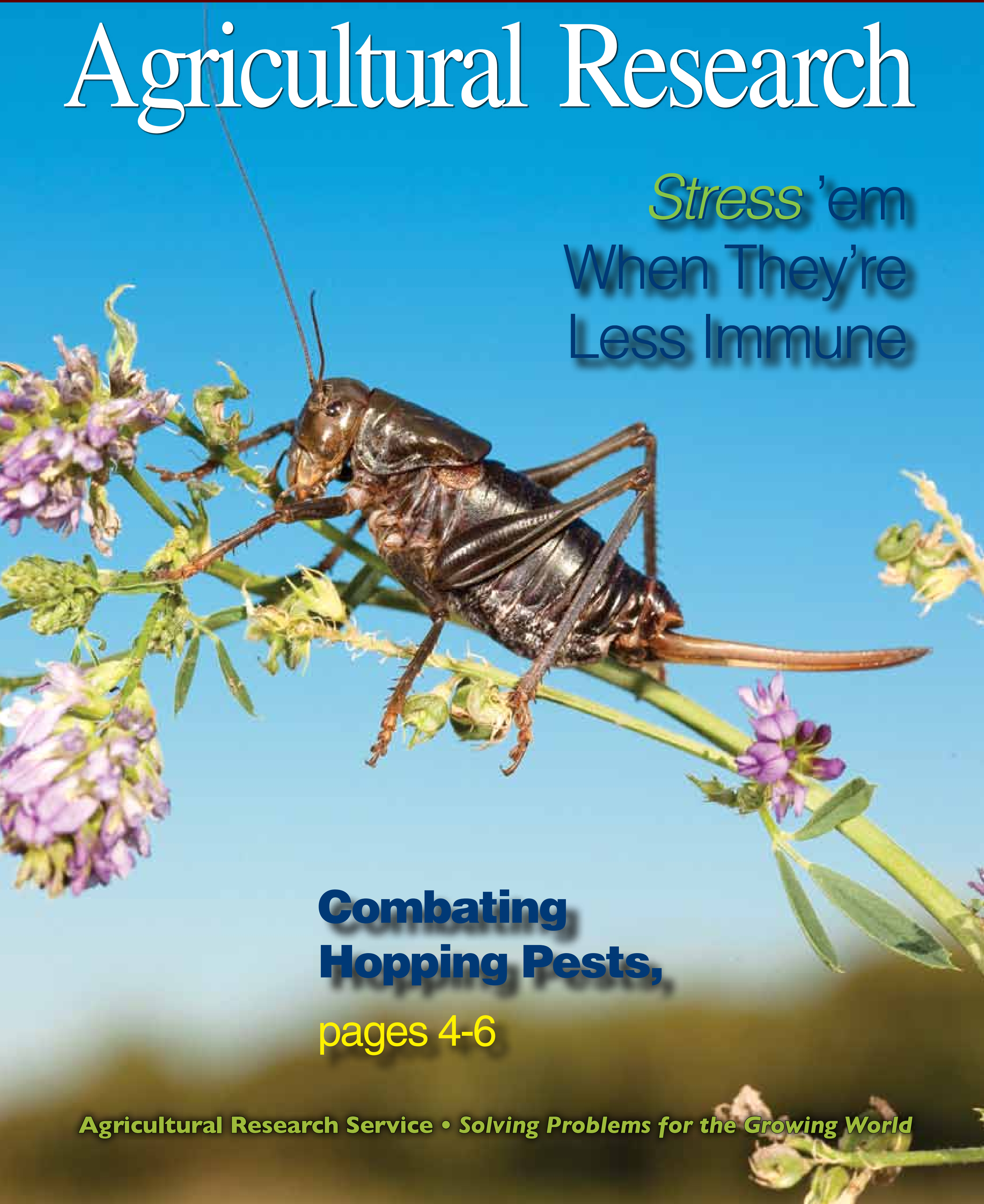
U.S. Department of Agriculture · Agricultural Research Service · January 2011

Agricultural Research



Stress 'em When They're Less Immune

Combating Hopping Pests,

pages 4-6

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

Innovative Ways To Fight Insect Pests

If left uncontrolled, insect pests would eat or ruin about half of all crops grown in the United States. Insects and ticks that bite livestock cause discomfort, reduce production efficiency, and transmit important diseases like bluetongue and equine piroplasmosis. Biting pests are a threat to public health because they can transmit malaria, dengue, chikungunya, West Nile virus, and more.

New insecticides are constantly needed for all uses—crops, livestock, and public health—but companies tend to produce more new active ingredients for crops than for public health and livestock products, which have smaller markets and are more difficult to develop.

Several Agricultural Research Service (ARS) laboratories, in collaboration with others, are using innovative methods to find new insecticides for livestock and humans, particularly for our troops overseas. The search for safer, next-generation insecticides for the military and the public is being done in partnership with the U.S. Department of Defense (DOD) and industry. The Deployed War-Fighter Protection Research Program (DWFP) has provided funds and promoted industry collaborations and partnerships to advance this important initiative. Administered by the Armed Forces Pest Management Board, DWFP also funds a competitive grants program. While the DWFP program is specifically geared towards protecting our troops, the results can also be useful for the public and for agriculture.

STEPHEN AUSMUS (D2024-1)

Female boll weevil on a cotton flower. The pollen that sticks to her is a great forensics tool. See story, page 20.

Using DWFP funding, ARS's national program on Veterinary, Medical, and Urban Entomology (#104) has created a "virtual laboratory" from several ARS locations that have expertise in pesticide development. The virtual lab's activities include discovery, evaluation, development, and marketing. Our entomologists in Oxford, Mississippi, extract natural products for insecticidal or repellent activity, then perform a simple screening assay developed by ARS to prioritize the compounds. Our Gainesville, Florida, insect scientists screen thousands of compounds from chemical libraries and have invented new technology based on insect physiology. Our Beltsville entomologists apply synthetic chemistry expertise to chemically modify existing compounds and to invent new ones.

Gainesville scientists are working closely with the U.S. Navy Entomology Center of Excellence, Jacksonville Naval Air Station, Florida, to develop devices to make compounds more useful on a large scale outdoors.

Recently, ARS and the military have partnered with the IR-4 project to help with registration of products by the U.S. Environmental Protection Agency (EPA). IR-4 deals with registration of specialty crop pesticides but has only recently taken on the task of assisting us with registration of public health pesticides.

