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Agricultural Research

What Makes a Tomato Taste Great?

Story on pages 12-13

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

FORUM

On Guard Against Foreign Animal Diseases

Since the establishment of the U.S. Department of Agriculture by President Abraham Lincoln in 1862, much has changed in the battle against animal diseases that threaten our food security and public health. Along with more people, animals, and agricultural products crossing international borders comes a higher risk of an intentional, accidental, or natural occurrence that may result in an outbreak of a foreign animal disease.

Scientists work to control and prevent diseases that occur in the United States, but they also work diligently to upgrade traditional tools and develop new technology to mitigate infectious-disease outbreaks that are occurring elsewhere. Foot-and-mouth disease and African swine fever are two examples.

To address these concerns, the Agricultural Research Service, the chief scientific research agency of USDA, maintains a comprehensive animal health biodefense research program dedicated to protecting livestock against potential harmful biological agents. The goal is to ensure the safety of the nation's agriculture and food supply through improved disease detection, prevention, control, and—when feasible—eradication.

ARS's Animal Health national program has several research components, one of which is biodefense research to support federal and state regulatory agencies as they prepare for potential foreign-animal-disease outbreaks. This component supports President Barack Obama's national strategy for countering biological threats by targeting priority diseases that pose the greatest economic and public-health threats to the United States. To improve our response to foreign-animal-disease outbreaks, we develop diagnostics, vaccines, and biotherapeutics that our scientists have engineered for controlling and eradicating specific diseases. If these countermeasures meet the required standard, they may be added to the National Veterinary Stockpile—the nation's repository of vaccines, personal protective equipment, and other critical veterinary products.

One disease agent of high priority is classical swine fever virus, a highly infectious virus of pigs that threatens the U.S pork industry. Scientists in the ARS Foreign Animal Disease Research Unit at the Plum Island Animal Disease Center are investigating this disease. Among their many accomplishments, they have shown that the virus contains three heavily glycosylated—or sugar-coated—proteins, which play a critical role in virulence and immune responses. This and other discoveries provide essential information for developing the next generation of vaccines.

Research has also been renewed to study another pig disease, the deadly African swine fever, that's threatening global economies.

Continued research at Plum Island on foot-and-mouth disease (FMD), one of the most economically devastating diseases affecting animals worldwide, has resulted in major breakthroughs. For example, researchers have discovered the primary site where FMD virus infection begins—a region in the back of the cow's throat. This advancement will aid in developing countermeasures and effective tools to block viral transmission.

Global eradication of FMD and other foreign animal diseases has been proposed for many years. While some parts of the world, such as South America, are on the verge of eradicating FMD with currently available tools, others require new tools and sustainable approaches relevant to their methods of livestock production. There is a clear need for alternative control methods, particularly vaccines that can address the major shortcomings of our current stock. New vaccines, diagnostics, and control strategies could help break the endemic transmission cycles of viruses that continue to circulate and remain active in particular regions.

To help achieve these goals, international collaborations are essential. The Global Foot and Mouth Disease Research Alliance—a worldwide association of animal research organizations—was formed to generate and share knowledge and discoveries among global partners and to develop better tools to combat FMD.

No one can predict with certainty the cause of the next animal-disease outbreak. But we can prepare by escalating our ability to rapidly detect, identify, and characterize new and emerging animal pathogens. We continue our work with collaborators to address major problems that limit control and eradication of foreign animal diseases. This includes understanding the ecology, pathogenesis, and transmission of diseases in relevant animal species and, importantly, developing new and cost-effective technologies to rapidly detect, control, and eradicate the most damaging animal diseases.

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KEITH WELLER (K6085-5)

Aerial view of Plum Island, an 840-acre island located 1.5 miles off the northeastern tip of Long Island, New York. The island is home to ARS's Foreign Animal Disease Research Unit, where ARS scientists collaborate with colleagues from the Department of Homeland Security and USDA's Animal and Plant Health Inspection Service to protect America's livestock from foreign animal diseases.

Story begins on page 4.

Cover: U.S. consumers want tomatoes to be available year-round, and they want the garden-fresh taste. ARS scientist Joanne Labate and colleagues are exploring tomato's genetic diversity to help breeders develop tastier tomatoes and promote consumption of this healthful vegetable. Story begins on page 12. Photo by Peggy Greb. (D3025-1)

KATHLEEN APICELLI (D2996-1)

Guarding the Country Against Foreign Animal Diseases

The MS Plum Island makes the crossing between New York's Orient Point and Plum Island several times a day, carrying passengers and cargo as part of normal Plum Island Animal Disease Center operations. Two other boats—the MS Shahan and JJ Callis—are in the fleet of passenger and cargo marine vessel supporting daily work and operations at the center.

A deadly animal virus is on the loose, treading through Russia and knocking on the doors of Eastern Europe and Asia. After its introduction into the Republic of Georgia and the Caucasus region in 2007 and spread into Russia, the virus that causes African swine fever (ASF) was spotted for the first time last year in Ukraine, putting European and Asian countries on high alert. The virus moves quickly, killing 100 percent of infected pigs within a week in some instances.

Standing between a possible U.S. invasion by foreign animal diseases like ASF is the Plum Island Animal Disease Center, situated off the northeastern tip of Long Island, New York. For almost 60 years, the center has served as somewhat of a fortress, where a small force of scientists tackles dangerous diseases that threaten the health of livestock and world economies.

In 1954, the U.S. Department of Agriculture's Agricultural Research Service took over Plum Island from the U.S. Army to establish laboratories where scientists could find ways to prevent and control exotic diseases that threaten U.S. livestock production and global food security. The primary objective was to develop methods to detect and prevent foot-and-mouth disease (FMD)—an economically devastating disease. FMD was eradicated from the United States in 1929, but today it is spreading throughout Asia and Africa. Recent outbreaks have occurred in the United Kingdom, Bulgaria, Japan, and South Korea.

Working with other agencies, scientists in the ARS Foreign Animal Disease Research Unit (FADRU), at Plum Island, also keep diseases like ASF, classical swine fever, and vesicular stomatitis at bay. In 1984, a USDA Animal and Plant Health Inspection Service (APHIS) team took over diagnostic work, leaving the basic research to ARS scientists. Another change occurred in 2003, when Plum Island operations were transferred to the

Research associate Paul Lawrence prepares a gel to analyze expression of foot-and-mouth disease virus (FMDV) proteins as part of microbiologist Elizabeth Rieder's research on novel FMDV vaccines.

KATHLEEN APICELLI (D2998-1)

U.S. Department of Homeland Security (DHS), which works with USDA in the advanced development of vaccines and diagnostic tests to help control and respond to disease outbreaks.

Tracking FMD: The Infection Site

The Plum Island center provides a safe setting for studying FMD, which affects cloven-hoofed animals like cattle, swine, sheep, goats, and deer. The highly contagious disease is rarely fatal in adult animals, but it debilitates its victims and can cause large-scale death of young animals. Incursions of FMD cause profound economic consequences through isolation from trade, loss in milk production, severe lameness, and massive depopulations. When the virus has been introduced in FMD-virus-free countries, millions of animals—infected and uninfected—have been euthanized after outbreaks to prevent the virus from spreading.

A breakthrough in FMD research came in 2010 when veterinary medical officer Jonathan Arzt identified the site where FMD virus initiates infection in cattle. Working with FADRU research leader Luis Rodriguez and microbiologist Juan Pacheco, Arzt found that the virus selectively infects epithelial cells in the back of the cow's throat.

"Now that we've determined the actual route the virus takes in infected cattle, we can try to develop new vaccines and biotherapeutics to target and prevent virus infection of the primary site and potentially control and eradicate FMD," Arzt says. "Blocking the initial site of infection is the best way to achieve complete protection."

There are seven FMD virus serotypes—O, A, C, Asia-1, SAT-1, SAT-2, and SAT-3. Scientists detected the infection site using serotype O and have since had the same success with serotype A.

"The work with serotype A elevates our level of confidence even higher," Arzt says. "We now know that what we found with serotype O was not an anomaly or isolated finding."

In other work, scientists have helped build an infrastructure for testing, biosecurity measures, and identification of FMD virus strains in Vietnam and Pakistan. "Our goal is to help them discover how FMD outbreaks are initiated under natural conditions so they can protect their own herds, but it also gives us an advance look at the FMD virus strains that are currently emerging and circulating," Arzt says.

KATHLEEN APICELLI (D2999-1)

Above: In efforts to develop an effective vaccine against African swine fever virus (ASFV), graduate student Erin Howey (foreground) performs immunofluorescent screening of porcine tissues for ASFV with veterinary medical officer Jonathan Arzt.

Below: Disease protection and prevention of livestock of various species, including cattle, is a prime function of ARS scientists and collaborators at the Plum Island Animal Disease Center.

KEITH WELLER (K4117-15)

The First Line of Defense

In the last 15 years, FADRU scientists have proven that interferons—proteins made and released by host cells in response to the presence of viruses or other pathogens—protect against FMD. Interferons act as antiviral agents that kill the virus or stop it from multiplying and reproducing.

"They are the first line of defense and response against viral infection," says microbiologist Teresa de los Santos. "They protect animals immediately, giving the

KATHLEEN APICELLI (D3011-1)

Microbiologist Teresa de los Santos uses an automatic liquid handling system to prepare multi-well plates to study immune responses of swine to foot-and-mouth disease virus infection using real-time PCR.

vaccine time to induce the immune response needed to fight the disease."

