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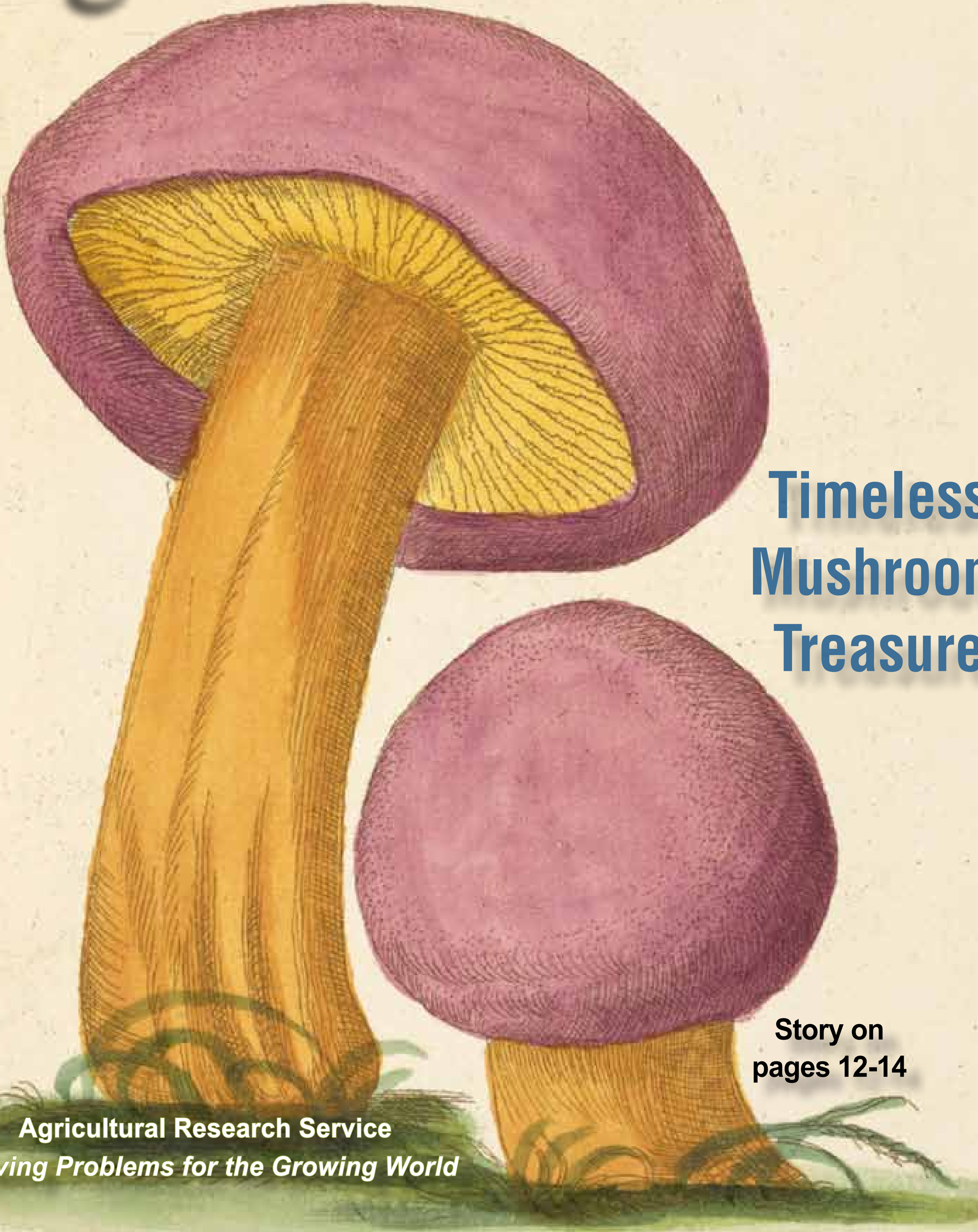

Timeless Mushroom Treasure

Story on pages 12-14

Agricultural Research Service

Solving Problems for the Growing World

FORUM

Breeding Rootstocks To Help Apple Growers

Today's apple growers face a host of challenges. Some challenges come from nature and some from our own changing tastes. Walk through any supermarket produce section or stop by any farmer's market and, depending on the season, you are likely to see a vast assortment of apple varieties. We want apples that not only ship and store well, but taste good when eaten fresh; baked into pies and pastries; or used in jellies, candies, or other products. This unprecedented variety is the result of decades of efforts by breeders, nurseries, and growers in response to changing consumer tastes and demands.

But such bounty comes at a price. Some of our most popular apple varieties are susceptible to a number of diseases and pathogens, which makes producing them a challenge. Gala, Honeycrisp, and Fuji are susceptible to fire blight, a bacterial disease that can kill a tree. Apple replant disease is found in orchard soils used to grow the same crop year after year. Many of the nation's most productive orchards have produced apples for decades, making the disease a serious problem. Many of the apple rootstocks traditionally used were bred in England decades ago and are not equipped to deal with today's demands.

Fortunately, a unique apple rootstock-breeding program in Geneva, New York, is helping growers meet these modern-day challenges. Agricultural Research Service researchers at the Plant Genetic Resources Unit in Geneva, along with their partners at Cornell University, have bundled resistance to several threats into new rootstock varieties. The accomplishments are described in this issue on page 4. To better understand the significance of the work, and the stakes involved, it helps to know more about how apples are produced.

Apples are a $3.1-billion-a-year industry in the United States. They are grown commercially in 29 states, and they rank second, behind oranges, as the nation's most consumed fruit, when fresh and processed uses are combined. Apple trees in commercial orchards, like other fruit trees, are bred in two parts: the fruit-bearing scion at the top and the rootstock that forms the foundation and roots. The dual approach makes the fruit and root systems easier to breed. The scion determines the variety of apple. The rootstock determines the tree's overall size, when it will first bear fruit, and its ability to resist many diseases. Rootstocks forage the soil for nutrients, which are critical to the tree's survival, health, and productivity.

Along with fire blight and replant disease, other major threats to apple producers include woolly apple aphids and winter cold; in some apple-producing areas, winter temperatures can dip to –20°F. But of all these, the most serious threat may be fire blight. The disease is bacterial, and once a tree is infected, no pesticide of any kind will help. Fire blight likes hot, humid conditions, and when it gets into a 2- to 4-year-old "juvenile" tree, it can kill it quickly. Unfortunately, some of the most popular varieties of apples, including many from Washington State, which produces half of the nation's apples, are susceptible to it. The best defense is to use resistant varieties and rootstocks.

Nurseries often buy rootstocks when they are about the size of a pencil, graft scions onto them, and grow the plants for a few seasons before selling them. Typically, orchard operators buy some new trees from nurseries every year, and over the course of about 20 to 25 years they gradually replace all of their trees. The grower's choice of rootstock is as critical as the apple variety produced. Sometimes growers are reluctant to switch rootstocks. They know that a rootstock that performs well in one type of soil may not be as successful in another. They are learning, however, that they can reduce their risks by using Geneva rootstocks. There are about 2.4 million new Geneva rootstock trees available to growers each year, but by some estimates, growers would use about 10 million if they were available.

Individual rootstock plants, however, are grown from mother plants, and it takes about 4 to 5 years to deliver quality rootstocks. While the nation's nurseries cannot replace all of their rootstock plants overnight, they are moving as quickly as possible to provide Geneva rootstocks to the growers who want them.

The development of apple rootstock in Geneva is a success story. The demand for it serves as testimony to the role played by ARS scientists in helping apple growers provide the nation with this tasty and nutritious fruit.

Sally M. Schneider
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Natural Resources and Sustainable Agricultural Systems
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Solving Problems for the Growing World

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ELIJAH TALAMAS (D3241-1)

A *Trissolcus euschisti* wasp (about 1.5 millimeters long) that has emerged from a stink bug egg. This wasp species is a promising biocontrol for brown marmorated stink bug. Story begins on page 18.

Cover: A watercolor titled "*Agaricus ferratis* (Agaric with Serrated Gills)," created by James Bolton, a noted naturalist who lived in England in the late 18th century. Bolton was the author and illustrator of the first published English-language work devoted to fungi. His manuscript of that work, including his original watercolors, is part of the Special Collections at the National Agricultural Library, in Beltsville, Maryland. Story begins on page 12. J. BOLTON WATERCOLOR (D3253-1)

Rooting Out the Best in Apple Rootstocks

Honeycrisp apple trees with Geneva rootstocks in a large nursery in Washington State. These trees will be shipped all over the United States for planting in large commercial orchards, small family farms, and backyards.

With 600 acres of apple trees in Walden, New York, Jeff Crist faces a number of threats from nature each year.

Fire blight, a bacterial disease that attacks blossoms, shoots, and limbs, has been known to kill off entire orchards. Replant disease, which reduces growth of new trees in soils that support the same type of tree year after year, can cause major losses. Aphids and cold temperatures can also cut into apple production.