One of the important inventions to come out of this program is from Gainesville: the concept of molecular pesticides. These are short sequences of nucleic acids that normally regulate cellular synthesis of proteins. Designed to stop specific proteins, molecular pesticides can target particular pests, therefore sparing the environmental stress of killing all insects. These pesticides are persistent enough to be useful, but do not build up in the environment. Since the mode of action depends on the sequence of nucleic acids, there is hope that any insecticide resistance could be countered very quickly with a new mode of action.

Entomologists at Oxford have developed new, highly effective repellent compounds. These originated from American beautyberry, a plant that has traditionally been used to ward off flies from mules. They have also discovered an entirely new chemical class of insecticides by making extractions from fungal species that kill insects.

Beltsville researchers invented compounds that can be put into cuffs and collars of uniforms. Currently, uniforms are treated with a synthetic pyrethroid, but this only protects the skin directly beneath the cloth. Exposed skin nearby is fully susceptible to bites. The new compounds would create a barrier around the entire soldier, decreasing the need to apply inconvenient repellents.

But not every invention is a chemical. At College Station, Texas, ARS agricultural engineers have developed new ways to test equipment and evaluate pesticides and application techniques. At Gainesville, high-throughput systems developed there allow researchers to combine experiments with mosquito larvae, data processing, and structural analyses to quickly screen and test each chemical's potential as an insecticide. They have examined more than 2,000 compounds and found more than 200 to be effective against mosquito larvae and about 28 effective against adult mosquitoes. Gainesville scientists are also collaborating with the University of Florida to examine the U.S. Department of Agriculture's historical archives of insecticide data by quantitative structure-activity relationship modeling to predict and make new insecticides. This approach was used successfully to predict and make good repellent candidates.

Meanwhile, Beltsville scientists are figuring out exactly how repellent compounds work. It's exciting biology in itself, but these discoveries will be the key to the invention of truly innovative compounds that alter biting insect behavior.

Finding and developing novel compounds that kill mosquitoes is a long-term and worthwhile endeavor, with final registration dependent on successful completion of a large battery of EPA-mandated tests. Although the DOD-ARS insecticide development program is moving into only its second 5-year funding period, results so far indicate that several compounds with new modes of action show promise as future mosquito controls for the public.

Daniel Strickman
National Program Leader
Medical and Veterinary Entomology
Beltsville, Maryland

January 2011
Vol. 59, No.1
ISSN 0002-161X

Agricultural Research

Solving Problems for the Growing World

Agricultural Research is published 10 times a year by the Agricultural Research Service, U.S. Department of Agriculture (USDA). The Secretary of Agriculture has determined that this periodical is necessary in the transaction of public business required by law.

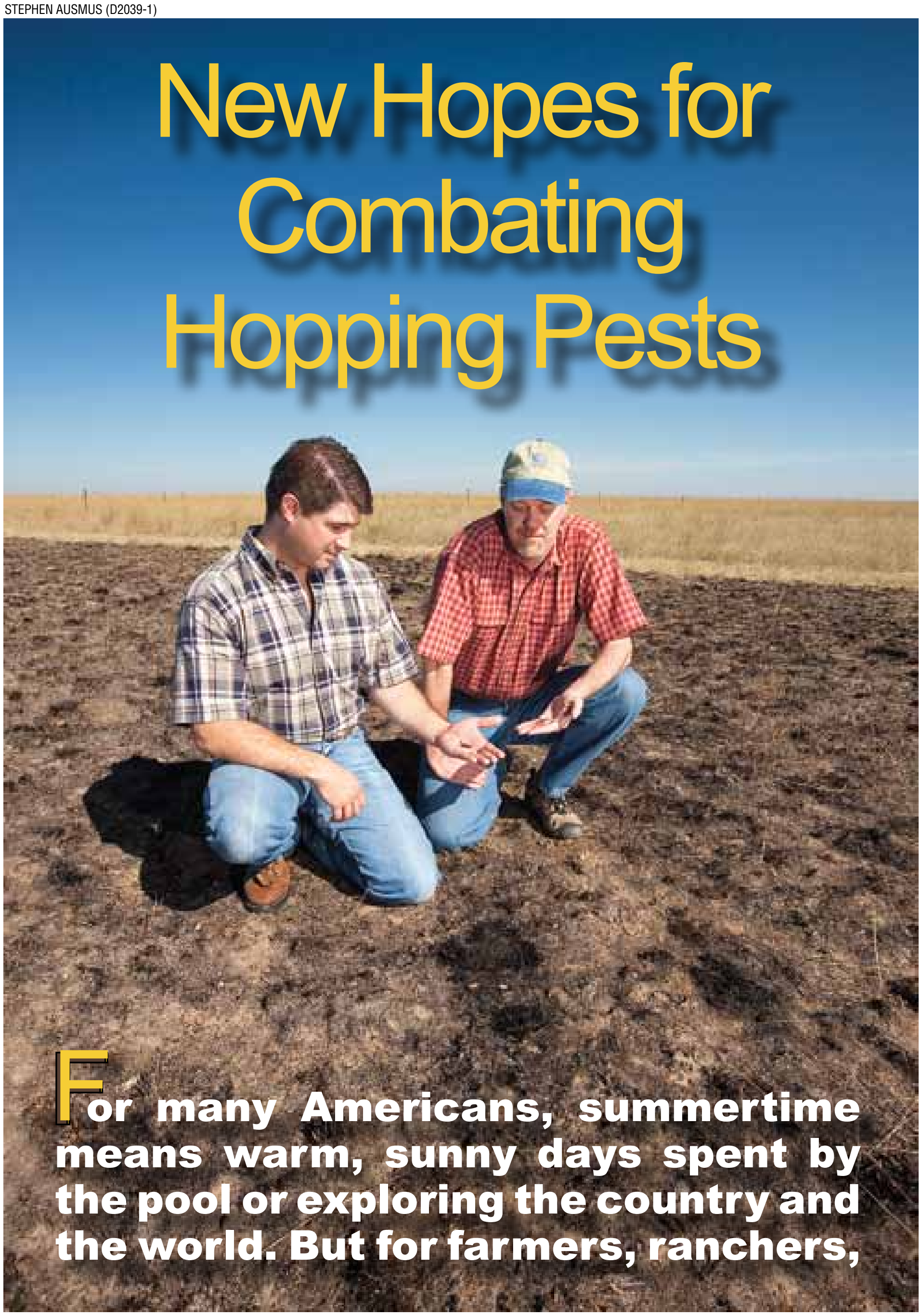
STEPHEN AUSMUS (D2075-1)

A common grasshopper, *Phoetaliotes nebrascensis.* Story begins on page 4.