This is potentially a very important tool for controlling FMD outbreaks, because FMD spreads very rapidly; by the time animals are vaccinated, the virus may have already spread to other herds.

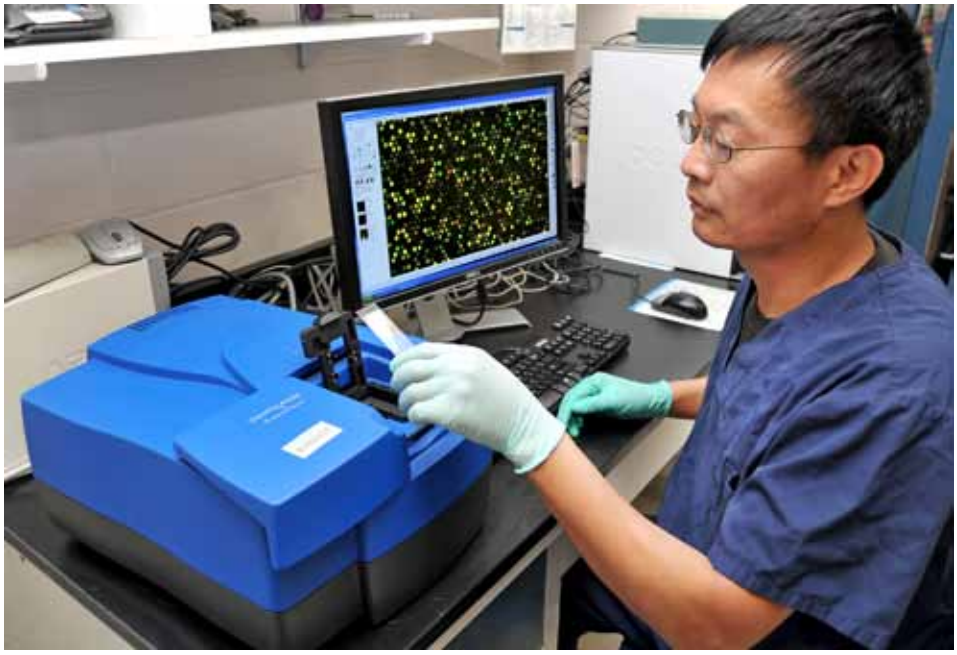
There are three families of interferons—type I (interferon alpha-beta), type II (interferon gamma), and type III (interferon lambda). Retired ARS chemist Marvin Grubman demonstrated that type I is very effective in controlling FMD virus infection in swine.

"We can inoculate swine with a viral vector containing the gene coding for swine type I interferon and then challenge them with FMD virus 1 day later and protect them," Grubman says. "Type I interferon protection could last approximately 5 days, so we still have a couple of days to cover before the vaccine immunity kicks in at about day 7 post vaccination."

Vaccines require 7 days to induce protection against FMD, leaving vaccinated animals susceptible to infection during that time. Therefore, scientists are trying to cover that window of susceptibility by combining interferons with vaccines.

By combining type I and type II interferons, Grubman produced another patented antiviral vaccine-delivery technology that rapidly blocks FMD virus in pigs. In combination with a vaccine, it provides thorough protection from day 1 until the vaccine immune response kicks in. However, this approach has not been as successful in cattle.

De los Santos and FADRU computational biologist James Zhu have discovered a solution to the problem of rapid protection of cattle. The Plum Island team was the first to report and identify a type III interferon in cattle. They also demonstrated that type III interferon is effective against



Computational biologist James Zhu scans a microarray containing over 44,000 features of the bovine genome. Differential gene expression is used to understand the response of cattle to foot-and-mouth disease virus infection.

KATHLEEN APICELLI (D3012-1)

FMD virus in cattle as early as 1 day after vaccination.

"We first discovered that a member of the type III interferon family could actually inhibit FMDV replication in cell cultures," de los Santos says. "We then inoculated cattle with a viral vector that delivered bovine type III interferon and challenged with FMD virus 24 hours later. We saw a significant delay in the appearance of clinical signs in animals that received type III interferon as compared to those given type I interferon or no treatment. In other experiments, where cows were naturally exposed to FMD virus, the type III interferon treatment was even more protective."

Scientists also constructed a mutant virus—called "SAP-mutant"—which has a mutation in one of the virus's proteins, named "leader." They used the SAP-mutant to develop an attenuated (weakened) FMD vaccine, which when administered to pigs can protect them against challenge with virulent FMD virus.

Working with Zhu and other colleagues, de los Santos used a "reverse genetics" approach to understand the basis of attenuation of the SAP-mutant. Unlike classical genetics, which seeks to find the genetic basis of a trait or phenotype, reverse genetics attempts to find what trait appears as the result of a particular gene mutation.

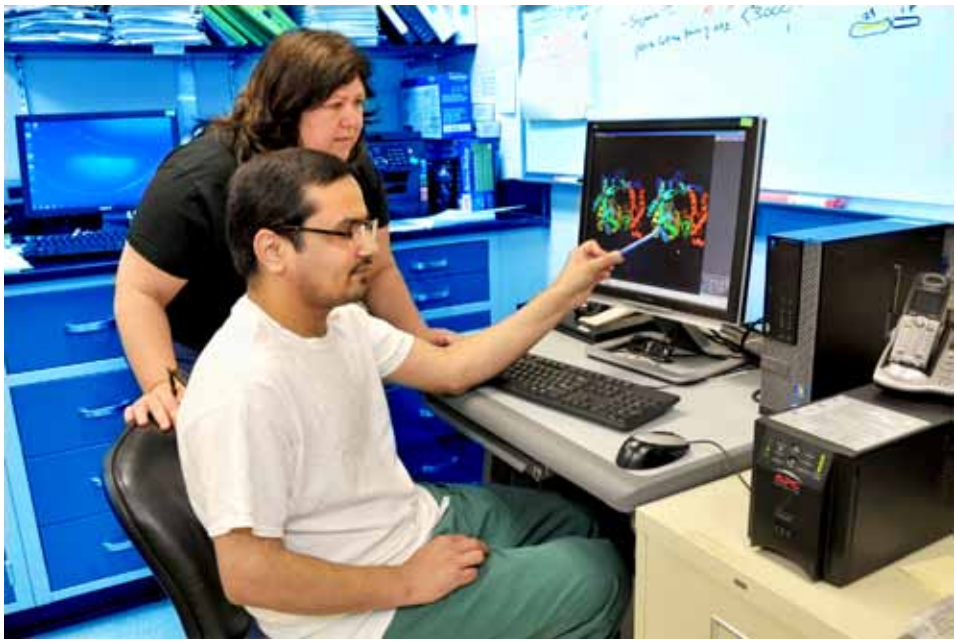
"We learned that the SAP-mutant virus was unable to interact with some host proteins involved in innate immunity," de los Santos says. "Therefore, the virus was less infectious and could potentially serve as the basis for an effective live attenuated FMD vaccine candidate."

Producing FMD Vaccines Safely

In other research, a team led by microbiologist Elizabeth Rieder has designed new technology for producing FMD vaccine without the need of virulent virus.

"We cloned the FMD virus genetic material in a plasmid [a small DNA molecule], which allows us to introduce mutations and produce deletions in the virus and understand the functions of particular parts of the virus genome," Rieder says.

KATHLEEN APICELLI (D2995-1)



Molecular biologist Elizabeth Rieder and research associate Devendra Rai view the effect of mutations on the three-dimensional structure of the FMDV polymerase protein in studies to develop FMD vaccines.

Researchers identified a sequence that, if removed, renders the FMD virus harmless to animals while still leaving it capable of growing in cell culture. "It has allowed us to understand how the virus amplifies itself, interacts with a host animal, and inhibits its defense mechanism and how different parts of the virus genome function," Rieder says. "This is important basic knowledge we can use in developing vaccines or biotherapeutics."

Scientists used this technology to produce a new FMD vaccine. This technique is safer than current technology, which uses naturally occurring (wild-type) virus, because the attenuated FMD virus doesn't cause disease in animals, Rieder says. In addition, the virus used in the vaccine has been labeled with unique markers to differentiate it from wild-type virus found in outbreaks.

A patent has been filed for the new technology, which is being developed for vaccine production by a private company.

Insights From Human Technology

Immunologist William Golde and his team were the first to try—in swine—a technology used by physicians to type humans for organ transplants. The team used it in swine and cattle to specifically

In research on immune system response to FMDV infection and vaccination, immunologist William Golde looks at cattle white blood cells to be characterized by fluorescent antibody cell sorting.

KATHLEEN APICELLI (D2997-1)

measure immune responses to FMD vaccines.

The technology, called "major histocompatibility complex (MHC) tetramers," allows scientist to follow immune responses mediated by individual T-cells. MHC is a molecule located on the surface of a cell that mediates interactions of white blood cells with other body cells.

"The human technology translated very readily to swine and cattle," says Golde. "Combining it with new technologies to track individual B cells [antibody-producing cells], we've been able to track immune responses to vaccines and to infections with greater accuracy."

Golde's other work includes testing an automated, needle-free, vaccine-delivery device called "DermVac," which induces stronger immunity with less FMD vaccine. Animals vaccinated by this method, with just a fraction of the recommended vaccine dose, were protected from disease when infected with FMD at 7 or 28 days after vaccination.