"The challenges from a horticultural standpoint are always there. You just have to try to do everything possible to minimize them," says Crist, a fourth-generation farmer whose family has grown apples in the region for about 100 years.

Crist and other apple producers are better equipped to face those threats because of a one-of-a-kind program in Geneva, New York, operated by Agricultural Research Service scientists and Cornell University. The program, overseen by Gennaro Fazio of the ARS Plant Genetic Resources Unit in collaboration with Cornell scientists, produces new rootstock varieties that will play a major role in the continued success of a $3.1-billion-a-year apple industry in the United States. Apples rank second, just behind oranges, as the nation's most consumed fruit, when fresh and processed uses are combined.

Commercial apple growers routinely replace trees every 20 to 25 years, and this year, about 1 in 5 of the new trees shipped from nurseries to growers—some 2.4 million trees—is on Geneva rootstock. Rootstocks from Geneva are also being used in some European countries, as well as in Brazil, Chile, New Zealand, South Africa, and Uruguay. Geneva rootstocks are in high demand because they have proved to be effective at overcoming many of the obstacles growers face in producing a bountiful harvest.

"The Geneva program has made major contributions. It's given us new tools to combat fire blight, replant disease, woolly apple aphids, and winter cold, among other problems," says Phillip Baugher, who is chairman of the research committee for the U.S. Apple Association and president of Adams County [Pennsylvania] Nursery, which propagates 600,000 apple trees annually.

Two-Part Trees

Most commercial apple trees are bred in two parts: the fruit-bearing scion and the rootstock, which forms the foundation and roots. The two parts are completely different and address different needs, so the dual approach makes breeding easier. The scion determines the variety of apple.

A typical rootstock. Millions of trees of some of America's favorite apple varieties are grown on Geneva rootstocks.

SARAH BAUER (D3228-1)

Each tagged set of these Gala apples was grown on a different type of Geneva rootstock. The variation among the sets illustrates how the rootstock can affect fruit color and size and, ultimately, eating qualities.

The rootstock determines the tree's size, when it will begin to bear fruit, and how well it resists soil diseases. Rootstocks also transport nutrients from the soil to the scion, which is critical to the tree's survival, health, and productivity.

"Certain scions may do better with certain rootstocks. Figuring out the right pairings is part of the challenge for growers. They have to mix and match," Fazio says.

Breeding apple rootstocks is a time-consuming process. Each rootstock is field tested for about 10 years in different environments to evaluate its strengths and weaknesses in the climates and soils of the 29 states where commercial apple trees are grown. Along the way, many rootstocks fall prey to soil diseases or perform poorly when exposed to different climates.

One example shows the time sometimes required: Three rootstocks released by ARS and Cornell scientists in 2010 were based on crosses of trees done in 1976. The work, however, pays off. Since the 1970s, the Geneva breeding program has produced trees that are 10 to 20 percent more productive and are better at taking up certain nutrients from the soil.

Focus on Fire Blight, Early Bearing, and Dwarfing

Efforts start with a rigorous screening process at an ARS site based on the Cornell University campus in Geneva that includes apple rootstocks and trees from throughout the world. Seeds made from the hybridization of plants with promising characteristics are germinated in a greenhouse and inoculated with a series of pathogens. The pathogens usually kill 70 to 80 percent of the seedlings. Surviving rootstocks are then screened with DNA markers associated with aphid resistance and productivity. The remaining seedlings are then field screened in Geneva and sent

PEGGY GREB (K10813-1)

PEGGY GREB (K10810-1)

Commercial apple trees are usually produced by grafting a scion onto a rootstock. The scion determines the apple variety, and the rootstock determines tree size, disease resistance, and more. Far left: A section of the rootstock's trunk is chipped out where the scion will be attached to it. Near left: A completed graft.

PEGGY GREB (K10803-1)

PEGGY GREB (K10805-2)

Left: ARS geneticist Gennaro Fazio examines a limb of a Gala apple tree heavily damaged by fire blight. Above: Close-up of the leaf damage.

to nurseries and research sites across the country and overseas to be evaluated for cold tolerance, response to different soil conditions, and disease resistance.

One major achievement in the rootstock-breeding program has been advancing resistance to fire blight. Significant fire blight outbreaks in New York, Michigan, and Washington State have caused millions of dollars in annual losses over the years. It's also a concern in Europe and New Zealand. A fire blight epidemic in New York and Michigan in 2001 and 2002 wiped out entire orchards. In the Northwest, many growers refused to plant new orchards until their apples could be grafted onto resistant rootstock.

All rootstocks released from Geneva in recent years are tolerant or immune to fire blight. In the wake of the fire blight outbreaks, rootstocks from Geneva are proving instrumental in allowing apple producers to plant new varieties that are very fire blight susceptible, Fazio says. "Growers were leaving land fallow, waiting for our rootstocks to become available, and when the nurseries began propagating our releases, some fruit companies ordered tens of thousands of trees with our rootstocks," he says.

Researchers in Geneva have also been instrumental in developing trees with improved dwarfing and early-bearing characteristics, which are both critical traits to apple producers. Early bearing reduces the time required for a young tree to produce fruit. Early bearing is determined by several genes, which makes breeding for it extremely challenging. The new Geneva rootstocks, however, have reduced the time it takes for a traditional large tree to start bearing fruit from 5 years to 2 years, and they are fire blight resistant as well.

Dwarfing produces trees that grow to about 10 feet instead of 20 to 30 feet. The smaller trees take up less space, and that increases yield per acre. Commercial apple growers began to adopt dwarf trees in the 1930s, and since then, yield per acre has quadrupled. Dwarfing can also improve fruit quality, cut back on insecticide use, and reduce harvest-related injuries.

Like their full-size counterparts, dwarf trees are susceptible to a number of pests and diseases, so growers still face tough decisions about the types of rootstocks and scions to use in their orchards. With more trees per acre, the stakes are higher. A grower who may have once spent about $3,000 per acre for about 200 trees now buys 5 to 10 times as many trees and spends 5 times as much per acre.

"The density of the plantings has increased, so the amount of investment for every planting is that much greater, and every decision you make about planting becomes that much more important," Crist says.

Examining Rootstocks' Role in Nutrient Uptake

Researchers in Geneva have also focused on finding ways to improve the rootstock's ability to take up soil nutrients, which is a key to tree health and productivity. Improved nutrient uptake could also increase the nutrient content of the fruit and reduce the need for fertilizer, which would reduce nutrient runoff.

In one study, Fazio and his colleagues measured nutrients in the leaves of Gala and Golden Delicious apple trees grafted to different sets of rootstocks. They analyzed the DNA of the rootstocks to search for genes associated with enhanced uptake of specific nutrients, such as calcium and phosphorus. Calcium deficiencies cause postharvest disorders, and phosphorus is a commonly applied fertilizer.

"If two rootstocks are transporting different concentrations of the same nutrients, one may have more efficient nutrient uptake or transport genes than the other, and identifying those genes will help with selection of more efficient, improved rootstocks in the future," Fazio says. Results, published in 2013 in *Aspects of Applied Biology,* showed that certain genes are likely to enhance the uptake of specific nutrients, including calcium and phosphorus.

In another study, Fazio and his colleagues analyzed genetic markers in two populations of rootstocks to see whether genes associated with certain markers play a role in dwarfing, early bearing, and fruit productivity. The rootstocks they analyzed had been studied and characterized at Geneva for up to 7 years. They discovered the location of a new genetic marker *(Dw2)* and described its relationship to a previously discovered marker *(Dw1),* shedding light on how genes associated with both markers and their locations affect those key characteristics. The results were published in 2014 in the *Journal of the American Society for Horticultural Science*.

Researchers at Geneva are aware that the stakes are high for growers and others interested in quality apples. "Our goal is to give growers more choices, so that they have healthier trees, increased productivity, more nutritious fruit, and hopefully a future with reduced use of fertilizer and the nutrient runoff that goes with that," Fazio 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.