Cover: The Mormon cricket is a voracious feeder that wipes out acres of grasses and field crops in no time. When it's young, though, it grows so fast that its immune system cannot keep up. This may be the best time to use biocontrol fungi to target the insect pest. Story begins on page 4. Photo by Stephen Ausmus. (D2020-1)

New Hopes for Combating Hopping Pests

STEPHEN AUSMUS (D2039-1)



Ecologist Lance Vermeire (left) and entomologist Dave Branson inspect a burn site in Terry, Montana, to determine how grasshopper populations are affected by late summer or early fall fires.

For many Americans, summertime means warm, sunny days spent by the pool or exploring the country and the world. But for farmers, ranchers, scientists, and state pest control organizations in the western half of the country, summer also means a chance of infestations of hopping pests, particularly grasshoppers and Mormon crickets.

Each adult female grasshopper can lay multiple egg pods—each containing many eggs—in one summer, which could greatly increase the population the next summer, after the eggs hatch. This compounding effect could lead to drastic yield losses for farmers and ranchers as grasshoppers, who can eat their body weight daily in vegetation, leave less grass on the rangeland for livestock and sometimes move into crops and feed on wheat and alfalfa.

State and federal pest control agencies spend millions of dollars each year to control grasshopper and cricket populations. During a particularly bad infestation, the cost can skyrocket. Coupled with the loss of revenue for farmers and ranchers, a grasshopper infestation could cost our country billions of dollars per year.

That's why a team of scientists in the Pest Management Research Unit at the ARS Northern Plains Agricultural Research Laboratory, Sidney, Montana—working with state, federal, and university collaborators—is researching ways to better manage grasshopper outbreaks.

Population Management Is Key

Entomologist David Branson is playing with fire. But it's not a bad thing. "We're researching how controlled burns of rangeland areas can be used to manage grasshopper populations, either through changing food availability or mortality of grasshoppers and unhatched eggs," he says.

Branson is working with ecologist Lance Vermeire, with the ARS Fort Keogh Livestock and Range Research Laboratory in Miles City, Montana, and ecologist Gregory Sword, a former ARS researcher who is now with Texas A&M University.

The researchers have conducted several studies examining how grasshopper populations are affected by late summer and early fall fires, which occur after the insects lay some or all of their eggs. It has been commonly assumed that insects living or hibernating in the soil would be protected from grassland fires.

In one laboratory study, Branson and Vermeire sought to determine whether increased soil temperature from fire affects grasshopper eggs laid at different depths. They compared egg mortality rates of two common pest species of North American grasshoppers—*Ageneotettix deorum,* which lays shallow egg pods, and *Melanoplus sanguinipes,* which lays deeper, more vertically oriented egg pods.

The scientists used a propane heater to simulate the increase in soil temperature during rangeland fire. They found that 80 percent of the shallow-laid eggs of *A. deorum* were killed. On the other hand, none of *M. sanguinipes*' deeper-laid eggs were killed.

"The study shows that fire can be used to selectively manipulate the population dynamics of a common pest grasshopper species," says Branson. "But we can't rely on controlled burns alone to manage grasshopper populations. It's just one tool in the arsenal."

Branson and colleagues are also examining the effect livestock grazing could have on grasshopper populations. Since grasshoppers have to compete with livestock for food, grazing could be used to prevent or moderate damaging grasshopper outbreaks.

Branson and Sword recently completed a study of grasshopper community responses to fire and livestock grazing. "In contrast to chemical control, these types of habitat manipulations have the potential to keep grasshopper populations below economically threatening levels," says Branson.

The 5-year study, which took place in the northern Great Plains mixed-grass prairie, examined the effects of livestock grazing and fire on grasshopper population density and community composition. The scientists studied the effects of three different livestock grazing systems—twice-over rotational grazing, season-long grazing, and ungrazed control—in burned and unburned habitats.

Despite overall low grasshopper densities during the study, Branson and Sword found that grasshopper diversity was significantly higher with twice-over rotational grazing. They also found both fire and grazing significantly affected grasshopper populations and community composition. For example, grasshopper densities were higher in burned pastures with season-long grazing.

More long-term studies during periods with greater grasshopper densities are needed to better assess the effects of grazing or fire on grasshopper population dynamics.

Crickets Create Chaos, Too

The grasshopper isn't the only insect wreaking havoc in the Northern Plains states. Its cousin, the Mormon cricket, is also plaguing farmers and ranchers. The two insects are closely related and belong to the insect order Orthoptera.

BETH REDLIN (D2047-1)

Mormon crickets' rapid growth weakens their immunity.

Prior to testing a fungus's effect on Mormon cricket populations, ARS entomologist Stefan Jaronski (left) works with technician Chris Reuter (center) and entomologist Nelson Foster, both with APHIS, to use an APHIS-developed sprayer that simulates aerial fungus application to a small area of ground.

Like grasshoppers, Mormon crickets can wipe out acres of grasses and field crops through their voracious feeding. But these insects are also a danger to drivers. Cricket populations can build up so much that crushed insect bodies on highways can create road slicks, resulting in hazardous driving conditions.

Ecologist Bob Srygley is examining the cricket's immune system to better understand how the insect is defending itself from attack. His goal is to identify the best opportunity in a cricket's life cycle to use biocontrol methods that will reduce cricket populations.

But accomplishing his goal won't be easy. You see, Srygley wants to rear Mormon crickets in the laboratory so he doesn't have to wait to conduct tests on field populations. The problem: Rearing second-generation Mormon crickets in a laboratory hasn't been done before.

"Determining the rearing requirements for the cricket, like identifying critical temperatures for egg and nymph development, will help us better understand its reproductive process," says Srygley.

In the meantime, Srygley is conducting tests on crickets caught in the field. He has recently examined the role the cricket's diet plays in its immune system and how the immune system changes with development.

"We've found that young crickets want to grow quickly, so they sacrifice the growth of their immune system to allocate more resources for physical growth," says Srygley. "Based on enzyme assays of the last few nymphal instars and adults, we've also found that the immune system is weakened with each molt. When the insect reaches the adult stage, it simply adds mass without molting further and no longer needs to compromise its immune system. So it seems ideal to apply biocontrol agents during the insect's immature or early-adult life stages."

Srygley also conducted field experiments comparing protein-fed crickets

STEPHEN AUSMUS (D2043-3)

One goal of ARS scientists is to successfully rear Mormon crickets in the laboratory to better understand immune system development as the insect grows. Shown left to right are four of the insect's life stages: egg, first instar nymph, third instar nymph, and adult female.

with carbohydrate-fed crickets. He found bands of crickets in Utah whose diets lacked enough protein. As a result, the insects were deficient in an enzyme key to combating fungi. Similarly, Srygley found bands of crickets in Nevada whose diets lacked enough carbohydrates, which led them to be deficient in an enzyme involved in combating bacteria.