"We achieved effective protection against FMD with less than the recommended vaccine dose," Golde says, "showing that the vaccine's potency was enhanced when delivered with this device."

A Classic Case of Pig Virus

Although classical swine fever (CSF) has been eradicated in the United States, the contagious, sometimes fatal disease is present in wildlife in Europe, where infected wild boar can transmit it to domestic pigs.

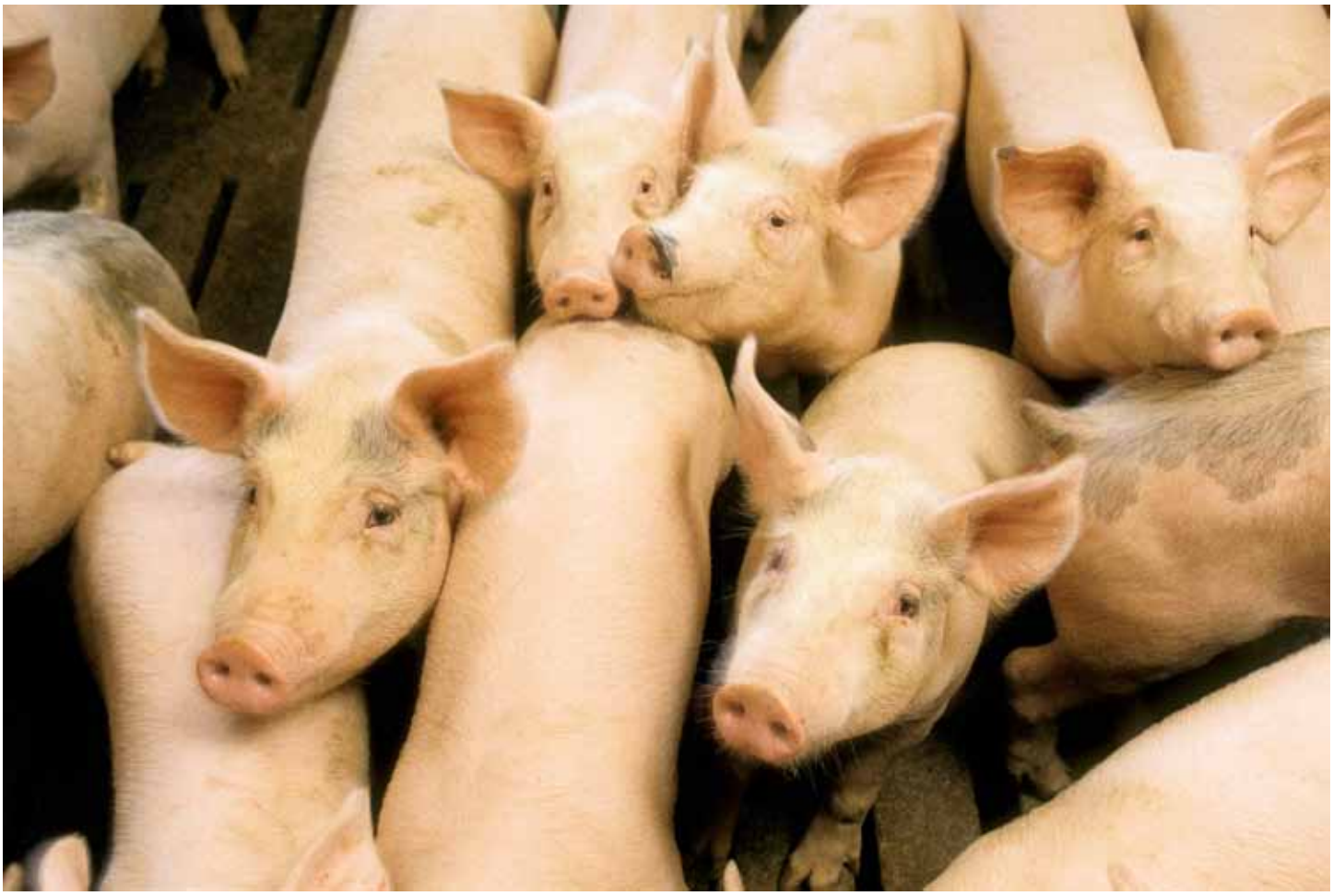
"Once the disease is detected, all animals in close proximity of infected pigs must be destroyed, creating a huge economic problem," says microbiologist Manuel Borca. "Vaccination is banned in Europe, but it would be considered if a marker vaccine is developed." A marker vaccine allows vaccinated animals to be distinguished from those infected with wild-type virus.

Under the leadership of Borca, scientists at Plum Island have been using different strategies to develop virus strains for vaccines that meet these criteria. They identified 10 to 12 different areas of the virus genome that, if mutated, cause the virus to become attenuated.

Using this approach, a CSF virus was genetically manipulated not only to attenuate it, but also to introduce a genetic marker that can distinguish it from wild-type virus. Borca received a patent for this marker virus, which produces early immunity within the first week of vaccination.

Earlier CSF work at Plum Island included the development of a rapid diagnostic test, based on real-time PCR (polymerase chain reaction), which detects the virus in infected animals 2 to 4 days before clinical signs appear. "The test was further validated using field samples in the Dominican Republic," Borca says. "We showed that it works in field conditions."

Although foot-and-mouth disease was eradicated from the United States more than 80 years ago, it continues to spread throughout other countries. New ARS discoveries are providing vital information to help fight this economically devastating disease, which affects cattle and other cloven-hoofed animals.



REGIS LEFEBURE (K5657-3)

KATHLEEN APICELLI (D2994-1)

Veterinary medical officer Jonathan Arzt (left) and microbiologist Luis Rodriguez discuss distribution and characterization of FMDV-positive cells at the primary site of infection in the bovine nasopharynx, a potential target for more effective FMD vaccines.

Spread of a Deadly Swine Disease

An outbreak of African swine fever (ASF) in the Republic of Georgia and surrounding countries in 2007 prompted ARS to renew efforts to find ways to prevent or control this serious disease, for which there is no cure or vaccine. Concern over ASF is heightened by its unrelenting spread towards Europe since 2007. Its clinical signs are similar to those of CSF, but ASF is more uniformly fatal. Pigs suffer from high fever, hemorrhages, vomiting, and loss of appetite.

"African swine fever has many strains that are extremely infectious," Borca explains. "All infected animals die within a week."

All attempts to produce effective vaccines have been unsuccessful so far because of the complexity and large size of the ASF virus.

"It has a large genome of more than 150 genes, making everything more complicated," Borca says. "It is more difficult to research the ASF virus than the smaller viruses, like CSF."

In earlier years, scientists at Plum Island made significant progress in understanding the role of particular ASF virus genes in causing disease. Borca was part of a team that was able to characterize several genes that are important in virulence.

"If you eliminated some of those genes, the virus became somewhat attenuated," Borca says. "This approach was used at that time to develop less-virulent viruses that could be tested as vaccine candidates."

Animals given the attenuated viruses and later challenged with a virulent virus were protected against ASF, he says.

After a 7-year lapse in ASF studies due to limited funding, research has been reinitiated by ARS scientists, under Borca's leadership, using these same techniques.

"In the last 2 years, we have produced recombinant viruses, using the ASF strain that is killing thousands of animals in the Caucasus region, by eliminating the genes previously identified as important for attenuating the virus," Borca says. "We are now testing those viruses in animals to see if they are attenuated and if they could be used as vaccine candidates."

A Blistering Battle

Another livestock invader is vesicular stomatitis virus (VSV), which rarely occurs in the United States but can infect humans and some wildlife species.

"It looks like foot-and-mouth disease—blisters on the tongue, mouth, and fleshy parts of the feet," Rodriguez says. "However, VSV affects horses, and FMD does not."

No effective vaccines are available for the disease, and the virus is transmitted by insects. "Black flies and sand flies are involved, as are the tiny culicoides insects, also called 'no-see-ems,'" Rodriguez says.

Working with scientists in Mexico, Rodriguez recently traced the origin of a large number of 2008 outbreaks in northern Mexico to southern Mexico. He identified the virus strain and predicted that it might spread to the United States. Indeed, this virus was the cause of outbreaks in New Mexico in 2012.

"We discovered that the virus that occurred in the United States actually comes from Mexico, but we don't know how it gets here," he says. "We think it's by insects and have found that if we protect animals from bug bites, we can decrease the impact of the outbreak."

Teamwork's the Key to Success

While ARS scientists at Plum Island work with universities, industries, international organizations, and governments, their innovative technologies and discoveries are enhanced by APHIS and DHS support. The main goal is to prevent and control foreign animal diseases by developing improved tools for diagnosing diseases and creating safe, faster acting vaccines and biotherapeutics. APHIS confirms diagnostic tests and technologies for surveillance, detection, and response to disease threats. DHS assists in developing vaccines and other countermeasures required for an effective response to a foreign-animal-disease outbreak on U.S. soil. This diligent teamwork at Plum Island helps protect livestock, humans, and food supplies from diseases that put America at risk.—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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Understanding Signals and Controlling Growth in Plants

KEITH WELLER (K3954-7)

ARS is using *Arabidopsis* as a model plant to study signaling pathways in plants. These pathways are helping them identify sensors that control nutrition and energy within the plant.