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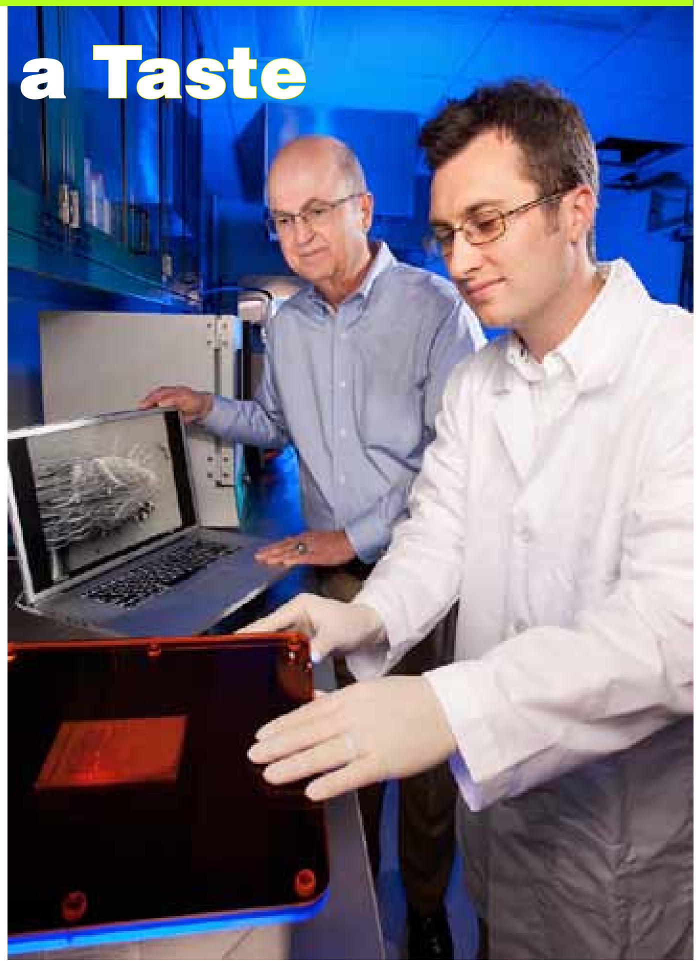
Mosquitoes Reveal a Taste for Repellents

PEGGY GREB (D3239-1)



Entomologist Joseph Dickens (left) and postdoctoral associate Jackson Sparks use a gel to separate genes in the mouthparts of the yellowfever mosquito that are involved in detection of DEET.

The more scientists learn about mosquitoes' sense of smell and taste, the better they're able to develop ways to protect us against mosquito bites, which can sometimes transmit dangerous pathogens that cause diseases such as malaria, yellow fever, and West Nile virus.

Previous research has shown that DEET, the most common active ingredient in insect repellents, interacts with the mosquito's sense-of-smell (olfactory) receptors, located on its antennae and mouthparts. These receptors pick up carbon dioxide and other odors emitted by humans and animals, leading the mosquito to a host.

Recently, Agricultural Research Service scientists in Beltsville, Maryland, showed that insect repellents also affect the mosquito's sense-of-taste (gustatory) receptors. Entomologist Joseph Dickens and his colleagues in the Invasive Insect Biocontrol and Behavior Laboratory discovered that a taste receptor located on a mosquito mouthpart is sensitive to DEET.

"We know that DEET deters blood feeding by interactions with olfactory receptor cells," Dickens says. "Mosquitoes become confused and fly away. The fact that DEET is also interacting with a specific taste receptor might indicate that there is a sensory pathway for taste that helps inhibit feeding."

After discovering that certain nerve cells respond to DEET and other repellents, scientists looked for the receptors responsible for this reaction. Experiments involved recording responses of yellowfever mosquitoes to different insect repellents—DEET, IR3535, picaridin, and citronellal—and a feeding deterrent, quinine.

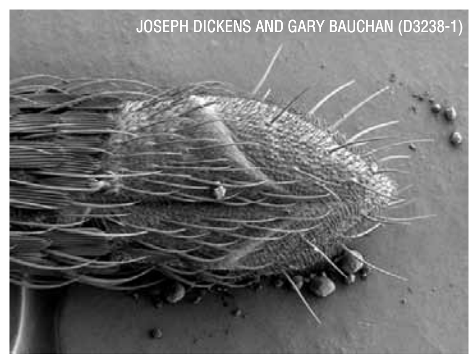
At the tip of the mosquito's "beak"—or proboscis—is a pair of flaps called "labella" that make the first contact with a person's skin. These flaps have tiny hairs that serve as chemical-sensing organs. Electrodes were placed over a single hair to record electrical impulses from nerve cells within the hair.

"We found that at least three sensory cells were activated in the hair," Dickens says. "One was sensitive to increasing concentrations of salt—sodium chloride. Another one was sensitive to increasing concentrations of sugar, and another cell was responsive to DEET and quinine."

Dickens and postdoctoral research associate Jackson Sparks are working to distinguish other receptors located on the mouthparts and legs of mosquitoes and determine their molecular genetic functions. "We're getting an idea of the specific receptors at a gene level involved in the response to DEET," Dickens says, "and we have candidate genes that we're going to examine."

The research, which was published in *Naturwissenschaften* in February 2013, has given scientists a better understanding of how mosquito repellents work, and it could help provide methods to uncover novel and improved chemicals to better manage insects and other arthropods.

"DEET works, but it's only effective at high concentrations," Dickens says. "Products to keep mosquitoes from biting generally have 20 percent DEET. Some even contain 50 percent or more. We're trying to discover alternatives that are many times more active than DEET so that less chemical has to be applied to the skin."—By **Sandra Avant,** ARS.



JOSEPH DICKENS AND GARY BAUCHAN (D3238-1)

Sensory hairs at the tip of the proboscis, or beak, of the yellowfever mosquito have cells that respond to a feeding deterrent and the mosquito repellent DEET. Magnified about 500x.

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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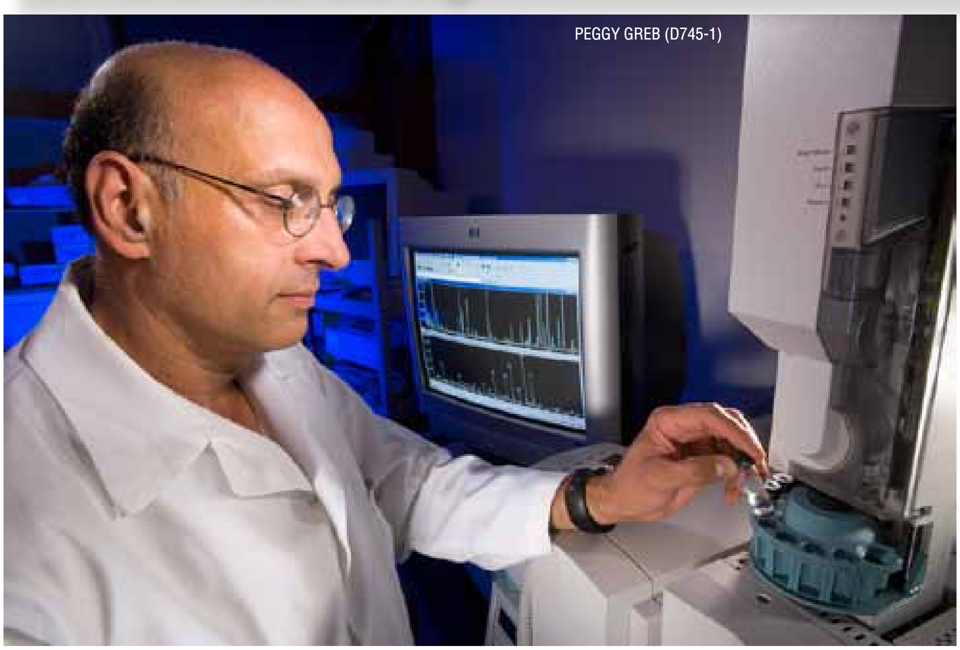
WOLFGANG HOFFMANN (D764-1)

Switchgrass for Ethanol

Where It Came From—and Where It's Going



Top: Geneticist Michael Casler harvests switchgrass seed in a program to develop high-yielding new cultivars that are well suited to bioenergy production in different environments. Bottom: Molecular biologist Gautam Sarath analyzes switchgrass cell-wall samples for their lignin content. The data will be used to identify elite switchgrass plants for improvement through breeding.

Switchgrass was originally valued as a forage crop, but its more recent claim to fame has been as a potential feedstock for bioenergy production. Now, after decades of combined research, Agricultural Research Service scientists have announced the release of Liberty, a variety of switchgrass specifically developed for bioenergy generation.

Says ARS geneticist Ken Vogel, who recently retired from the ARS Grain, Forage, and Bioenergy Research Unit in Lincoln, Nebraska, "With Liberty, we were able to combine the high yields of the southern lowland varieties with the winter hardiness of the northern upland types. Right now, Liberty can yield 8 tons of biomass per acre, and with further breeding, we have the potential to get to 10 tons per acre—maybe as soon as 5 years from now."

The release of Liberty is a significant milestone for ARS. It's also a key accomplishment for CenUSA Bioenergy, a project funded by the U.S. Department of Agriculture's National Institute for Food and Agriculture that is tasked with developing perennial bioenergy production systems in the Midwest. Other ARS researchers who contributed to Liberty's development include agronomist Rob Mitchell, molecular biologist Gautam Sarath, and geneticist Michael Casler. Mitchell and Sarath both work at the ARS laboratory in Lincoln, and Casler works at the U.S. Dairy Forage Research Center in Madison, Wisconsin. Casler, Mitchell, and Vogel have all played significant leadership roles in CenUSA Bioenergy.