Srygley plans to further study this phenomenon by creating carbohydrate- and protein-deficient cricket populations in his laboratory. He hopes to be able to use this knowledge about the cricket's nutrient and enzyme deficiencies to help identify biocontrol agents to battle the pest.

Fungal Foes May Be Our Heroes

So what happens when an outbreak occurs? Currently, the main recourse is to use chemical sprays. But entomologist Stefan Jaronski and colleagues are examining other options, such as fungal biocontrol agents.

Jaronski is working with colleagues at the USDA Animal and Plant Health Inspection Service (APHIS), Utah State University, and the University of Wyoming to evaluate several domestic fungi. The fungi were found through an exploratory program led by Utah State professor Don Roberts. They have identified two candidate fungi that could be used to control grasshopper and Mormon cricket populations.

Field tests of the fungi, *Metarhizium robertsii* DWR 346 and DWR 356, began last summer in Montana, Wyoming, and Utah. The scientists are comparing the fungi with a commercial isolate that is registered by the U.S. Environmental Protection Agency to control tick and beetle populations. After a few years of testing, the researchers will be able to determine whether the fungi can be deemed suitable biocontrol agents.

Jaronski and his APHIS colleagues, led by entomologist Nelson Foster in Phoenix, Arizona, are working to receive permission to conduct field tests on a fungus called "Green Muscle." The product was developed by CABI, an international not-for-profit organization aimed at solving agricultural and environmental problems, and commercialized in Africa for locust control. A related strain has been commercialized in Australia as "Green Guard" for control of locusts and wingless grasshoppers.

"Green Muscle is *Metarhizium acridum,*" says Jaronski. "It's related to the domestic fungi we're currently testing but is specific to Orthoptera. With Green Muscle, we won't have to worry about it infecting other insects as we do with many of the domestic fungus candidates."

And that's just the beginning of Green Muscle's advantages.

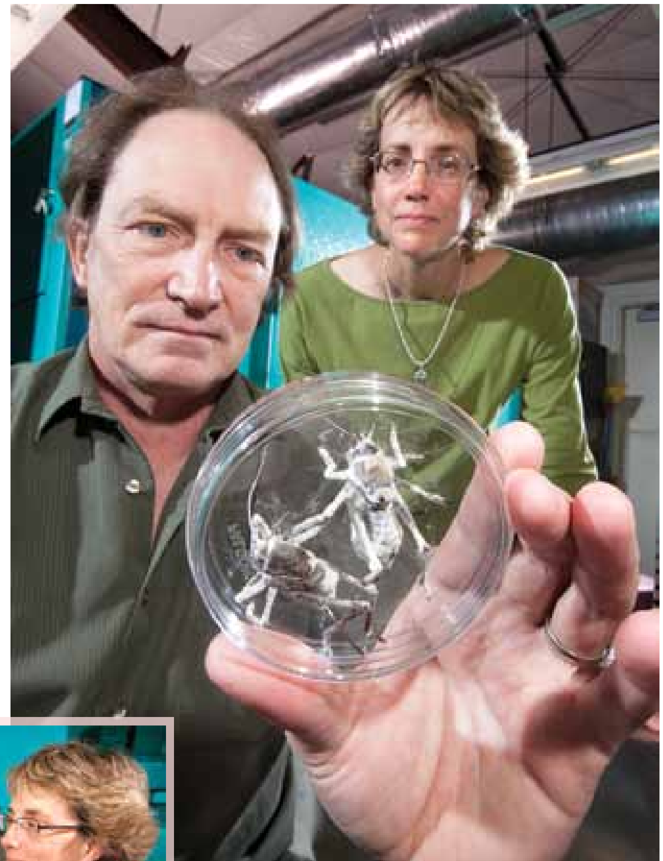
STEPHEN AUSMUS (D2045-3)

Using a dissecting microscope, Laura Senior and Robert Srygley screen Mormon cricket eggs for fertility. The egg in the middle has a prominent eye spot that shows the egg is developing; the other two are infertile.

Normally, when the grasshoppers' and crickets' immune systems detect invasion by a fungus, they deploy blood cells and generate melanin to encapsulate it and keep it from growing. Green Muscle can evade recognition and, if detected and encapsulated, can grow and burst through the insect's defenses.

Grasshoppers and crickets also use "behavioral fever" to prevent a fungus from growing inside them. Most fungi stop growing at 32-34°C, while the insects—especially diseased ones—can heat their bodies up to 38-40°C by extra basking in the sun. The heat exposure stops the fungus from growing, helping to keep the insects alive. But *M. acridum* fungi like Green Muscle have better temperature tolerance and don't experience heat shock. As a result, grasshoppers and crickets succumb to infection after 5-10 days.

STEPHEN AUSMUS (D2044-2)



Ecologist Robert Srygley (left) and technician Laura Senior inspect a male and female Mormon cricket exposed to the insect-killing fungus *Beauveria bassiana*.

It takes just a few hundred spores of Green Muscle to successfully infect a grasshopper. Other fungi require several thousands of spores per insect to ensure infection. This feature means Green Muscle should allow users to reduce their current pest control costs significantly.

"We're really trying to cover all our bases here," says Jaronski. "When prevention fails, we need to use targeted, suppressive, environmentally safe tools to effectively manage an outbreak."—By **Stephanie Yao,** ARS.

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

David Branson, Robert Srygley, and Stefan Jaronski are with the USDA-ARS Northern Plains Agricultural Research Laboratory, 1500 North Central Ave., Sidney, MT 59270; (403) 433-9406 [Branson], (406) 433-9420 [Srygley], (406) 433-9486 [Jaronski], david.branson@ars.usda.gov, robert.srygley@ars.usda.gov, stefan.jaronski@ars.usda.gov. ✻