Imagine being able to slow down or speed up the growth rate of a crop, manipulate when it flowers, or extend its lifespan. An Agricultural Research Service scientist and his Canadian colleague have found a new signaling mechanism in plants that may allow them to do just that.

Autar Mattoo of the ARS Sustainable Agricultural Systems Laboratory in Beltsville, Maryland, Raju Datla of the Canadian Research Council, and collaborators are analyzing the cellular machinery that controls the growth rate, longevity, nutrition uptake, and metabolism of experimental plants via the "TOR signaling" pathway. Potential long-term applications of this research may include crops with early maturation and shorter growing seasons, ornamentals that last longer, and trees with more desirable growth and fruiting patterns.

Key Signaling Pathway Revealed

TOR-mediated signaling has been widely studied in yeast, animals, and humans. The TOR (target of rapamycin) pathway is a nutrition-and-energy sensor that plays a major role in mediating the signals that control growth, development, and lifespan in these diverse systems. TOR-mediated signals couple energy and nutrient availability with the protein-synthesizing machinery of the cell, the ribosome. Rapamycin, an immunosuppressant drug first discovered as an antifungal compound, shuts down the TOR pathway, thereby slowing growth and development.

Previous work showed that in yeast, mice, and humans, rapamycin functions by binding to a protein known as "FKBP12" (FK506 binding protein 12). But rapamycin doesn't bind effectively to FKBP12-related proteins in the model plant *Arabidopsis* or in crop plants, possibly because of differences in the protein structure.

"The question was: Could plants be engineered to respond to rapamycin, thus

enabling researchers to analyze TOR pathway functions in plants?" Datla says.

To address this question, the team developed transgenic *Arabidopsis* plants that produced the yeast version of the FKBP12 protein and selected lines for treatment with rapamycin to monitor the plants' responses at the growth, developmental, metabolic, and gene-expression levels. As anticipated, the plants containing the yeast FKBP12 gene responded to rapamycin by growing more slowly, producing shorter roots and shoots, and living longer than their normal counterparts. For example, leaves on transgenic plants were still green and growing 70 days after seed germination, whereas nonengineered plants had completed their life cycle and senesced.

Rapamycin treatments also affected gene expression, turning off or "down-regulating" genes associated with photosynthesis and cell growth. Consequently, rapamycin-treated transgenic plants failed to respond to increased light and displayed growth rates 10 times slower than those of nonengineered plants when lighting was intensified. Rapamycin treatment also altered the plant's metabolism—how it processed amino acids, organic acids, polyamines, carbon, and nitrogen.

TOR Linked to a Critical Player in Plant Growth

Previous research suggests that RPS6, a key component of the protein-synthesizing machinery in the cell, plays an important role in plant growth. This protein is known to regulate cell size, growth, and lifespan, so the researchers wanted to investigate its role in TOR signaling. They silenced the gene that expresses the RPS6 protein and observed effects that were similar to those of treating transgenic plants with rapamycin: smaller leaves, shorter roots, delayed flowering, and longer lifespans. The mutant plants also showed the same response to light and nutrition deficiencies as the transgenic plants treated with rapamycin.

In other experiments, they crossed the transgenic *Arabidopsis* lines with lines in which the RPS6 protein gene was silenced. They found that the protein had to be functioning for rapamycin to shut down the TOR pathway and have its full effect, slowing down growth and development.

Thus, "For TOR inhibition to manifest fully, the plant needs functioning RPS6 genes," Datla says. The results are described in a paper in the December 2012 issue of *The Plant Cell*.

Regulatory Implications

While a transgenic crop or agricultural product is likely decades away, and would require regulatory approval, the implications of the researchers' discovery are intriguing. The study focused on slowing down plant development, delaying flowering, and extending lifespan. But the researchers say the work shows that by manipulating either the TOR pathway or the gene responsible for expressing the RPS6 protein, these processes could also be accelerated.

"These results may enable researchers to develop new strategies for accelerating plant growth, causing early flowering, and extending or reducing the lifespan of an engineered crop," Mattoo says.—By **Dennis O'Brien**, ARS.

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

*Autar Mattoo is with the Sustainable Agricultural Systems Laboratory, 10300 Baltimore Ave., Building 001, Beltsville, MD 20705-2350; (301) 504-6622, autar.mattoo@ars.usda.gov.**

Autar Mattoo (foreground) and postdoctoral fellow Rakesh Upadhyay are studying plant genes involved in metabolic pathways related to growth and longevity. Here they are using quantitative polymerase chain reaction to analyze tomato genes.

PEGGY GREB (D3023-2)

SUSAN M. SHEFFER (D3017-1)

Tapping Tomatoes for Taste

Homogenized tomato fruit sample used for measuring acids, sugars, and vitamin C at the ARS Plant Genetics Resources Unit in Geneva, New York. Researchers there are studying how these various compounds interact to create flavor in tomatoes. This knowledge may help in future efforts to breed a tastier tomato.

Tomatoes are a $2 billion crop in the United States, and we demand a lot from them. No matter where we live, we want fresh, delicious tomatoes to be available year-round. Large-scale producers ship tomatoes long distances, and that makes firmness and long-term storage top priorities for tomato breeders.

Consumers, however, value garden-fresh taste. A common complaint is that tomatoes in supermarkets lack the flavor of locally grown varieties. To improve flavor, breeders need to know more about the varieties that hold the greatest potential for enhancing taste.

Joanne Labate, an Agricultural Research Service molecular biologist, and Larry Robertson, curator of the ARS Plant Genetic Resources Unit vegetable collections in Geneva, New York, joined with Dilip Panthee of North Carolina State University to explore tomato's genetic diversity in a comprehensive study designed to help breeders develop tastier tomatoes.

The team raised many varieties of tomatoes and analyzed them for the compounds that play a role in determining flavor. They also had volunteers evaluate their field-grown varieties for flavor and other sensory characteristics. The study, published in *Plant Genetic Resources: Characterization and Utilization* (2013), represents one of the most comprehensive efforts to identify the sources of genes for boosting flavor among both commercial tomato varieties and breeding lines.

"Commercial tomato varieties have a narrow genetic base. To find ways to improve their flavor, we need to broaden that base and begin looking among our entire stock of tomatoes for new sources of beneficial genes," Labate says.

A Cross Section of the World's Tomatoes

What we refer to as "flavor" in a tomato is actually the interaction and ratios of sugars, organic acids, and volatile compounds derived from amino acids, lipids, and carotenoid precursors. Fructose and glucose are the major forms of sugar. Citric acid is the dominant acid in the ripe tomato fruit, though malic acid is also present.

The researchers wanted to determine the variability of these four compounds from one variety to the next and see how that variability contributes to flavor.

They grew 173 varieties on test plots in North Carolina. The varieties were selected from the approximately 6,000 accessions in the ARS Tomato Germplasm Collection in Geneva because they represent a cross section of the world's tomato diversity. Some were commercial varieties, some were heirlooms, and others were lines used by breeders. All were part of a core collection of tomatoes kept in Geneva that had never been evaluated for fruit-quality traits.

Tomatoes were classified into one of three categories: plum or roma; cherry or grape; or the traditional large, round types. Ten volunteer taste testers were trained in sensory analysis and asked to rate each variety on a scale of 1 to 5 in four sensory areas: odor, taste, flavor, and texture. Taste tests were conducted over 6 weeks. The researchers also measured each variety's firmness and measured juice for levels of sugar, citric acid, and vitamin C.

A Key Ratio: Acid Versus Sugar

The result is a treasure trove for breeders: a comprehensive set of rankings on flavor qualities, sweetness, vitamin C content, sugar and acid content, and other characteristics. The findings show that although thousands of compounds go into determining flavor and other characteristics, two play a key role in determining overall flavor: sugar and acid. While the amount of acid varied only slightly in the tomatoes tested (from 0.2 to 0.64 percent), there was a wide variety in sugar content (3.4 to 9.0 percent). That's important because, for ripe tomatoes, the greater the ratio of sugar to acid, the sweeter the tomato. The sweeter the tomato, the more flavor it contained.

The findings are good news for breeders because they show a broad range of possibilities for adjusting sugar levels and developing more flavorful tomatoes. Breeding to enhance flavor shouldn't be that difficult because both sugar and acid content can be reliably and inexpensively measured, the researchers say.

The North Carolina fields used by the researchers also affected the characteristics observed in the study tomatoes, such as size, shape, and taste. But genes strongly influence such traits, and traits such as flavor are likely to show up consistently in a number of different environments if superior varieties with associated molecular markers are used in breeding, the researchers say.

"The growing environment will affect the expressed traits, but being able to identify and select superior tomatoes for breeding might increase the frequency of favorable alleles being transferred into different varieties and ultimately improving them," Robertson says.

A Statistical Approach

In another study, Labate and Robertson used new statistical genetic approaches to determine when incorporating genes for desirable traits led to inadvertently adding other genes with unintended consequences. The phenomenon, known as "linkage drag," is a common problem for plant breeders, but it is a particular challenge for tomato breeders because so many of the tomato's key horticultural traits originated from wild relatives. Tomatoes originated in Peru, Chile, Ecuador, and Mexico, and they were brought to colonial America through Europe. During the early 20th century, they were crossed with wild species to make the tomatoes we have today.