Another Name for Success

Liberty made a previous appearance as "KxSNETO2," one of the switchgrass types used in a 16-year breeding study by Vogel and others to increase long-term yields. In the study, breeding for increased biomass yield in upland switchgrass resulted in average gains of 4 percent every year, while biomass yields in lowland types increased an average of 1 percent every year. However, the lowland-upland hybrid KxSNETO2 increased biomass production as much as 43 percent.

Their results convinced the researchers that switchgrass biomass yield is a moderately heritable trait that can be readily improved using conventional breeding methods. However, they did not observe any genetic links between biomass yield and quality. This suggested that breeders might be able to combine different traits to mitigate production challenges posed by climate change, weather, and pests.

Another plus with the new cultivar is that gains in yield were achieved without an increase in nitrogen fertilizer use, which helped lower expected farm-gate production costs by around $20 to $30 per ton. And with the increased yields, each hectare of switchgrass could potentially be used to produce from 700 to 1,500 more liters of ethanol.

PEGGY GREB (D748-1)

In Nebraska, technician Marty Schmer harvests switchgrass to evaluate yield. The results will help guide breeding and management efforts to increase switchgrass yields.

Teamwork and Time

It took a multi-location effort to get to this point. For years, Casler and others have been studying the evolutionary history of switchgrass, which now flourishes in a range of North American environments. Lowland ecotypes are found on flood plains, wetlands, and other low-lying areas, while upland ecotypes are found in flood-free areas subject to frequent droughts.

Since switchgrass has successfully adapted to these different environments, its genome contains a diverse array of traits, many of which could be useful in breeding efforts. But switchgrass types can have as many as eight sets of chromosomes in each cell, so natural crossbreeding over thousands of years has produced some varieties that contain an unwieldy genetic mix.

To identify evolutionary patterns in the complex genome, Casler and his colleagues examined the genetic makeup of 480 individual plants from 67 lines and cultivars, including upland, lowland, and hybrid types. Using advanced genetic techniques, the team identified several distinct switchgrass "clades" (groups of organisms descended from a common ancestor) in North America. The researchers located two lowland clades in the eastern Gulf Coast and southern Great Plains and three upland clades in the central Great Plains, northern Great Plains, and eastern savanna.

The team determined that despite switchgrass's renown as a prairie dweller, the primary center of origin for modern switchgrass was along the eastern Gulf Coast between 1 and 1.5 million years ago. A less significant center of origin and diversity was located along the western Gulf Coast. Meanwhile, a western mountain ecotype became the source of upland ecotypes that migrated to the arid and semiarid Great Plains, which then became a secondary—not the primary—center of switchgrass diversity.

The scientists surmise that after these types were established, repeated rounds of North American glaciations affected switchgrass dispersal and development by forcing diverse types into relatively close proximity. By increasing the opportunity for different types of switchgrass to mate, the glacial intervals created favorable conditions for much of the gene flow between upland and lowland ecotypes.

These "crossing cages" are used to make up to 100 switchgrass hybrid crosses per year in the breeding program at Madison, Wisconsin.

MICHAEL CASLER (D3242-1)

PEGGY GREB (D2614-1)

Geneticist Ken Vogel (left) and molecular biologist Gautam Sarath compare switchgrass plants produced by mating plants from upland and lowland ecotypes with parent plants.

Conversely, as the plants migrated northward, the opportunities for genetic mixing declined, and upland types adapted to frost and longer days and started flowering earlier. Meanwhile, lowland varieties remained relatively intolerant of cold and continued to flower much later in the summer. Many “hot spots” of genetic diversity still thrive in the southeastern United States and along the Atlantic Seaboard.

After sorting through their results, the scientists identified eight regional gene pools with traits that might be beneficial in breeding switchgrass varieties for different production environments. Results from these studies have been published in *Genetica* and *Crop Science*.

Room for Improvement

Vogel and Casler also evaluated how previous switchgrass breeding for in vitro dry matter digestibility (IVDMD) altered biomass composition, forage quality, and potential ethanol yield. IVDMD is a laboratory test that mimics the rumen processes of cattle to measure forage digestibility. It is a good indicator of ethanol yield from switchgrass via a process known as “simultaneous saccharification and fermentation,” which researchers hope will become a cost-effective method for extracting and fermenting plant sugars into ethanol.

The researchers used six breeding generations of switchgrass in their study. The first four generations had only been bred to improve IVDMD, while the last two generations had been bred to improve winter survival as well.

The analysis showed that the six types had notable differences in a range of variables linked to forage quality and ethanol yield traits. The study also showed that forage digestibility is affected by many traits that could be improved with breeding.

For instance, breeding solely for increased IVDMD initially lowered forage and biomass yields, but yield levels stayed the same after the scientists began improving the plant’s winter-survival characteristics. And while the first several rounds of breeding increased biomass digestibility and ethanol yields, both characteristics declined in the improved varieties when breeding selection began for winter survival.

Researchers often focus on modifying tough cell-wall lignin characteristics to improve the conversion of plant biomass to liquid fuel. However, as a result of their study, the ARS scientists determined that many other cell-wall and cell constituents influence this process.

In addition, the scientists demonstrated that breeding to improve IVDMD significantly changed the concentration of almost all the cell-wall components. These changes altered the way carbon was distributed between lignin and carbohydrates throughout the biomass, but did not notably alter total overall biomass carbon concentrations.

The researchers were surprised to find that breeding for winter survival in later generations led to decreased IVDMD, which indicated a clear association between the two traits. They believe that selecting for IVDMD might result in plant vascular structural changes that reduce winter survival.

The team concluded that breeding to improve IVDMD also affects plant characteristics that are important for ethanol production. The results confirmed that traits affecting biomass composition can also affect plant hardiness—information that plant breeders can use to guide their work in developing switchgrass varieties for forage or for bioenergy. Results from these studies were published in *Crop Science* in 2013.

Mitchell and Sarath, who have been working with Vogel for over a decade, will continue the switchgrass studies at Lincoln and the collaborations with Casler and other ARS scientists. Meanwhile, Vogel is pleased that with the release of Liberty, the plant he began studying so long ago is finally living up to its promise. “It’s nice to retire on a high note,” he says.—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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Starting With the Seeds

For a plant that can survive the challenges of a prairie environment, switchgrass seeds can be surprisingly delicate. Fewer than 50 percent of switchgrass seeds planted in drilled plots germinate and emerge as seedlings, and more than 90 percent of those seedlings will die within a year. But the competitive environment of the prairie provides a real-world laboratory that fosters development of genetic traits that favor survivorship, such as high rates of root growth or shoot growth.

ARS geneticist Michael Casler analyzed 5-year survivorship rates of switchgrass varieties planted at four locations in Illinois and Wisconsin. Although he concluded that most survivorship is random, he determined that survivors at the northernmost location were hardier, with better value for breeding, and their progeny displayed better survivorship and better yields.

“From an agronomic and breeding point of view, we want to know if survivorship has a positive genetic correlation with any important agronomic traits,” Casler says. “From a practical standpoint, we can use the survivors to support our breeding programs.”

Results from these studies were published in *Crop Science* in 2013.

KEITH WELLER (K3627-16)

Developing Tools To Fight Marek's Disease

ARS scientists are developing new tests and vaccines that are making it easier to detect viruses in chickens and protect them from the cancer-like diseases some of them cause.

New tests and vaccines are making it easier to detect viruses in chickens and protect them from the cancer-like diseases some of them cause.

One of the latest tools developed by scientists at the Agricultural Research Service's Avian Disease and Oncology Laboratory (ADOL) in East Lansing, Michigan, is a modified polymerase chain reaction (PCR) test to detect unique genetic sequences of both Marek's disease and avian reticuloendotheliosis viruses in various tissues from chickens.

Marek's disease is caused by a highly contagious herpesvirus that primarily affects young adult chickens. It causes severe production losses and usually death and is spread from bird to bird by contaminated dust and dander in chicken houses. Avian reticuloendotheliosis, a retrovirus, infects a broader host range—chickens, turkeys, ducks, geese, and quail. It can also cause tumors, production losses, and death in affected birds.

Diagnosis of these diseases includes taking tissue from the bird's organs, preserving it in formalin, and examining it under a microscope.

"If a definitive diagnosis cannot be reached by gross and microscopic examinations, additional tissue must be obtained from affected chickens and sent to the laboratory for PCR or other tests," says Aly Fadly, ADOL research leader. "These tests, called 'immunohistochemistry' and 'virus isolation,' require use of fresh or frozen tissue."

Most, if not all, laboratories are equipped to preserve tissues in formalin and to process them further for microscopic examinations, Fadly adds. Now, the modified PCR allows any laboratory to extract virus DNA from preserved tissues to detect Marek's disease and reticuloendotheliosis.