New Red Imported Fire Ant Enemies in Place for Fight

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

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

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

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

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

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

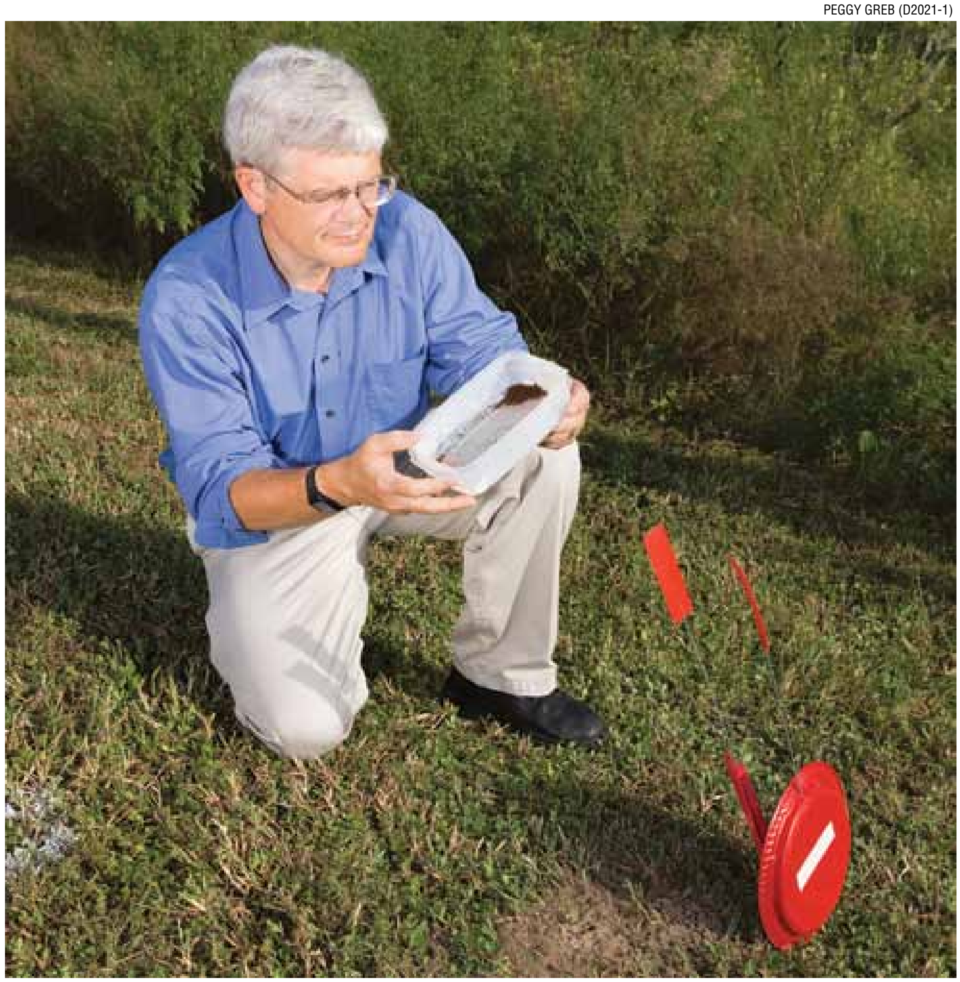
This research is part of Veterinary, Medical, and Urban Entomology, an ARS national program (#104) described at www.nps.ars.usda.gov.

Sanford Porter is in the USDA-ARS Imported Fire Ant and Household Insects Research Unit, Center for Medical, Agricultural, and Veterinary Entomology, 1600-1700 S.W. 23rd Dr., Gainesville, FL 32608; (352) 374-5914, sanford.porter@ars.usda.gov. *

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

SANFORD PORTER (D2023-1)

PEGGY GREB (D2021-1)



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

PEGGY GREB (D2053-1)

Citrus Chemical Call to Arms Found Could Improve Use of Nematode “First Responders”

A *Diaprepes* weevil feeds on a citrus leaf.

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

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

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

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

The weevil also poses an aboveground threat to Florida’s citrus crop, valued at $993 million during the 2008-2009 season. As an adult, the pest feeds on leaves, giving them a notched appearance. “Notching is one of the first things you see when entering an infested grove,” says Jared G. Ali, a University of Florida researcher collaborating with Alborn, who is in the ARS Chemistry Research Unit in Gainesville, Florida.

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

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

PEGGY GREB (D2051-1)

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

PEGGY GREB (D2057-1)

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

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

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

Biological Alternatives

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

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

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

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

PEGGY GREB (D2054-1)

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

PEGGY GREB (D2055-1)

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

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

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

Catching the Action in Real-Time

To determine what signaling compounds citrus roots released when attacked by grubs, the scientists used a specialized glass chamber called a "root-zone olfactometer."

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

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

The team's research could one day lead to new varieties developed from rootstocks shown to be adept at recruiting nematodes. So far, they have tested the signaling capacities of 5 rootstocks and hope to screen as many as 20 more by the end of 2011.

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

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

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

Hans Alborn is in the USDA-ARS Chemistry Research Unit, Center for Medical, Agricultural, and Veterinary Entomology, 1600-1700 S.W. 23rd Dr., Gainesville, FL 32608; (352) 374-5714, hans.alborn@ars.usda.gov. *

Lignin + Nootkatone = Dead Ticks

Derived from essential oils of plants such as grapefruit, vetiver grass, and Alaskan yellow cedar, as well as by chemical synthesis methods, nootkatone is commonly used in foods, cosmetics, and pharmaceuticals. But scientists have also demonstrated nootkatone's potential to kill ants, termites, mosquitoes, cockroaches, and ticks, including *Ixodes scapularis* (blacklegged tick), whose bite can transmit bacteria that cause Lyme disease in humans and other animals.

According to the Centers for Disease Control and Prevention (CDC), there were 29,959 confirmed cases of Lyme disease in 2009—the latest year for which statistics are available. Afflicted individuals experience fever, headache, fatigue, and skin rashes. Left untreated, Lyme disease can affect the joints, heart, and nervous system.

For people in regions of the Northeast and Midwest where blacklegged tick populations are endemic, the threat of Lyme disease necessitates sharp-eyed vigilance and a willingness to take preventive measures. These include wearing light clothing to reveal crawling ticks, removing leaf litter where they might hide, creating mulch barriers, spraying insecticides, and using repellents.

Biobased Pesticide

For some folks, though, concerns about environmental or personal exposure to chemicals make spraying a measure of last resort. Interest in alternatives has prompted research on natural tick controls.

Although low doses of nootkatone have proven effective against different tick species in the laboratory, the essential oil rapidly turns to vapor when applied in the field. And while nootkatone is environmentally benign and nontoxic to humans, early formulations caused discoloration or other signs of toxicity in plants.

Now, however, a solution to both of these problems could be at hand.

As part of a 3-year cooperative project awarded by the CDC, entomologists Kirby Stafford and Robert Behle are testing a

KIRBY STAFFORD III (D2049-2)

Nootkatone application in Connecticut by Craig Boland, owner of Grassman, LLC, a company contracted to apply the substance to the perimeters of homeowners' yards. Most ticks are found in the area where the forest meets the yard.