The researchers were able to identify latent genes that migrated into breeding lines along with genes deliberately introduced to confer desirable traits. The results, published in *BMC Plant Biology* (2012), showed, for instance, how a gene introduced for disease resistance carried with it a gene that influenced fruit ripening. The work sets the stage for more detailed studies on the effects of linkage drag on tomato breeding efforts, elimination of undesirable genes from breeding stocks, and identification of more desirable sets of genes for tomato breeders.

The researchers are optimistic that both studies will provide guidance to breeders interested in developing more flavorful tomatoes. The tomato genome sequence, published in 2012 by ARS scientists and collaborators, is also enabling breeders to use molecular markers to identify and lock in tomato genes that are responsible for desired traits such as flavor.—By **Dennis O'Brien,** ARS.

One of numerous varieties of tomatoes grown and analyzed for compounds that play a role in determining flavor.

DILIP PANTHEE (D3018-1)

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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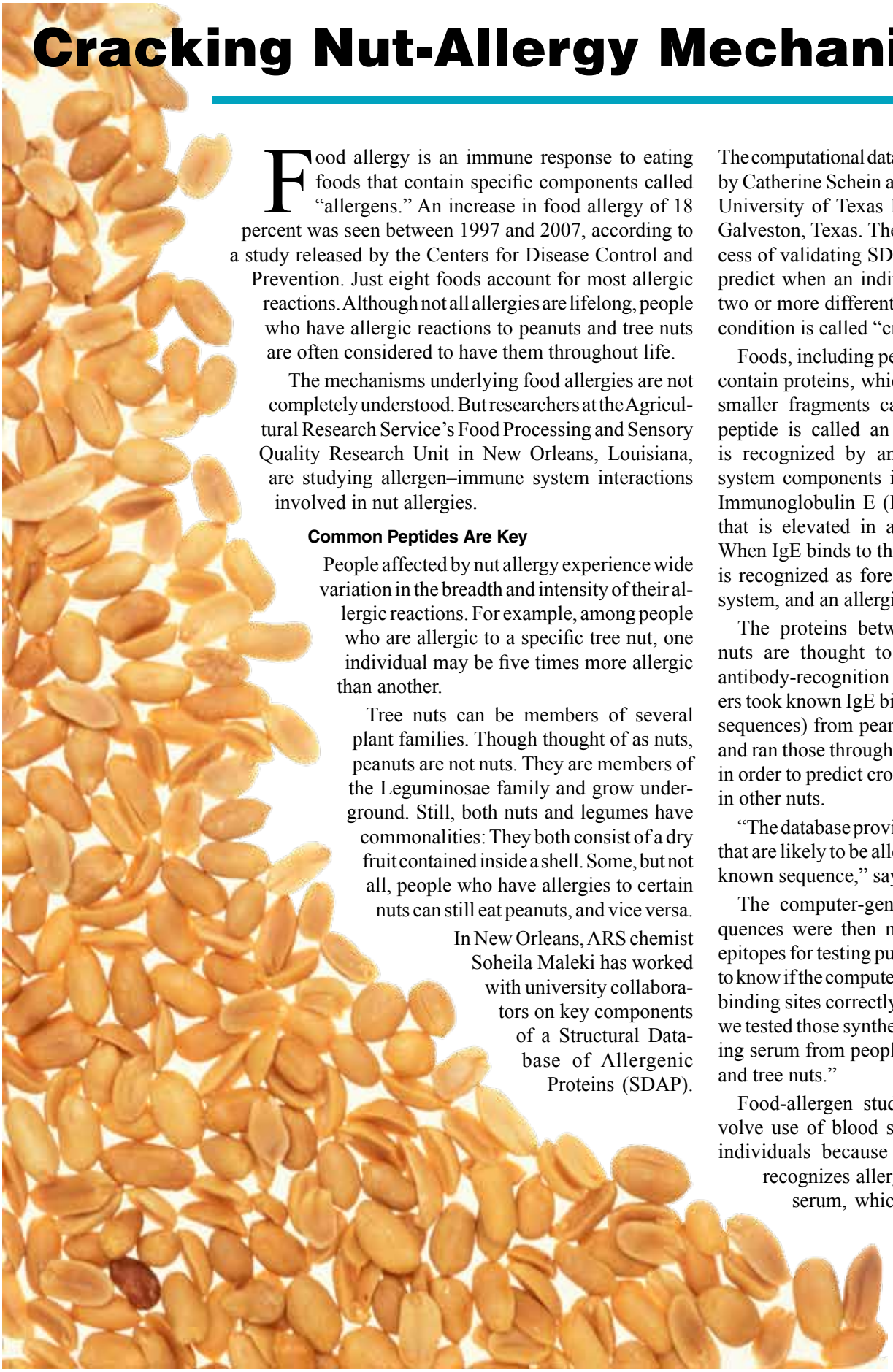
Cracking Nut-Allergy Mechanisms



Food allergy is an immune response to eating foods that contain specific components called "allergens." An increase in food allergy of 18 percent was seen between 1997 and 2007, according to a study released by the Centers for Disease Control and Prevention. Just eight foods account for most allergic reactions. Although not all allergies are lifelong, people who have allergic reactions to peanuts and tree nuts are often considered to have them throughout life.

The mechanisms underlying food allergies are not completely understood. But researchers at the Agricultural Research Service's Food Processing and Sensory Quality Research Unit in New Orleans, Louisiana, are studying allergen–immune system interactions involved in nut allergies.

Common Peptides Are Key

People affected by nut allergy experience wide variation in the breadth and intensity of their allergic reactions. For example, among people who are allergic to a specific tree nut, one individual may be five times more allergic than another.

Tree nuts can be members of several plant families. Though thought of as nuts, peanuts are not nuts. They are members of the Leguminosae family and grow underground. Still, both nuts and legumes have commonalities: They both consist of a dry fruit contained inside a shell. Some, but not all, people who have allergies to certain nuts can still eat peanuts, and vice versa.

In New Orleans, ARS chemist Soheila Maleki has worked with university collaborators on key components of a Structural Database of Allergenic Proteins (SDAP). The computational database was developed by Catherine Schein and colleagues at the University of Texas Medical Branch, in Galveston, Texas. The team is in the process of validating SDAP's ability to help predict when an individual will react to two or more different types of nuts. This condition is called "cross-reactivity."

Foods, including peanuts and tree nuts, contain proteins, which are digested into smaller fragments called "peptides." A peptide is called an "epitope" when it is recognized by antibodies—immune system components in the bloodstream. Immunoglobulin E (IgE) is an antibody that is elevated in allergic individuals. When IgE binds to the epitopes, the food is recognized as foreign by the immune system, and an allergic reaction occurs.

The proteins between cross-reactive nuts are thought to have similar IgE antibody-recognition sites. The researchers took known IgE binding sites (epitope sequences) from peanut and nut proteins and ran those through the SDAP database in order to predict cross-reactive epitopes in other nuts.

"The database provides other sequences that are likely to be allergenic based on the known sequence," says Maleki.

The computer-generated binding sequences were then made into synthetic epitopes for testing purposes. "We needed to know if the computer predicted the novel binding sites correctly," says Maleki. "So we tested those synthesized sequences using serum from people allergic to peanut and tree nuts."

Food-allergen studies commonly involve use of blood serum from allergic individuals because their serum's IgE recognizes allergenic epitopes. The serum, which was provided by

ARS (K7236-21)

cooperators at the University of California Davis, allowed the team to match previously unknown epitopes within the major allergenic proteins known to be common to a variety of nut and peanut allergies.

The authors found that similar immunoglobulin epitopes on allergenic proteins, as defined by SDAP, could account for some of the cross-reactivity between peanuts and tree nuts. The finding indicates that SDAP can be useful for predicting previously unidentified cross-reactive epitopes, based on their similarity to known IgE epitopes.

"The novel sequences we found and validated using the database are similar, but not identical, to the sequences we fed into the software," says Maleki. "We were able to confirm sites that the immune system sees and binds but that we could not have predicted otherwise."

The study was funded by the U.S. Environmental Protection Agency and the National Institutes of Health and was published in *Allergy* in 2011.

Increasing Diagnostic Reliability

Previously, Maleki had assessed the diagnostic reliability of standard peanut-allergy tests. She found that while people generally eat peanuts that have been heat treated (via roasting or boiling), the extracts that are commonly used to diagnose peanut allergies are from *raw* peanuts. She and colleagues hypothesized that raw peanut proteins undergo specific changes during roasting that may contribute to increases in allergenic properties. (See story on page 16).

Since then, Maleki and colleagues have published a series of studies that shed light on the molecular differences between raw and heat-treated nuts in terms of their inherent peptides that trigger human allergic reactions.

The major allergenic proteins (or allergens) of peanut are known as "Ara h 1," "Ara h 2," and "Ara h 3." For one study, Maleki looked into how the peanut-roasting process alters how well an allergic individual's immunoglobulins bind to peanut allergens. The team compared the reaction by human IgE antibody to the heated and unheated forms of Ara h 1. The study showed that roasting-induced side reactions, such as browning, increased the amount of IgE that recognizes and binds to Ara h 1—when compared to the amount that binds to Ara h 1 from raw peanuts.

"This result partly accounts for the increased allergenic properties observed in processed, roasted peanuts," says Maleki. The study was published in 2012 in *Molecular Nutrition and Food Research*.

