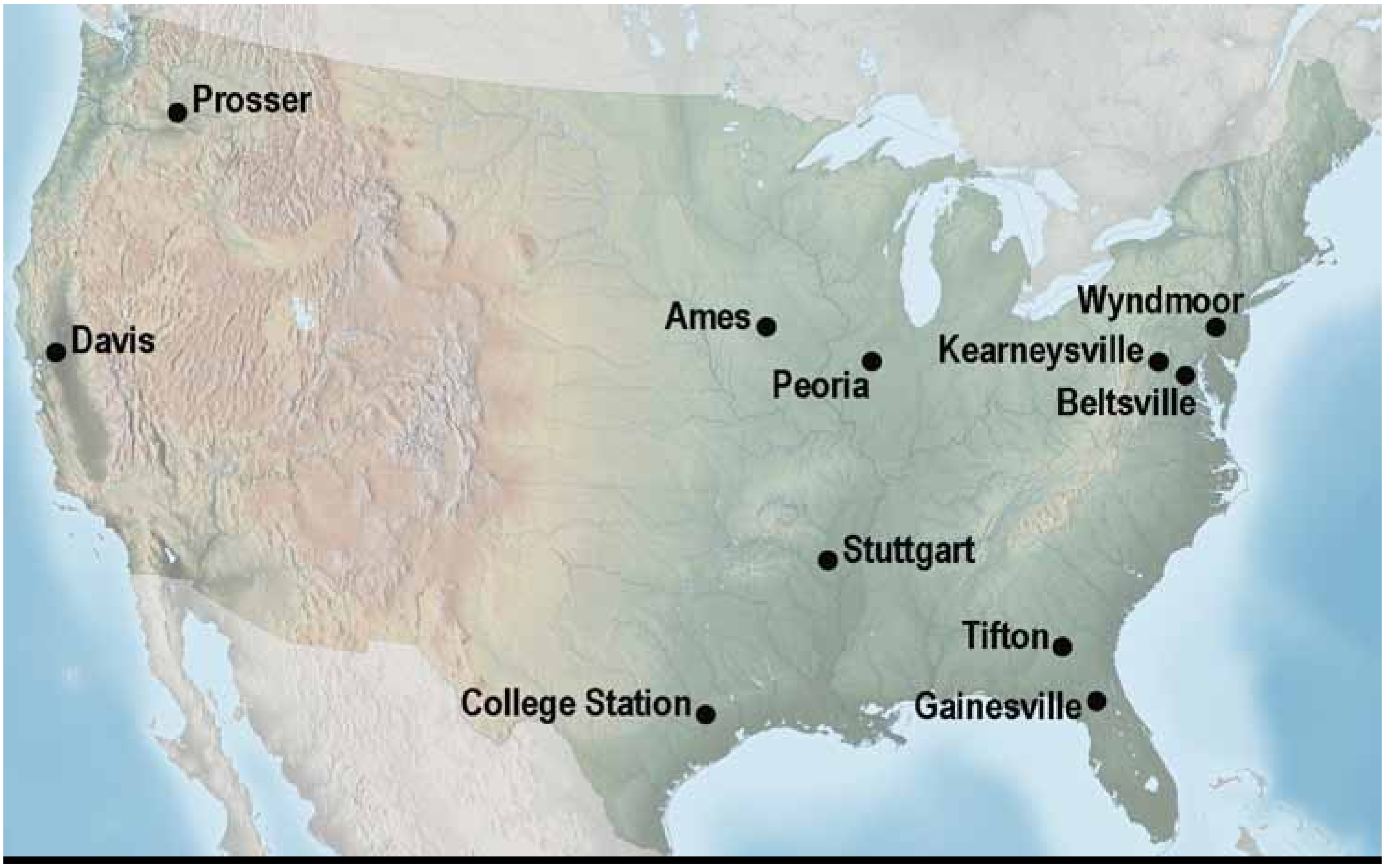
Laugero and coresearchers Matthew Tryon at the ARS nutrition center, Rashel DeCant of the University of California-Davis Department of Nutrition, and Cameron Carter of the university's Departments of Psychiatry and Psychology documented the study in a peer-reviewed scientific article published in 2013 in the journal *Physiology & Behavior*. ARS and the UC-Davis Imaging Research Center, in Sacramento, funded the study.—By **Marcia Wood**, ARS.

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

Kevin Laugero is in the USDA-ARS Obesity and Metabolism Research Unit, Western Human Nutrition Research Center, 430 W. Health Sciences Dr., Davis, CA 95616; (530) 752-4173, kevin.laugero@ars.usda.gov.

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

Locations Featured in This Magazine Issue



Locations listed west to east.

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

Davis, California

3 research units ■ 127 employees

Vegetable and Forage Crops Production Research Unit, Prosser, Washington

1 research unit ■ 41 employees

Southern Plains Agricultural Research Center, College Station, Texas

4 research units ■ 105 employees

Ames, Iowa

8 research units ■ 398 employees

Stuttgart, Arkansas

2 research units ■ 63 employees

National Center for Agricultural Utilization Research, Peoria, Illinois

7 research units ■ 254 employees

Tifton, Georgia

3 research units ■ 76 employees

Center for Medical, Agricultural, and Veterinary Entomology, Gainesville, Florida

4 research units ■ 150 employees

Appalachian Fruit Research Station, Kearneysville, West Virginia

1 research unit ■ 63 employees

Henry A. Wallace Beltsville Agricultural Research Center, Beltsville, Maryland

27 research units ■ 806 employees

Eastern Regional Research Center, Wyndmoor, Pennsylvania

6 research units ■ 213 employees