KIRBY STAFFORD III (D2049-1)

Spray residue of nootkatone on leaves.

spray-dry procedure that encapsulates nootkatone in lignin. In nature, lignin serves as a kind of molecular mortar that holds together the cell walls of plants. In this case, the researchers used lignin as a semipermeable packaging in which to extend nootkatone's residual activity and slow its environmental loss.

The lignin-encapsulation technology was originally developed and patented for use with other pest-control agents. Investigations by Behle and colleagues at ARS's Crop Bioprotection Research Unit in Peoria, Illinois, determined that the technology could similarly protect nootkatone, improving its effectiveness as a tick control.

Targeting Tiny Tick Nymphs

Stafford, who is with the Connecticut Agricultural Experiment Station (CAES) in New Haven, first began field testing nootkatone in 2008 and later sought Behle's formulation expertise when it became apparent the essential oil lacked sufficient residual activity to kill host-seeking nymphs and those hiding in leaf litter.

"We found that when you apply nootkatone, it doesn't last more than 3 days in the field before control breaks down," says Stafford. This can leave too little time for the oil to move down into leaf litter, where nymphs that aren't seeking hosts like to hide, adds Stafford, who conducted the outdoor trials with CAES postdoctoral researcher Anuja Bharadwaj.

Controlling nymphs is critical because their small size allows them to evade detection long enough to transmit *Borrelia burgdorferi,* the spirochete bacterium that causes Lyme disease. According to Stafford, about 90 percent of all cases of Lyme disease can be attributed to feeding by nymphs, which are most active from late May through July.

Protecting Plants, Too

In greenhouse experiments at Peoria, Behle worked with visiting scientist Lina Flor-Weiler and others to evaluate lignin-encapsulated (LE) nootkatone and compare it to emulsified concentrates (EC), a formulation previously used by other groups to apply the oil under field conditions.

For plant-toxicity tests, the researchers sprayed the leaves of 3-week-old cabbage plants, 7-day-old oat plants, and microscope slide covers with either EC or LE nootkatone at five concentrations. These were based on field application rates of 1.6 grams per meter square. After 2 days, small circular areas where the leaves had been treated were cut, weighed, and visually inspected.

The researchers observed that EC-treated areas generally weighed less and showed more damage than those treated with LE nootkatone, indicating the latter formulation's reduced toxicity to plants. Tests for residual activity on slide covers, used as controls for comparison, showed that 95 percent of the EC nootkatone had disappeared by 5 days versus 45 percent for the LE mixture, indicating it had substantially slowed the oil's volatility.

In tests for lethality to ticks—which included *I. scapularis* and three other species—the insides of small glass vials were coated with five different concentrations of nootkatone. In each vial, the researchers placed 10 unfed nymphs and recorded their survival 24 hours after exposure to the treatments.

Each of the four tick species (*I. scapularis, Amblyomma americanum, Dermacentor variabilis,* and *Rhipicephalus sanguineus)* succumbed to nootkatone. But the lone star tick, *A. americanum,* required a slightly higher dose than the others, possibly due to its larger size.

"This is the first report that directly compares toxicity of nootkatone to four target species of tick," the ARS-CAES team notes in a paper accepted for publication in the *Journal of Economic Entomology*.

Home Defense

In 2009, trials conducted on residential properties in Connecticut also showed promise. For those tests, Stafford and Bharadwaj sprayed LE nootkatone along the perimeter of the homeowners' properties where the yards met the forest, with the treatment area extending 3 feet into the yards and 3 feet into the bordering forest. This buffer zone, says Stafford, "is where 82 percent of ticks are normally found."

The trial, which included nontreated homes as controls, ran from June through July. Although the researchers were not able to detect surface residues of the LE nootkatone, they did detect traces of the oil that had found its way below the leaf litter. They observed no signs of burning or other plant damage. Most importantly, "We did not recover any live ticks from the treated sites for the rest of the summer," says Stafford, who adds that final field efficacy data are pending.

In June 2010, they began a third round of tests, treating nine total residential properties—five of them using another nootkatone-encapsulating formulation Behle devised.

Stafford ventures that if the costs of obtaining nootkatone can be reduced, the essential oil could be especially attractive to a burgeoning organic lawn care movement in the Northeast. Rather than a stand-alone defense, however, nootkatone would be integrated with other measures as part of a "biorational" approach to insect control.—By **Jan Suszkiw,** ARS.

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

Robert W. Behle is in the USDA-ARS Crop Bioprotection Research Unit, National Center for Agricultural Utilization Research, 1815 N. University Ave., Peoria, IL 61604; (309) 681-6310, robert.behle@ars.usda.gov. *

SCOTT BAUER (K9409-1)

A nymph-stage blacklegged tick on a leaf. Infected nymphs transmit the pathogen that causes Lyme disease. About 90 percent of all cases of Lyme disease can be attributed to nymph feeding.

Newcastle Disease Virus Vaccine on the Horizon

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

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

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

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

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

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

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

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

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

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

PEGGY GREB (D2048-1)

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

Qingzhong Yu is in the USDA-ARS Endemic Poultry Viral Disease Research Unit, 934 College Station Rd., Athens, GA 30605; (706) 546-3628, qingzhong.yu@ars.usda.gov. *

Ultraviolet Light Boosts Carrots' Antioxidant Value

DOUG WILSON (K7191-4)

ARS studies suggest that a moderate, 14-second dose of UV-B can boost fresh, sliced carrots' antioxidant capacity by about threefold.

Exposing sliced carrots to UV-B, one of the three kinds of ultraviolet light in sunshine, can boost the antioxidant activity of the colorful, crunchy veggie. That's according to preliminary studies by Tara H. McHugh, a food technologist and research leader at the ARS Western Regional Research Center in Albany, California, and her team.

Found mainly in fruits and vegetables, antioxidants are natural compounds that may reduce risk of cancer and cardiovascular disease.

The carrot investigation, conducted by McHugh, postdoctoral associate Wen-Xian Du, and others, suggests that a moderate, 14-second dose of UV-B can boost fresh, sliced carrots' antioxidant capacity by about threefold. The dose is energy-efficient and does not significantly heat or dry the carrots.

Scientists have known for at least a decade that exposing plants to UV-B may cause what's known as "abiotic stress." That's what likely happened with the sliced carrots, McHugh says.

Plants respond to abiotic stress by revving up their production of two natural enzymes, polyphenylalanine ammonia-lyase and chalcone synthase. As production of those enzymes increases, levels of phenolics, compounds synthesized by the enzymes, also increase. Some phenolics are antioxidants.