In another study, Maleki and colleagues in Spain showed that a combination treatment of heat and high pressure (autoclaving) applied to peanuts significantly reduced allergic reaction. Autoclaving involves a higher moisture environment, similar to steaming or boiling, than roasting. As result, autoclaving does not initiate the browning effect that comes with roasting. The less allergenic reaction to the combination-treated peanuts was confirmed by skin-prick tests applied to volunteers known to have peanut allergies.

"Proteins become unfolded with autoclaving," says Maleki. "If you unfold the protein, you may reduce allergenicity." The study was published in 2012 in *Food Chemistry*.

Insights into allergen-immune system interactions will help with preventing and diagnosing serious food allergy.—By **Rosalie Marion Bliss,** ARS.

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

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People tend to eat peanuts that have been roasted or boiled, while the extracts commonly used to diagnose peanut allergies are from raw nuts. An ARS chemist has studied raw and cooked peanuts and revealed peptide differences that may be responsible for allergic reactions.

JACK DYKINGA (K4297-14)

Casting a Wider Net To Detect Peanut Allergy

Allergists take several steps to pinpoint the source of allergy symptoms. In addition to conducting tests during which skin is pricked or blood is drawn, allergists also conduct a detailed medical history when screening an individual for food allergy.

Skin-prick tests involve using extract from the suspected allergenic food in a device that dispenses a tiny amount during the prick. The blood-draw test involves measuring the amount of antibodies in the blood sample that recognize allergenic protein fragments called "epitopes." When an antibody called "immunoglobulin E" (IgE) binds to epitopes, the food is recognized as foreign by the immune system, and an allergic reaction occurs.

In the case of peanuts, commercial skin-prick allergy tests use extracts prepared from raw peanuts. However, Agricultural Research Service researchers in New Orleans, Louisiana, have found that roasted peanuts bind higher levels of IgE than raw peanuts.

At the unit, ARS chemist Soheila Maleki led a team that prepared individual extracts from raw, roasted, or boiled peanut samples. The scientists also procured three commercial extracts derived from raw peanuts under unknown methods. The team included colleagues from Louisiana State University in Shreveport.

Each of the 6 extracts was used in skin-prick tests on 19 study volunteers who had shown symptoms of peanut allergy and 4 volunteers who could eat peanuts without any symptoms.

Each of the individually prepared laboratory extracts—raw, roasted, or boiled—led to both false positives and false negatives. False positives occurred in nonallergic patients, and false negatives occurred in allergic patients.

"None of the three custom-made extracts tested individually showed optimal diagnostic reliability in terms of patient sensitivity and specificity," says Maleki. At least three of the volunteers with a history of severe reaction to peanuts had a variety of reactions (including one false negative) to one or more of the three commercial extracts tested.

Maleki and colleagues suggest that future testing include a mix of extracts from raw, boiled, and roasted peanuts to enhance diagnostic capability.

The study was published in *Annals of Allergy, Asthma, and Immunology* in December 2010.—By **Rosalie Marion Bliss,** ARS.

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Assessing the U.S. Population's Sodium Intake

PEGGY GREB (D2457-1)

An ARS food-survey tool proves accurate in estimating the amount of sodium consumed by volunteers.

Sodium intake has become a hot topic as public policymakers address regulatory proposals aimed at lowering the amounts in foods. In 2010, the Institute of Medicine issued a report recommending that new national sodium standards be implemented by the federal government. Several major food manufacturers have long been implementing sodium-reduction strategies through self-regulation.

The usefulness of proposed regulatory steps will depend on accurate and practical methods to monitor the U.S. population's sodium intake. In the late 1990s, researchers at the Agricultural Research Service's Beltsville Human Nutrition Research Center developed the Automated Multiple Pass Method (AMPM), an innovative surveying tool used to obtain information on the amounts of foods and beverages consumed by a survey volunteer during a 24-hour period.

The researchers also conducted a study to evaluate the accuracy of the method, using each volunteer's 24-hour urinary excretion to measure biomarkers of nutrient status. That study, involving 524 male and female volunteers aged 30 to 69 years, confirmed the accuracy of this innovation in food-consumption survey methods.

Now, Donna Rhodes, a nutritionist with the Beltsville center, has used this data to assess sodium intakes. The accuracy of sodium intake was calculated as the ratio of reported dietary intake to that estimated from the urinary sodium excretion. Estimates of sodium intake included salt added in cooking but not salt added at the table.

The results showed that the AMPM-derived dietary sodium-intake estimates (based on volunteers' reported food consumption) were 93 percent accurate among men and 90 percent accurate among women when cross-checked against the urinary sodium-excretion data.

Results from this study are significant because they demonstrate that the AMPM is a valid tool for estimating sodium intake as well as energy intake. The current USDA-ARS national food and nutrient intake survey uses the automated tool for both in-person and telephone interviews.

"The automated tool will continue to accurately estimate the population's sodium intakes from foods as food-composition databases produced by ARS are routinely updated to reflect changes in the salt content of foods consumed," says Rhodes.

The study was accepted for publication by the *American Journal of Clinical Nutrition*.—By **Rosalie Marion Bliss,** ARS.

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Sweeping Away Scotch-broom Shrubs

The people who brought Scotch-broom *(Cytisus scoparius)* from Europe to the United States thought the woody legume and its bright yellow blossoms would be an ideal ornamental for California gardens and could help stabilize fragile hill slopes. But they inadvertently introduced an invader.

The vigorous perennial shrub acclimated easily to the new terrain. It now thrives throughout the Pacific Northwest and the eastern United States, displacing native ecosystems and altering soil chemistry via nitrogen fixing wherever it goes. On their native turf, individual Scotch-broom plants produce up to 9,600 seeds annually, but that outputs soars up to 14,000 seeds per plant in nonnative ecosystems—and seeds can stay viable for years.

Resource managers at the Joint Base Lewis-McChord (Fort Lewis) military installation near Tacoma, Washington, are trying to restore native prairie ecosystems that once thrived in the region. But they have been thwarted by flourishing thickets of Scotch-broom, which can grow to heights of 6 feet or more. So a team of researchers, including Agricultural Research Service entomologists Ray Carruthers and Angelica Reddy, investigated options for using an integrated weed control strategy to control the exotic survivor. Carruthers is the research leader at the ARS Exotic and Invasive Weeds Research Unit in Albany, California.

To date, the plant has been contained at Fort Lewis with some success by using controlled burns, mowing, or releases of a biological control agent, the Scotch-broom seed weevil. The mowing and burning control strategies had been applied to many Fort Lewis sites for at least 20 years.

In their study, the scientists evaluated three management strategies: weevil seed predation alone, weevil seed predation combined with mowing, and weevil seed predation combined with burning. They surveyed pods and plants for two seasons to measure seed production, seed predation by weevils, weevil numbers in pods, and seed bank densities.

There were no observed statistical differences among the three management strategies in the number of seeds per pod, weevil counts per pod, or proportion of seeds eaten by the weevils per pod or per plant. But combining weevil predation with either burning or mowing significantly reduced the number of pods per plant, mature seeds per plant, and seed bank densities relative to weevil predation alone. The combination strategies also reduced the per-plant production of healthy, mature seeds.

More research is needed to validate the best possible strategies for controlling Scotch-broom. However, in light of these findings, the researchers think that using 3-year rotation fires with seed predation could provide more effective long-term management of Scotch-broom at Fort Lewis.

Other work there showed that burning did not disrupt native prairie plants, and sometimes Scotch-broom that was mowed came back even more vigorously—an outcome that could result in an endless loop of ineffective eradication efforts.

The scientists, who published the results in 2012 in *Invasive Plant Science and Management,* received an award from the Weed Science Society of America in 2013 for the "Outstanding Paper in Invasive Plant Science and Management."—By **Ann Perry,** 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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RAYMOND I. CARRUTHERS (D3037-2)

Left: Flowering Scotch-broom *(Cytisus scoparius)* plants are invasive weeds affecting native plants in Washington State and other parts of the United States. Below: An adult Scotch-broom seed weevil *(Exapion fuscirostre),* a potential biological control for the weed, emerging from a Scotch-broom seed.

ANGELICA M. REDDY (D3015-1)

COLTON YODER (D3004-1)

Delaying Drainage From Prairie Potholes Protects Water Quality

After a storm, a tile riser drains a pothole in a wheat field. Tile risers are perforated pipes extending a foot or more above the soil. Water flows into the holes in the pipe and then typically flows unimpeded and unfiltered into subsurface drains and eventually into streams, causing pollution. An ARS scientist has shown that a blind inlet (opposite page) provides a solution.

Midwest prairies are some of the most productive farmlands in the United States, and prairie potholes—depressions left behind in the landscape when glaciers made their last retreat around 12,000 years ago—contain some of the prairies' most fertile soils. The potholes are prone to ponding because they have no natural drainage outlet, so farmers often install tile risers to drain away excess water. These risers, which are vertical perforated pipes that extend a foot or more above the soil, also connect directly to subsurface tile drainage networks that are spread throughout the Midwest.

"For farmers, the advantage with risers is that water drains quickly," says Agricultural Research Service soil scientist Doug Smith, who works at the ARS National Soil Erosion Research Laboratory in West Lafayette, Indiana. "But they don't like having to drive around the risers or having to make sure they don't hit them with field equipment. And the risers are hard to see when the crop is up."