"This simple test offers poultry breeders, growers, and diagnosticians an effective alternative to current cumbersome biological and molecular tests that require use of frozen or fresh tissues," he adds. "It also lowers costs."

To help prevent the spread of Marek's and other avian diseases, commercial flocks are routinely vaccinated. "However, we are seeing the emergence of more virulent viral strains of Marek's disease virus," Fadly says. "Therefore, there is a critical need for improved vaccines to protect birds against highly virulent virus strains."

While working at ADOL, former ARS scientist Sanjay Reddy developed a novel Marek's vaccine called "CVRM2." He used state-of-the art molecular technology to generate CVRM2 from the best available commercial vaccine—CVI988—and a "promoter." A promoter is a region of DNA that initiates gene transcription—the process by which genetic information is copied from DNA to RNA. The RNA sequences are then translated into amino acids to form specific proteins, and these proteins stimulate the immune system of the vaccinated host.

In laboratory and field trials, the new vaccine was as effective as or superior to the commercial vaccine in protecting chickens against highly virulent Marek's disease viruses.

ARS recently approved a license agreement with a private company to develop a commercial Marek's disease vaccine from CVRM2.

Results of both the vaccine and modified PCR studies were published in *Avian Diseases* in 2013. —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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Timeless Works of Fungi

Mushroom hunters—and their more scientific colleagues, the mycologists—are accustomed to seeking their prizes in unusual places. But finding mushroom treasures inside the National Agricultural Library (NAL) is a unique encounter even for them.

For preserved in NAL's Special Collections is a truly irreplaceable specimen: *Icones Fungorum circa Halifax Sponte Nascentium,* James Bolton's original, hand-drawn manuscript of his highly regarded published work, *An History of Fungusses, Growing about Halifax*.

The manuscript is 6 volumes with 242 exquisite watercolors of fungi, mostly life-size, with extravagant detail about where Bolton collected the specimen and what he discovered of its biology.

PHOTO USED WITH PERMISSION FROM DAN MOLTER (CREATIVE COMMONS 3.0 LICENSED)

J. BOLTON WATERCOLOR (D3254-1)

James Bolton, who had no formal training in taxonomy or art, drew so accurately that, for the first time, mycologists in different countries could be sure they were talking about the same mushroom, making his book an essential reference until cameras became practical, about 100 years later.

Left: Bolton's watercolor of *Agaric violaceus,* drawn about 1784. Above: The same mushroom species, photographed in 2008.

A self-taught naturalist and artist, Bolton came from a family of weavers in Yorkshire, England. His original patron, Margaret Cavendish Bentinck, Dowager Duchess of Portland, had the largest natural history collection in Great Britain. After her death in 1785, Henry Noel, Earl of Gainsborough, to whom the manuscript is dedicated, supported Bolton's work.

Bolton is credited with describing hundreds of fungi, many new species or new British records, although the number was later reduced by taxonomic reclassifications.

When published, in 1788, *An History of Fungusses* established Bolton's place in mycological history. Because of the exactness of his depictions, it became a standard resource for mushroom identification. It is as regularly quoted today as it was by Elias Magnus Fries, the founding father of mushroom taxonomy, in the 1800s, according to Royal Botanic Garden Edinburgh head of mycology (retired) Roy Watling. The late 1700s and early 1800s were dynamic times for mycology, with experts contending in identifying new species and expanding and resolving taxonomic order.

Bolton "was criticized as being both a 'lumper' and a 'splitter,' but we now think of him as being mainly the latter. He designated *Bolbitius titubans* and *B. vitellinus* as two separate species and recognized all five different species of *Armillaria* (honey fungus), when these had been put together by the rest of the early mycologists. The ultimate tribute to his outstanding talents is that his ideas have been confirmed by modern mycology," Watling says.

The published version of Bolton's manuscript fills just 4 volumes, each nearly the same dimensions as the manuscript, with 182 plates, 40 of them grouped with more than 1 illustration to a plate. But what NAL Special Collections has that is unique is Bolton's original manuscript.

According to a 1932 *Transactions of the British Mycological Society* article written by famed U.S. Department of Agriculture plant pathologist and mycologist Cornelius Lott Shear, NAL purchased the manuscript for 1,000 Swiss francs from "an old bookseller in Zurich, Switzerland."

The published edition contains all of the illustrations that are in the manuscript, plus a few additional. In the manuscript, the watercolors are arranged in the order in which the fungi were collected and drawn. But when the watercolors were transferred to copper plates for printing, they were rearranged and renumbered, according to Shear. Bolton wrote, in the manuscript notes, that he made some of the drawings directly on the copper plates from fresh specimens for the published illustrations.

J. BOLTON WATERCOLOR (D3255-1)

Blue agaric *(Agaricus caeruleus).*

J. BOLTON WATERCOLOR (D3256-1)

Cherry colored agaric *(Agaricus aurentius).*

J. BOLTON WATERCOLOR (D3257-1)

Tough boletus *(Boletus tenax).*

Since Shear wrote of it in the third person, he probably was not the manuscript's purchaser. But NAL has not yet located the records of how it obtained the manuscript or what brought it to the library's attention in 1932.

Some think it could have been John Stevenson who discovered the Bolton manuscript or advocated for its purchase. Stevenson was in charge of the USDA National Fungus Collections from 1927 to 1960 at the Agricultural Research Service's Beltsville [Maryland] Agricultural Research Center.

Amy Rossman, who recently retired as research leader of the ARS Systematic Mycology and Microbiology Laboratory in Beltsville, which now administers the fungus collections, concurs with the Stevenson possibility.

"Stevenson was a renowned collector who amassed a wonderful library about

NAL has not yet located the records of how it obtained the manuscript or what brought it to the library's attention in 1932.

all things mycology," Rossman says. "He apparently even spent a lot of his own money for what he considered important."

Just before Rossman retired, a large cache of historical papers from her lab, including many from Shear and Stevenson, was turned over to NAL for proper storage and to provide access to researchers, says Susan Fugate, head of NAL Special Collections.

"As we organize the materials, we will be keeping a special eye out for any that mention the Bolton manuscript and how NAL came to acquire it," Fugate says.

"As we organize the materials, we will be keeping a special eye out for any that mention the Bolton manuscript and how NAL came to acquire it."
—Susan Fugate, NAL

Only in the Manuscript

The manuscript has significance far beyond its prized historical value. One unquestionable extra is that its pages preserve three actual specimens of fungi. Except for a specimen preserved at Kew Royal Botanic Gardens, and a few recently discovered in a brown paper packet at the Sunderland Museum, these are the only fungi collected by Bolton known to have survived.

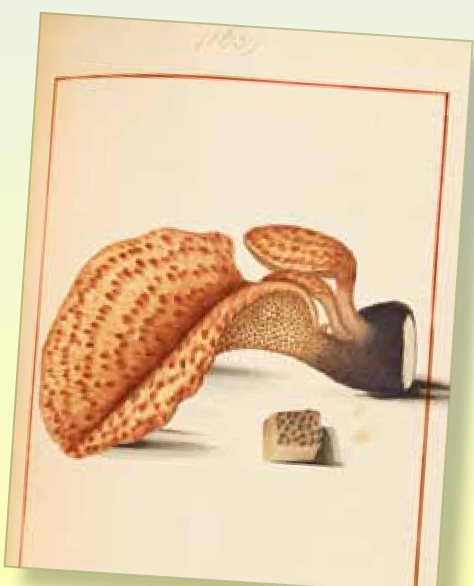
The description opposite each drawing in the Bolton manuscript "often makes fascinating reading, and frequently provides more detailed information than the rather brief comments found in the printed book," wrote John Edmondson in a memoir that accompanied an exhibition he organized on Bolton's work at the Liverpool Museum in 1995. NAL loaned the manuscript to the museum for the show, possibly the first time it had been publicly displayed since 1932.

Edmondson also raised the question of whether this is truly the "original" manuscript, as Shear claims. Edmondson holds that its watercolors are copies prepared from earlier drawings, and the published plates were based on those earlier drawings.

According to Shear, the last mention of the originals before 1932 was in *The Halifax Naturalist* in 1902 that said, "it is doubtful whether the originals for the *History of Fungusses* are still in existence. They were probably destroyed by fire when the old hall at Exeton [the Earl of Gainsborough's family seat] was burned in 1810."

Shear said an appeal to the Zurich bookseller for information about the manuscript's missing background brought only that it had been bought at a sale. But those who possessed it during the 141 years between the final volume's publication and its 1932 purchase must have cared for the manuscript well, as it is in excellent condition.—By **J. Kim Kaplan,** ARS.