Despite this and other knowledge about plants' responses to stress and to UV-B, the idea of using UV-B to quickly, safely, and conveniently enrich the antioxidant heft of fresh produce hasn't been extensively studied, McHugh says.

The carrot research, which McHugh has reported at annual meetings of the American Chemical Society and the Institute of Food Technologists, is helping fill in the knowledge gap. So will similar, ongoing studies at the Albany lab, funded by ARS and by a grant awarded to McHugh and Albany co-investigator Andrew Breksa in 2009 by USDA's National Institute of Food and Agriculture.

In earlier research, McHugh and colleagues worked with the Mushroom Council, based in San Jose, California, and Monterey Mushrooms, Inc., of Watsonville, California, to explore the potential of using UV-B to boost the vitamin D content of mushrooms. The idea was not new but, at the time, had yet to be commercialized.

Mushrooms are rich in ergosterol, which can be converted by sunlight into vitamin D. But since commercial mushrooms are typically grown in the dark, their vitamin D levels are usually low. McHugh and Monterey Mushrooms worked out a practical procedure for using UV-B at the packinghouse to capitalize on mushrooms' ergosterol. That resulted in the company's 2008 launch of its line of vitamin D-rich mushrooms, the first-ever commercial use of UV-B to increase mushrooms' vitamin D content.

The researchers documented some of their mushroom studies in a 2008 article in the *Journal of Agricultural and Food Chemistry*. Now, physiologist and research leader Charles Stephensen is looking at bioavailability, that is, how available the mushrooms' vitamin D is to our bodies.

Stephensen, based at the ARS Western Human Nutrition Research Center in Davis, California, is comparing availability of the vitamin from treated and untreated mushrooms and from vitamin D capsules. ARS and the Mushroom Council are funding the study.—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.

Tara H. McHugh is in the USDA-ARS Processed Foods Research Unit, Western Regional Research Center, 800 Buchanan St., Albany, CA 94710; (510) 559-5864, tara.mchugh@ars.usda.gov.

Charles B. Stephensen is in the USDA-ARS Immunology and Disease Prevention Research Unit, Western Human Nutrition Research Center, 430 W. Health Sciences Dr., Davis, CA 95616; (530) 754-9266, charles.stephensen@ars.usda.gov. *

Pilot scale UV-B treatment of carrot slices at ARS's Western Regional Research Center.

ROBERTO AVENA-BUSTILLOS (D2059-1)

DAVID NIELSON (D101-1)

Combining No-Till and the Right Rotations Stores More Precious Precipitation

STEPHEN AUSMUS (D099-22)

In an alternative cropping system plot, technician Brandon Peterson measures carbon dioxide loss due to tillage.

Scientists at the 102-year-old Agricultural Research Service Central Great Plains Research Station in Akron, Colorado, are in the 20th year of a major project determining which alternative crops farmers could use to eliminate—or at least reduce the frequency of—fallow fields.

The station serves an area that has twice the acreage of farmland that California has, including parts of Wyoming, Nebraska, and Kansas as well as Colorado. The long-term study, known as the "Alternative Crop Rotation" (ACR) project, provides data over four full 4-year rotation cycles, allowing scientists to average out the effects of different weather over the years.

Merle Vigil, an ARS soil scientist at Akron, and his colleagues constantly tailor the experiments in this project to meet the needs of farmers in the vast region the lab serves. The team meets annually with about 60 to 80 farmers to decide on needed research. The farmers are among the approximately 140 members of a farmer focus group.

The most diverse ACR project rotation uses wheat, corn, millet, and fallow in a 4-year cycle. It has the fourth highest economic return among the seven rotations being tested.

The project also includes 2- and 3-year rotations. All cycles include wheat, but they also include millet, corn, peas, sunflower, or triticale. The 2-year rotation uses the traditional 1 year of wheat, 1 year of fallow—done with both no-till and conventional tillage for comparison. The other cycles all use no-till.

Agronomist David Nielsen (right) uses a neutron probe as technician Martin Walker uses time domain reflectometry to assess soil water used by winter wheat in an alternative crop rotation study.

STEPHEN AUSMUS (D100-17)

Storing Precious Soil Water Is Key

Vigil gets farmers' attention when he tells them that storing water in just the top inch of an acre of land—an "acre-inch"—is worth $25 to $30 an acre. Vigil, ARS agronomist David Nielsen, and ARS soil scientist Joseph Benjamin—both also at Akron—made this calculation by using 10-year average crop prices in equations they developed to relate crop yields to stored water levels. Four to six tillage passes to kill weeds result in a loss of 3 acre-inches of water over 14 months of fallow. Those six passes cost $24 to $48 an acre in fuel and labor costs. "Adding that to the cost of water lost, that's $99 to $138 from your pocket," Vigil tells farmers.

The scientists have shown that using no-till practices in the conventional wheat-fallow rotation can increase net farm income. They have also shown that by combining no-till and no-fallow, farmers can capture much more of the precious 14 to 18 inches of rain or snowmelt that may occur each year in various parts of the Central Plains. In the case of one of the most profitable rotations, no-till wheat-millet, farm net income could increase by as much as $1,300 a year compared to no-till wheat-fallow on the hypothetical 160-acre farm used for economic analyses.

Although less fallow is good, the other three best rotations (wheat-millet-fallow, wheat-corn-millet-fallow, and wheat-corn-fallow) also had fallow as part of the rotation. That shows that it may not yet be practical to totally eliminate fallow.

Since the prairie sod was first broken in the 1800s, Plains farmers have only been growing wheat every 2 years. They leave fields unplanted for 14 months or more, because there is only enough water from precipitation to grow a wheat crop every 2 years.

"The idea is to store precipitation in the soil during the idle months," says Vigil. "That was a good idea then, but today it is not economically or environmentally sustainable for most soils in the region." Fallow loses 65 to 80 percent of precipitation to evaporation. Besides wasting water, fallow causes a decline in soil organic matter, leaves soil susceptible to wind erosion, and gives low economic returns.

Irrigation on Steep Decline

Capturing more rainwater is essential in a region that is so dry that competing urban uses promise to reduce the area's 9 million irrigated acres by about half a million within the next decade or two.

"Because no-till stores more water in the soil under crop residue, it permits increased cropping intensity from one crop in 2 years to three crops in 4 years—and in some cases, continuous cropping with no summer fallow," Vigil says.

Growing alternative crops in rotation with wheat and continuously cropping when conditions are favorable increase the capture and use of precipitation.