The tile risers are also a key component of a serious long-term—and widespread—environmental issue. Water drained from potholes by the risers is discharged via the subsurface drains into the nearest field ditch without any filtration or processing to remove excess phosphorus, nitrogen, or sediment. In these landscapes, Smith says, field ditches function as first-order streams. So there's a good chance the agricultural pollutants drained from the potholes in the St. Joseph River watershed in northeast Indiana will eventually reach Lake Erie and help nourish algal blooms that can turn the water a disconcerting shade of shamrock green.

Scientists earlier identified the connections between water from artificially drained potholes, nutrient loading, and water quality as part of research for the Conservation Effects Assessment Project, a nationwide effort to evaluate the effectiveness of conservation practices in improving water quality. Since 2002, Smith and others working in the St. Joseph River basin have identified direct correlations between the number and extent of artificially drained potholes in the watershed and nitrogen and phosphorus levels in nearby streams.

"We know that tile risers create water-quality issues throughout the Lake Erie Watershed, which includes the St. Joseph River Watershed," Smith says. "Now we must find out how to manage the problem."

Smith and West Lafayette soil scientist Stan Livingston conducted two studies to assess methods for reducing the transport of nutrient loads from prairie potholes. In the first study, a local farmer agreed to let the researchers set up tile risers and an alternative drainage system called a "blind inlet" in side-by-side potholes on a working crop field.

A blind inlet is similar to a French drain that channels excess water away from a building foundation. The scientists constructed their blind inlets by digging a square pit 3 feet deep at the lowest point of the pothole and then placing a layer of coarse limestone gravel in the bottom of the

pit. Septic tile lines were placed on top of the gravel layer and covered with another layer of limestone gravel. The second layer of gravel was covered by landscape fabric, and the rest of the hole was backfilled with coarse soil to facilitate infiltration.

Smith and Livingston found that water samples that exited the potholes through the risers consistently showed the highest levels of total phosphorus lost from the fields—as high as 1.73 ounces per acre during one storm event. In addition, almost all samples drained by the risers had higher levels of sediment, soluble phosphorus, total phosphorus, and nitrogen.

Phosphorus loads in samples channeled through the blind inlets were, on average, 78 percent lower than those in samples channeled through tile risers, and average sediment loads were 79 percent lower. Smith also noted that discharge rates from the potholes drained by blind inlets were lower, in large part because the gravel layers slowed down the flow of water. But the slower drainage didn't appear to damage the growing crops.

In the second study, in a 770-acre basin, Smith and Livingston replaced 13 tile risers with blind inlets. Then they compared sediment, phosphorus, and nitrogen loads in water samples collected from this basin to water samples collected from an adjacent 735-acre basin drained with tile risers.

The water samples were collected in the spring of 2010, when rainfall was significantly above average, so runoff levels from both basins were also much higher than usual. Compared to discharge in previous years, discharge in 2010 from the basin drained with tile risers increased 417 percent, and total phosphorus loading increased 737 percent. But in the basin drained with blind inlets, discharge only increased 64 percent, and total phosphorus loading only increased 92 percent.

"Blind inlets break the connectivity between pothole drainage and subsurface tiles," Smith says. Breaking that connectivity has a number of benefits, starting with simply reducing the volume of water eventually drained from the potholes.

While the water slowly percolates from the pothole through layers of gravel and soil, its nutrient load is available for plant uptake or denitrification, which reduces the amount of phosphorus and nitrogen that eventually ends up in the nearest field ditch. Since blind inlets trap sediment more effectively than risers—which only filter out large organic debris like cornstalks—they also help reduce loads of insoluble phosphorus that are adsorbed onto particulate matter.

Because of Smith's study, Indiana farmers who want to replace existing tile risers with blind inlets to improve water quality are eligible for financial assistance through the USDA Natural Resources Conservation Service's Environmental Quality Incentives Program Water Quality Initiative. A paper describing the research and results has been published in *Soil Use and Management*.

"Our results were what we were hoping for," Smith says. "We thought we'd see problems with tile risers, so we wanted to find something that we could use to treat the water-quality issues and that wouldn't hurt crop production. So I consider this project a success."—By **Ann Perry,** ARS.

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

*Doug Smith is with the USDA-ARS National Soil Erosion Research Laboratory, 275 South Russell St., West Lafayette, IN 47907-2077; (765) 494-0330, douglas.r.smith@ars.usda.gov.**

Construction of a blind inlet. Septic tiles are placed on top of a layer of coarse limestone gravel, covered in a second layer of gravel, encased in landscape fabric, and backfilled with coarse soil to facilitate infiltration.

STAN LIVINGSTON (D3005-1)

Water drained from potholes by tile risers is discharged via subsurface drains into the nearest field ditch without any filtering to remove excess phosphorus, nitrogen, or sediment.

DOUG SMITH (D2768-1)

Kitty Litter: Potential New Assignment for Leftover Dried Distiller's Grains

Sometimes ARS research really is the cat's meow.

Plant physiologist Steve Vaughn and colleagues have shown that dried distiller's grains, or DDGs—the leftovers after corn kernels are used to produce ethanol—can be processed further to make a promising, nearly 100-percent-biodegradable kitty litter.

With further testing, the litter may prove to be a more Earth-friendly choice than the popular but nonbiodegradable clay-based litters that today end up, for the most part, in landfills. At the same time, the DDG-based litter may provide a new and perhaps higher value market for the tons of DDGs that ethanol refineries primarily market as a cattle-feed ingredient.

In their research, Vaughn's group tested what are known as "x-DDGs." These are DDGs that, after being used for ethanol production, are treated with one or more solvents to extract any potentially useful natural compounds that remain.

The laboratory experiments yielded a suggested formulation composed of the x-DDGs and three other compounds: glycerol, to prevent the litter from forming dust particles when poured—or pawed; guar gum, to help the litter clump easily when wet; and a very small amount of copper sulfate, for odor control. The resulting litter is highly moisture absorbent, forms strong clumps that don't crumble when scooped from the litter box, and provides significant odor control, says Vaughn. He's based at the National Center for Agricultural Utilization Research in Peoria, Illinois.

The idea of using corn or other grains as the basis of an environmentally sound cat litter isn't new. But the Peoria team may be the first to as extensively study the potential of x-DDGs as the primary component of a litter and to make their results publicly available. Their peer-reviewed scientific article appeared in a 2012 issue of *Industrial Crops and Products.*

America has a continuing demand for kitty litter. Today, there are more than 95 million pet cats in this country, according to the American Pet Products Association. Most of these kitties are kept indoors, creating a need for about 1.18 million tons of cat litter every year. "Clumping" litters, made from sodium bentonite clay, are among the most popular, but they are not biodegradable.

Though the economics of producing an x-DDG-based litter weren't studied, they would likely depend on the profitability of compounds extracted from DDGs. The team tested both DDGs and x-DDGs and found that x-DDGs were the better choice for litterbox use.

The tests of x-DDGs are part of ongoing studies at the Peoria research center to develop new uses for crops and their byproducts. Vaughn did the work with chemists Mark A. Berhow and Jill K. Winkler-Moser at Peoria and Edward Lee of Summit Seed, Inc., Manteno, Illinois. —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.

*Steven F. Vaughn is with the USDA-ARS National Center for Agricultural Utilization Research, 1815 N. University St., Peoria, IL 61604; (309) 681-6344, steven.vaughn@ars.usda.gov.**

Tons of dried distiller's grains stored at the Big River Resources ethanol facility in West Burlington, Iowa. ARS scientists at Peoria, Illinois, have found that this ethanol byproduct can be processed further to create a new, nearly 100-percent biodegradable kitty litter.

STEVEN VAUGHN (D802-1)

Patented New Trap Irresistible to Insect Pests

Better control of red flour beetles and other costly, stored-product insect pests could be on hand, thanks to a new pitfall trap designed by Agricultural Research Service scientists.

Commercial traps now used are typically dome-shaped devices baited with pheromones or other attractants that lure the beetles into pits or onto glue strips. The new design, dubbed the "Terrestrial Arthropod Trap" and patented in October 2012 by ARS on behalf of the U.S. Department of Agriculture, takes this "fatal attraction" to a new level.

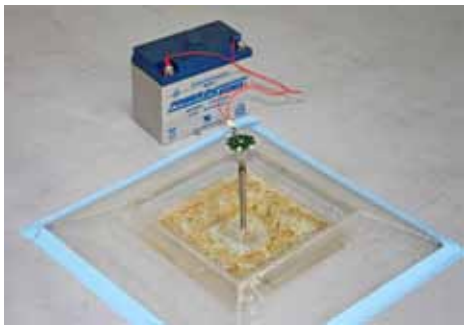
In addition to a pheromone dispenser, the pyramid-shaped trap sports three slender fins along each edge of its base to coax inquisitive beetles into crawling towards the opening of a central pit, where they can be captured, counted, and discarded. The new trap also features light-emitting diodes (LEDs), set to wavelengths of 390 nanometers. The LEDs attract beetles from long distances, ensuring that they get a whiff of pheromone the closer they approach. This can be especially useful in well-ventilated warehouse areas, where the ubiquitous odor of food can dilute or mask the scent of pheromone emanating from a trap.