Susan H. Fugate is head of Special Collections, USDA-ARS National Agricultural Library, 10301 Baltimore Ave., Beltsville, MD 20705; (301) 504-5876, susan.fugate@ars.usda.gov. *



J. BOLTON WATERCOLOR (D3260-1)

Scaly boletus *(Boletus squamosus).*

J. BOLTON WATERCOLOR (D3259-1)

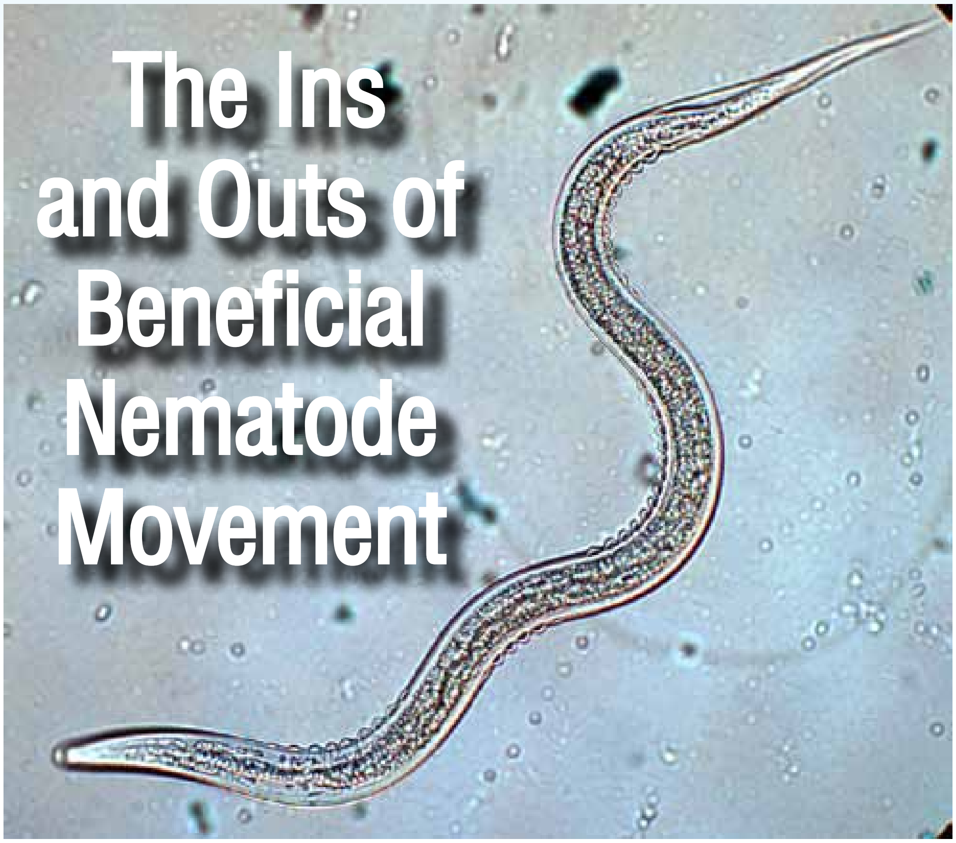
Bundled agaric *(Agaricus fascicularis).*

J. BOLTON WATERCOLOR (D3258-1)

Lacerating agaric *(Agaricus lacerates).*

The Ins and Outs of Beneficial Nematode Movement

JUAN MORALES-RAMOS (D3232-1)



ARS scientists are studying how small roundworms, such as this *Heterorhabditis indica* nematode, navigate through soil to find insect pests to attack.

Beneficial nematodes are small roundworms that can be used as environmentally friendly biopesticides. Agricultural Research Service scientists are interested in finding out how these tiny worms navigate through soil over vast distances to find insect pests to attack.

Nematodes are known to respond directionally to various cues, including electrical stimuli. In prior research, ARS entomologist David Shapiro-Ilan, plant pathologist Clive Bock, and collaborators found that the nematode *Steinernema carpocapsae* was attracted to an electrical current they applied to an agar dish. From this study, the researchers concluded that the worms rely on electricity, or electrical fields, to help them navigate in the soil. They then hypothesized that the nematodes may also use magnetic fields for the same purpose.

To test their hypothesis, Shapiro-Ilan and Bock, both with the ARS Fruit and Tree Nut Research Laboratory in Byron, Georgia, placed magnets on opposing sides of a petri dish containing agar and *S. carpocapsae* nematodes. One magnet was oriented toward the North Pole and the other magnet was oriented to the South Pole, creating a magnetic field of 0.1752 Tesla. (For perspective, a medical MRI uses a magnetic field of 1.5 to 3 Tesla.) The scientists wanted to see whether the nematodes would move directionally—either north or south—within the magnetic field.

"We did note a directional response of the nematodes, with more of them moving toward the South Pole than the North," says Shapiro-Ilan. "Magnetoreception can be important in facilitating or enhancing foraging ability in various organisms."

The finding that the nematode responds not only to electrical fields but also to magnetic fields was published in the *International Journal for Parasitology* in August 2013. The researchers believe this study was the first report of nematode directional movement in response to a magnetic field.

Next, Shapiro-Ilan and colleagues looked at the movement of six different entomopathogenic (insect-killing) nematodes and found that their movement was not random. Instead, the worms moved together as a group. "One might liken their movement to group behavior in other animals, such as a school of fish or a pack of wolves," says Shapiro-Ilan.

In the laboratory, tests were set up to observe nematode group movement in a wet soil environment. "In 20 of 24 analyses, nematodes demonstrated nonrandom, or coordinated, movement," says Shapiro Ilan. "Based on our findings, we contend that aggregated movement behavior may further contribute to a patchy distribution, or clumping, of natural or applied entomopathogenic nematode populations that is seen in crop fields."

These findings were published in the *International Journal for Parasitology* in October 2013.

Shapiro-Ilan and Bock acknowledge the contributions of James Campbell (ARS in Manhattan, Kansas), Daniel Kim-Shapiro (Wake Forest University), Teva Ilan (a student who worked in Bock's laboratory), Ed Lewis (University of California-Davis), and Paul Schliekelman (University of Georgia).

The findings of these studies have implications for understanding nematode foraging behavior and improving natural pest-control tactics. Knowledge of how and why beneficial nematodes find their prey is essential to optimizing their use in the future.—By **Sharon Durham,** 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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JERRY HEITSCHMIDT (D3210-1)

Moisture Meter Technology for In-Shell Peanuts Licensed

ARS engineers Samir Trabelsi (left) and Stuart O. Nelson (retired) test a sample of peanuts for moisture content using the new moisture-sensing meter they invented in Athens, Georgia.

A new meter developed by Agricultural Research Service scientists to measure the moisture of peanuts inside the shell, or pod, has been licensed by a manufacturer of agricultural-use instruments. The moisture-sensing meter was invented by engineers Samir Trabelsi and Stuart O. Nelson (retired) in the ARS Quality and Safety Assessment Research Unit, in Athens, Georgia.

"It is important that peanuts are dried to a kernel moisture content of less than 10.5 percent for storage purposes, because higher levels can lead to fungal growth," says Trabelsi.

The meter is based on patented low-power microwave sensing technology and an algorithm that produces a crop-specific moisture calibration equation—also developed by Trabelsi. The equation is used to customize an individual meter for use with a specific crop type.

The components of the microwave meter include a peanut-sample holder, a power source, a "mixer," and two antennas facing one another. The mixer compares the microwave signal transmitted by the first antenna into the peanut pods with the microwave signal received by the other antenna, after the signal has passed through the sample material.

"The microwave circuit measures the loss of energy and the change in the speed, or velocity, of the microwaves as they pass through the pods," says Trabelsi.

When a sample material is exposed to microwaves, part of the wave is transmitted and part is reflected, or not transmitted, providing the individual "electrical signature" of the sample material being tested. "Our patented calibration method uses this information to produce a moisture calibration equation that is programmed into the meter," says Trabelsi.

The Road to Market

Drying is an essential task that takes place at farms and at local peanut "buying points" right before the grading process. U.S. peanuts are required by the U.S. Department of Agriculture to be inspected at these buying points, and farmers take their peanuts there to be weighed, cleaned, inspected, graded, and ultimately purchased. Local buying points are under contract to peanut product manufacturers and shelling plants, where further grading takes place.

During peanut grading, inspectors determine quality factors such as peanut size, shell size, peanut damage, foreign-material content, and kernel-moisture content.

Representative samples of harvested peanuts are taken from the farmers' transport vehicles, and the peanuts must be removed from their shells before moisture content is determined. If the kernel moisture content is too high, the sample is marked "no sale," and the corresponding lot of peanuts has to be taken to the drying shed for further drying.

"We have been exhibiting prototypes of this technology to farmers and inspectors in various states for a few years," says Trabelsi. "This new meter is groundbreaking in terms of changing the way moisture has always been measured in peanuts and in terms of simplifying the process and reducing costs and labor."

During 2012, Trabelsi worked under an agreement with Dickey-John Corporation, based in Auburn, Illinois, a wholly owned subsidiary of TSI Incorporated, based in Shoreview, Minnesota, to build a work-

JERRY HEITSCHMIDT (D3211-1)

Above: Peanuts are normally dried to a moisture content of less than 10.5 percent for storage. Higher levels of moisture can lead to fungal growth (left) compared to properly dried, fungus-free peanuts (right). Below right: Postdoctoral engineer Micah Lewis visually inspects peanuts for quality factors before checking the peanut moisture content using the meter behind him.

JERRY HEITSCHMIDT (D3225-1)

ing prototype of the latest version of the patented in-shell peanut moisture sensor.

"The prototype, together with our marketing research, allowed us to evaluate the accuracy and effectiveness of the sensor and get a better sense of what we needed to do to bring the meter to commercial markets," says Beau Farmer, chief technology officer with TSI.

The technology allows government inspectors to skip the labor-intensive step of shelling peanut samples from lots prior to measuring moisture content. "A version based on the same technology could also be developed for farmers who want to test their peanut pods before sending them off for grading," says Farmer.

In addition to moisture content, the instrument can also reveal bulk density of the peanut pods. This is important because that measurement reveals the size and quality attributes of the peanuts inside the shell. "A higher density may indicate a higher peanut meat content," says Trabelsi.

Other Potential Uses

"The first generation of meters available commercially will be used to measure peanut moisture in cleaned peanut pods," says Trabelsi. But the researchers have also been working for several years on developing a sensor system that rapidly and accurately measures moisture content and density of cereal grains, oilseeds, and tree nuts for use on production lines and in processing applications.

Trabelsi's microwave moisture-sensing technology can be used to measure moisture in harvested grain, although the calibration equation will be different for cereal grains than for peanuts. The method can perform better than existing technologies and has the potential to benefit both the peanut and grain industries, according to Trabelsi and Farmer.

"The microwave moisture-sensing technology is a revolutionary application for in-shell peanut testing," says Farmer. "We wanted to develop a portable system for moisture measurements of unshelled or shelled peanuts that can be used at peanut-grading stations and possibly in the farmer's field. We are working hard on the underlying commercialization effort required to roll out these meters and satisfy market demand."—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.

*Samir Trabelsi is in the USDA-ARS Quality and Safety Assessment Research Unit, Richard B. Russell Agricultural Research Center, 950 College Station Rd., Athens, GA 30605; (706) 546-3157, samir.trabelsi@ars.usda.gov.**

Up Close and Personal With Tiny, Beneficial Wasps

Highly detailed image of the wasp *Trissolcus japonicus,* a primary candidate for biological control of brown marmorated stink bug. Here, the wasp appears to be huge, but it is actually only 1-2 millimeters long. The actual size is apparent from the dime on the left, which has several of the insects sitting on it.

Using specialized digital photography methods, Agricultural Research Service scientists and their collaborators are producing high-resolution images of members of the wasp superfamily Platygastroidea. Their goal is to improve the identification and taxonomic description of these tiny insects—including species with potential to biologically control important crop pests.

Of particular interest are 1- to 2-millimeter-long *Trissolcus* wasps that parasitize stink bug eggs. The larvae of such wasps hatch inside and devour the interior of the bug's eggs, killing them in the process. Some species attack the eggs of the brown marmorated stink bug (BMSB), *Halyomorpha halys,* an invasive species from Asia that's become established in 39 U.S. states and, in 2010, inflicted $37 million in damage to corn, soybean, grape, and other crops.

"One of the challenges of taxonomy for such small creatures is that taxonomists have had to rely on written descriptions and illustrations to understand what they have not seen firsthand," explains Elijah Talamas, an entomologist with the ARS Systematic Entomology Laboratory (SEL) in Washington, D.C. "In some cases this has worked very well, and high-quality illustrations are extremely useful. However, illustrators simply cannot capture all of the detail that a photograph can."

Toward that end, Talamas, together with ARS entomologist Matthew Buffington, has begun a project to photograph the collection of Platygastroidea wasp "holotypes" managed by colleagues at the Smithsonian Institution's National Museum of Natural History (NMNH) in Washington, D.C. Holotypes are the reference specimens on which species names and descriptions are based.

It Starts With a Slice

The process begins with positioning a holotype specimen under a specialized camera with a single-column lens attached to a vertical joist and taking stacks of photographs throughout the depth of the specimen. Each photograph contains a small part of the insect in focus, due to the small depth of field at high magnification. These "slices" are then combined into a single, highly detailed, digital image magnified up to 100 times the specimen's original size. The image is then uploaded to online databases, operated by Ohio State University (OSU) collaborator Norman Johnson, and linked to a description of the holotype and other information about it.

"Making the images freely available online makes it possible for anyone with an Internet connection to assess the morphology of the holotype specimens," says Talamas. "Each specimen has a unique

KIM HOELMER (D3223-1)


ELIJAH TALAMAS (D3227-1)

Above: In Newark, Delaware, ARS research specialist Ashley Colavecchio uses the stacking system to produce high-resolution images of wasps to improve the identification and taxonomic descriptions of the insects. Left: An image of a *Calotelea* wasp in amber that is about 20 million years old.

collecting unit identifier (CUID)—which allows a user to determine the specimen's origin on a species-distribution map. Taxonomists can refer to a particular specimen via its CUID without ambiguity."

Such capabilities are especially important in validating or correcting holotype names or descriptions that were made long ago. Talamas and colleagues published one such correction for *T. halyomorphae,* a promising biocontrol agent for BMSB, in the *Journal of Hymenoptera Research.* This species was described as new in 2009, but it was actually described more than 100 years ago by U.S. Department of Agriculture entomologist William Ashmead, and it is now called by its proper name, *T. japonicus*.

As an OSU graduate student under Johnson's mentorship, Talamas produced over 3,000 digitized images of Platygastroidea wasps, and he has produced hundreds more since joining SEL in March 2013.

Searching for Biocontrols

The documentation of *Trissolcus* species provides invaluable taxonomic support to a team headed by Kim Hoelmer at the ARS Beneficial Insects Introduction Research Unit in Newark, Delaware. There, under quarantine conditions, Hoelmer's group is examining the host specificity and safety of several Asian *Trissolcus* species with potential use in biocontrol-release programs against BMSB. (See "ARS Works Toward Control of Brown Marmorated Stink Bug," *Agricultural Research,* January 2013, pp. 18-20).

Being able to tell the Asian species apart from one another and from native *Trissolcus* wasps will be critical on several fronts, including monitoring the purity of numerous research cultures, tracking the wasps' spread from introduction sites, monitoring their behavior patterns in new environments, and gauging their effectiveness as biocontrol agents.

In a side project, using the NMNH collection, Talamas has begun photographing Platygastroidea wasp specimens entombed in amber dated 20-30 million years of age. There are many questions about the diversity and origins of the wasp superfamily, as well as its co-evolution with host insects, that are relevant to modern-day biocontrol pursuits.

"Right now, we don't have enough specimens in amber to show the co-evolution of Platygastroidea with stink bug hosts," says Talamas. "But we can begin to learn what groups were prone to extinction, which were more diversified, and conversely, which are present today but are not in the fossil record."—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.

*Elijah Talamas is with the USDA-ARS Systematic Entomology Laboratory, National Museum of Natural History, 10th St. & Constitution Ave., N.W., Washington, DC 20560; (202) 633-0998, elijah.talamas@ars.usda.gov.**

New Lure May Help Growers Combat Almonds' No. 1 Insect Pest

Navel Orangeworm

PEGGY GREB (D1719-1)

Left: An adult navel orangeworm moth. Females lay eggs on almonds, and larvae that hatch from the eggs feed on the nutmeat.

It's no wonder that almonds are one of America's favorite nuts. They taste great and are heart-healthy, too.

Most of the almonds grown in the United States today come from vast orchards in California. For the past 50 years, however, the Golden State's almond harvests have been threatened by what is today the number-one insect pest of almonds—the navel orangeworm.

The troubles begin when the female navel orangeworm moth lays her eggs on the exterior of the almond hull, also called its "husk." The husk protects the layers beneath it, notably the light-tan almond shell and the nutmeat that develops within it.

Female moths are attracted to almonds with husks that have split open—which happens naturally as the nut matures. Other sorts of damage also have appeal, such as entry holes left by other insects, or injury caused when a husk is slammed against a nearby branch on a windy day.

Each kind of injury may make it easier for the navel orangeworm larvae that hatch from the eggs to find their way to food (the nutmeat) and the shelter of the husk's interior.

But the harm that the larvae cause doesn't stop there. They wreak further havoc when they inadvertently bring spores of mold-forming *Aspergillus flavus* or *A. parasiticus* fungi along with them as they make their way to the almond kernel. The fungi are of concern because they can produce cancer-causing compounds known as "aflatoxins." Almond processors spend millions of dollars every year inspecting harvested almonds to ensure that any nuts that contain unsafe levels of these toxins don't end up in your shopping cart.