"Once the beetles are close enough for a lure to be effective, there is a further need for a trap designed specifically to appeal to the tactile and behavioral traits of the targeted pests," write the inventors in a patent description of their new trap design. Peter Teal leads the ARS Chemistry Research Unit in Gainesville, Florida. Lee Cohnstaedt is in ARS's Arthropod-Borne Animal Diseases Research Unit in Manhattan, Kansas. Adrian Duehl and Richard Arbogast were both formerly with ARS in Gainesville.

In timed laboratory trials, red flour beetles visited LED traps set to 390 nanometers 16 times versus 2 to 5 times for traps set to other wavelengths. Positioning the LEDs at the trap's top captured more beetles (55 total) than placing the diodes at the bottom (12 captured). Combining the LEDs with an attractant made the pyramid design even more effective, capturing 70 beetles versus 4 using a standard dome design.

The red flour beetle, *Tribolium castaneum,* is a primary target of the team's research because it eats both raw and processed cereal grains. It commonly infests flour mills but can also be found in warehouses, storage bins, and household food pantries, causing millions of dollars in losses annually.

The pyramid trap's success in laboratory trials is a testament to the researchers' efforts to study and exploit the beetle's natural behaviors and tendencies—from identifying optimal wavelengths that cue it visually to creating a crawl space between the trap's lid and base that appeals to the furtive pest's instincts, luring it to enter and fall into the pit inside.



PETER TEAL (D3009-1)

Small hive beetles, which can weaken honey bee colonies, were also attracted to the trap. In tests, researchers observed a 10- to 20-fold increase in captures of hive beetles compared to conventional traps used against the pest. Changes to the trap's design and to the combination of attractants and LED wavelengths can also be made to effectively target fleas, ticks, bed bugs, and mosquitoes.—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.

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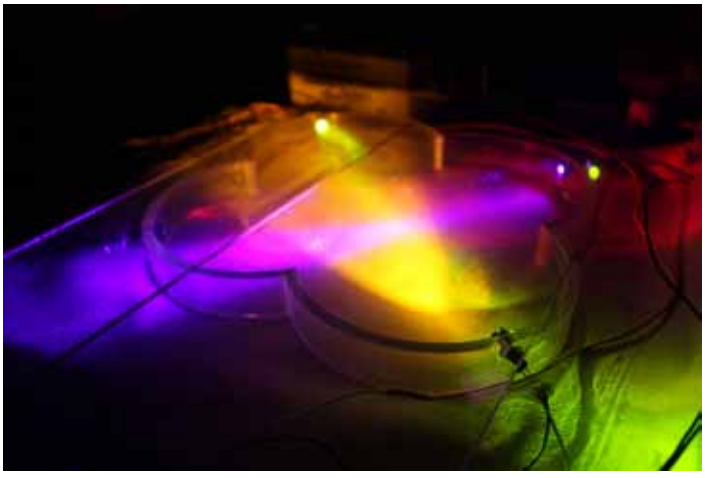
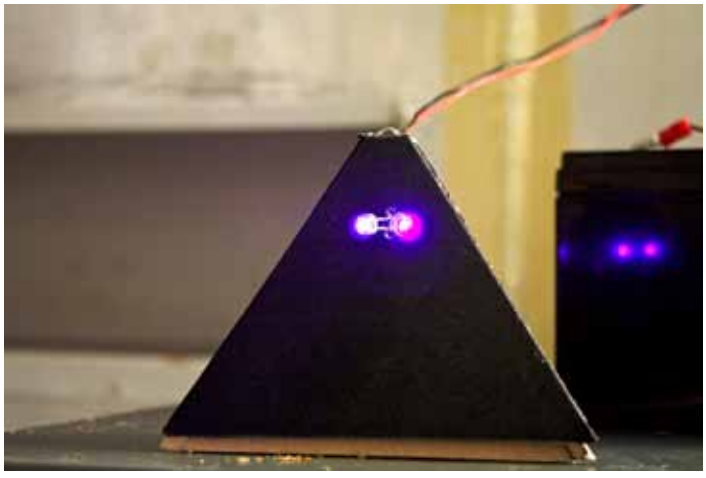
*Lee Cohnstaedt is in the USDA-ARS Arthropod-Borne Animal Diseases Research Unit, Center for Grain and Animal Health Research, 1515 College Ave., Manhattan, KS 66502; (785) 537-5592, lee.cohnstaedt@ars.usda.gov.**

Above: LED lights near the top of the new trap lure the pest. Bottom left: Different wavelengths and colors of light were tested to see which best attracted insects. Below right: The trap's exterior has an inner and outer layer with an opening at the bottom, where insects enter, crawl up between the layers, and fall into a trap at the top.



PETER TEAL (D3006-1)



PETER TEAL (D3008-1)

Models Suggest Canola Could Be New Biofuel Crop for the Central Great Plains

DAVID NIELSEN (D3014-1)

ARS scientists used data from this canola study in Akron, Colorado, to measure the crop's water use and yield and to validate a computer model to assess the potential profitability of future crops grown in Colorado.

Farmers in the semiarid central Great Plains have traditionally used a 2-year, no-till, dryland, winter wheat/fallow cropping system, which stretches scarce soil water supplies and minimizes crop failure from water stress. Now they are interested in growing crops during the fallow period, using water normally lost to evaporation.

To help assess production options, Agricultural Research Service agronomist David Nielsen is developing models that calculate the probability of raising profitable crops of spring canola, which produces seed oil that can be used to produce biodiesel. Nielsen works at the ARS Central Great Plains Research Station in Akron, Colorado.

Nielsen partnered with soil scientist Liwang Ma, research leader Laj Ahuja, and crop scientist Saseendran Anapalli, who all work at the ARS Agricultural Research Systems Unit in Fort Collins, Colorado. They began by adapting the CROPGRO computer model, which simulates plant growth processes under different weather, soil, and crop management conditions, to simulate spring canola production.

The team then added this CROPGRO canola cropping systems model to the Root Zone Water Quality Model 2 (RZWQM2), which was developed at Fort Collins. RZWQM2 simulates the effects of management on plant growth and the movement of water, nutrients, and chemicals within and around crop root zones. The researchers ran the combined model with 16 years of regional weather data, four different soil water levels at planting time, and other site-specific information to generate spring canola yield estimates for nine locations in Nebraska, Colorado, and Kansas.

The team found the simulated yields were consistent with regional precipitation and temperature patterns, factors that led to the highest average yields in the north-central area near Champion, Nebraska, and the lowest in the south-central area near Walsh, Colorado. When 75 percent of the soil water was available for crop use at planting, the model indicated that six of the sites had more than a 70-percent probability of producing a canola seed yield of at least 900 pounds per acre.

Using the average simulated yields at each location, the researchers ran an economic analysis that projected net returns. This analysis indicated that the most profitable location for canola production was Champion, with average net returns ranging from $67 to $189 per acre, depending on plant-available soil water levels. Simulations produced positive average net returns for five sites—Champion, Nebraska; Garden City, Kansas; McCook, Nebraska; Sidney, Nebraska; and Tribune, Kansas—even when only 25 percent of the soil water was available for crop use at planting.

Nielsen used these simulation results with Microsoft Excel to develop a simple decision-support tool for canola production and economic analysis.

"We need to carry out field studies," says Nielsen. "But farmers could use this decision-support tool to calculate the probability of producing the minimum spring canola yield they need to turn a profit—or they could use the seed oil they produce for their on-farm biodiesel production to run tractors and other equipment."—By **Ann Perry,** ARS.

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

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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

Albany, California
9 research units ■ 241 employees

Pullman, Washington
6 research units ■ 136 employees

Fort Collins, Colorado
7 research units ■ 141 employees

Akron, Colorado
1 research unit ■ 28 employees

Center for Grain and Animal Health Research, Manhattan, Kansas
5 research units ■ 125 employees

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

Southern Regional Research Center, New Orleans, Louisiana
7 research units ■ 171 employees

West Lafayette, Indiana
3 research units ■ 72 employees

Center for Medical, Agricultural, and Veterinary Entomology, Gainesville, Florida
4 research units ■ 134 employees

Geneva, New York
2 research units ■ 26 employees

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

Plum Island Animal Disease Center, New York
1 research unit ■ 31 employees

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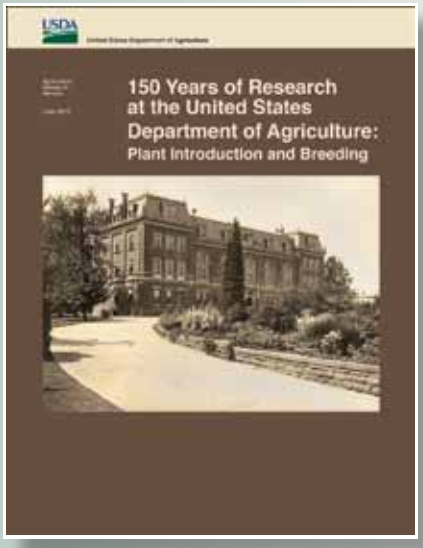
Spotlight On ...



150 Years of Research at the United States Department of Agriculture:

Plant Introduction and Breeding

One of the primary functions of the USDA when it was established in 1862 was "to procure, propagate, and distribute among the people new and valuable seeds and plants." This publication describes highlights from 150 years of the USDA's program on introducing and breeding new plants. Many of the "new" plants have now become standard crops, such as soybeans and blueberries. Click image for more information.