JOHN BECK (D3234-1)

Almond husks, or hulls, naturally split open as the nut matures on the tree. This opening makes it easier for navel orangeworm larvae to find their way to the nutmeat inside.

A Better Lure

Some almond growers hang small traps on tree branches to help detect incoming navel orangeworm moths and track their numbers. This way, the growers, and their pest control advisors, can determine the best times to apply insecticides.

Typically, these "monitoring traps" are baited with a lure that attracts the female moths. That lure is usually almond meal (almonds ground to a cornmeal-like texture) to which almond oil may have been added.

Now, a team of Agricultural Research Service and almond industry investigators, led by ARS chemist John Beck in Albany, California, has developed a promising new lure. According to Beck, the lure proved to be "at least seven times more powerful" than the almond-meal-based formulation in preliminary tests.

The new lure's effectiveness is due, in part, to its ability to attract both male and female moths. In so doing, it should provide a more accurate and useful picture of moth numbers within an orchard, Beck says.

The lure is a unique, carefully researched blend of five natural chemicals. Known to scientists as "aromatic volatiles," these compounds are emitted into the orchard air from damaged almond husks. Beck's team used leading-edge techniques to collect, isolate, identify, and measure the compounds, and used electroantennograph assays to select and compare finalists.

The electroantennograph tests enabled the researchers to evaluate, in the laboratory, the extent to which navel orangeworm moth antennae responded to a "whiff" of a candidate chemical.

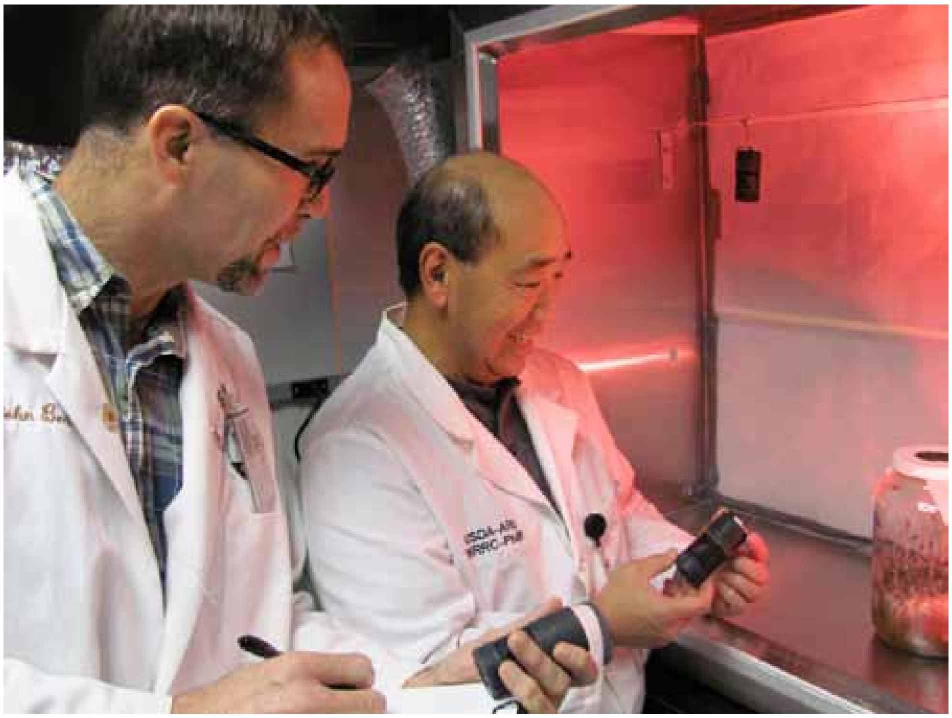
Three of the chemicals selected for the experimental blend—acetophenone, ethyl benzoate, and methyl

PEGGY GREB (D1720-1)

A navel orangeworm larva. This pest is the number-one insect enemy of almonds.

salicylate—are produced by the almond tree itself. The other two, conophthorin and 1-octen-3-ol, are emitted by spores of fungi that may dwell on almond trees,

COREY M. GRIFFITH (D3233-1)



Above: In Albany, California, chemist John Beck (left) and technician Wai Gee count eggs laid by a female navel orangeworm moth on an egg trap. Beck and Gee use egg and moth monitoring traps in laboratory and orchard tests to evaluate lures.

including *A. flavus* and *A. parasiticus.*

The blend resulted from about 7 years of laboratory and orchard research. Outdoor tests included a 3-year experiment that pitted the blend against the standard almond-meal lure. The scientists used more than 100 traps, distributed throughout 2 commercial almond orchards, for the research.

Each of the individual compounds in the blend was already known to exist in nature, and all were previously known to have some role in insect ecology. At least one—conophthorin—has been suggested for use in insect control, specifically, control of bark beetles that attack forest trees. But the five compounds had not—until now—been combined for use as an effective navel orangeworm lure. ARS is currently seeking a patent for the formulation.

New lures that ARS scientists are formulating may help growers keep pistachios (shown), almonds, and walnuts safe from navel orangeworms.

SCOTT BAUER (K7504-1)

More Discoveries Ahead

Much remains to be learned about the blend, its use, and some of its components. An example: Beck and colleagues want to guarantee that the lure is compatible with “mating disruption,” an environmentally friendly pest-control strategy used in many almond orchards. This well-established tactic relies on dispersing a synthetic version of the sex pheromone emitted by female navel orangeworm moths. Normally, male moths home in on the pheromone to find mates. But the oversupply of the pheromone can confuse them and disrupt mating.

“Our lure should be compatible with mating disruption,” Beck says, “but we want to be certain of that.”

In addition, the researchers want to make the lure more effective in pistachio orchards, which also are vulnerable to attack by the navel orangeworm. Their experiments have shown that the current blend works in pistachio orchards, but not as well as it does with almonds.

The team also plans to tailor the lure to work effectively in walnut orchards, where the insect is sometimes a major pest of that crop.

There’s more. The scientists are tackling the problem of how to keep the ratio of blend components stable. Maintaining the correct ratio is essential to the lure’s success, but it is complicated by the fact that the compounds evaporate at different rates.

Other work may yield a fast, easy way to dispense the lure. “It’s a liquid, and right now we’re using small plastic vials filled with about a half-teaspoon of it in the monitoring traps,” Beck says. “We’d rather use something like a small pouch that’s impregnated with the lure. All you’d have to do to start releasing the lure would be to peel the cover off the pouch.”

Perhaps the most intriguing study on their “to-do” list involves probing into what may turn out to be a mutually beneficial relationship that might have evolved between the moths and the molds. “We think that these *Aspergillus* molds, and some other mold species that can occur in almond orchards, may help the female

BRADLEY S. HIGBEE (D3236-1)

The small vial suspended in this monitoring trap dispenses an insect lure. ARS researchers have developed a new lure that attracts both male and female navel orangeworm moths to monitoring traps placed on almond tree branches. The traps help growers detect incoming moths and determine the best time to spray insecticides.

navel orangeworm moth by giving off aromatic volatiles that attract her to a damaged husk," Beck explains. "The moth is looking for a good place to lay her eggs. If the moth lays her eggs on a damaged husk, her larval young should benefit because they won't have to crawl far to find food and shelter. The mold benefits because its spores get a free ride on the larvae into the interior of the husk. That's a more hospitable environment than the exterior for spore development."

If such mutualism does indeed exist, the researchers might be able to exploit it in a strategy that harms moth and mold alike.

Beck, who is based at the ARS Western Regional Research Center in Albany, collaborated in the lure studies with ARS colleagues Doug Light, Noreen Mahoney, and Wai Gee—all at Albany; Dan Cook, with ARS in Parlier, California; Brad Higbee of Paramount Farming Co., LLC; and others.

Peer-reviewed scientific articles published in the *Journal of Agricultural and Food Chemistry, Journal of the Science of Food and Agriculture, Phytochemistry Letters,* and elsewhere, as well as a chapter in the book "Pest Management with Natural Products," document their studies.

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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
8 research units ■ 202 employees

Center for Grain and Animal Health Research, Manhattan, Kansas
6 research units ■ 105 employees

Lincoln, Nebraska
2 research units ■ 69 employees

Madison, Wisconsin
3 research units ■ 102 employees

East Lansing, Michigan
2 research units ■ 31 employees

Athens, Georgia
9 research units ■ 167 employees

Byron, Georgia
1 research unit ■ 27 employees

Geneva, New York
2 research units ■ 47 employees

Henry A. Wallace Beltsville Agricultural Research Center, Beltsville, Maryland
27 research units ■ 806 employees

Newark, Delaware
1 research unit ■ 19 employees