To date, the experiments have shown that in addition to soil water saved and increases in yields and economics, there are substantial improvements to soil with alternative crop rotations. Maysoon Mikha, an ARS microbiologist at Akron, has found that the past 15 years of no-till and continuous cropping with reduced fallow frequency have had positive effects on soil structure and functioning.

Even with traditional wheat-fallow, no-till increased soil organic matter by 24 percent at the 0- to 2-inch depth and 14 percent at the 2- to 6-inch depth, compared with conventional tillage with the same rotation.

Ways To Save Even More Water

The project has shown that no-till's value for storing precipitation in soil can be enhanced by changing harvesting equipment to leave even more residue on the soil surface. This includes use of a stripper header. The stripper header removes just the head of grain, leaving the rest of the plant standing to enhance precipitation storage and erosion protection. Traditional combine headers cut off most of the plant stalk with a sickle and then leave the stubble short.

Also, the scientists have recently shown that skipping one or more rows—rather than planting every row of a crop—conserves soil moisture and improves crop yields.

"We proved the value of stripper-header harvesting and skip-row planting in ancillary experiments and then made them part of the ACR project in recent years," Vigil says.

Nielsen says, "Including crops such as millet and triticale, grown for forage instead of grain, reduces the risk of total crop failure from a lack of rainfall during the critical growth stages of grain crops." He has found other ways to reduce the risks of drought, including estimating soil water in the spring to see if there is enough to warrant skipping fallow.

So What? Who Cares?

Vigil is famous with his colleagues for always making sure they can justify each research experiment by quickly and concisely answering, "So what? Who cares?"

Through careful research planned in cooperation with farmers and by adding newly successful techniques to the long-term rotation project—while dropping failures—they've answered these questions in dollars and cents and acre-inches of precious soil water saved, so Central Plains farmers really will care.—By **Don Comis,** ARS.

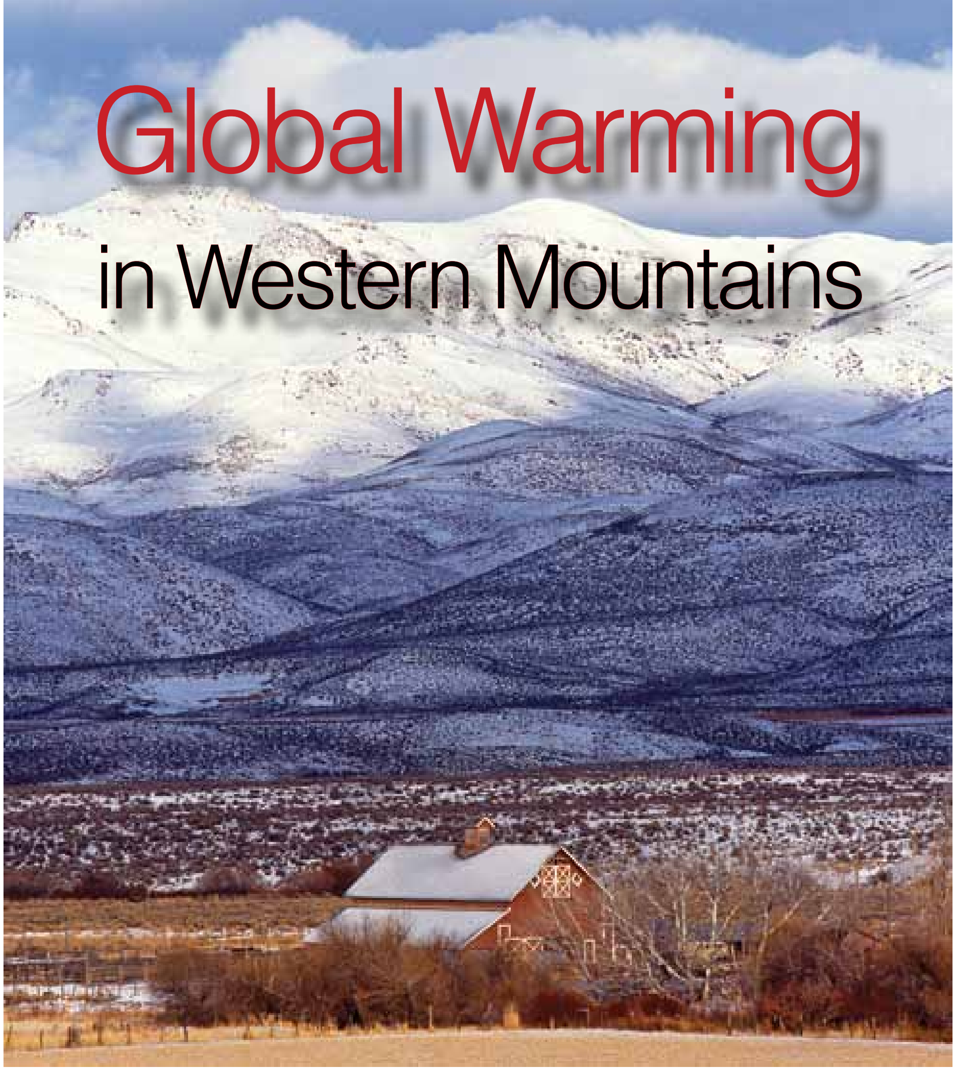
This research is part of Soil Resource Management, an ARS national program (#202) described at www.nps.ars.usda.gov.

Merle F. Vigil is with the USDA-ARS Central Great Plains Research Station, 40335 County Road GG, Akron, CO 80720; (970) 345-0517, merle.vigil@ars.usda.gov. *

Soil scientist Joseph Benjamin and technician Stacey Poland measure water-holding characteristics of soil using pressure cells.

STEPHEN AUSMUS (D097-29)

Global Warming in Western Mountains



Site of the 90-square-mile Reynolds Creek Experimental Watershed in the Owyhee Mountains, about 50 miles southwest of Boise, Idaho. SCOTT BAUER (K5060-3)

More than 50 years of records in the western mountains of the United States show that while it is now significantly warmer, total annual precipitation has not changed. But a system that was once dominated by winter snowfall now experiences a mix of rain and snow, with more streamflow in winter and less in spring. As a result, there is less water for ecosystems and agriculture during the spring and summer growing season. These changes make forecasting and managing western water resources more difficult and present a serious challenge to agriculture in the region.

Over the past five decades, mean temperatures rose 2˚–3˚C, according to Agricultural Research Service (ARS) data from the Reynolds Creek Experimental Watershed (RCEW). Warming has occurred across a range of elevations, with the greatest increases in minimum temperatures. The impact of this has been less snow and more rain, again at all elevations, but with the most profound changes at lower elevations, which saw a shift from 44 percent snow to 20 percent.

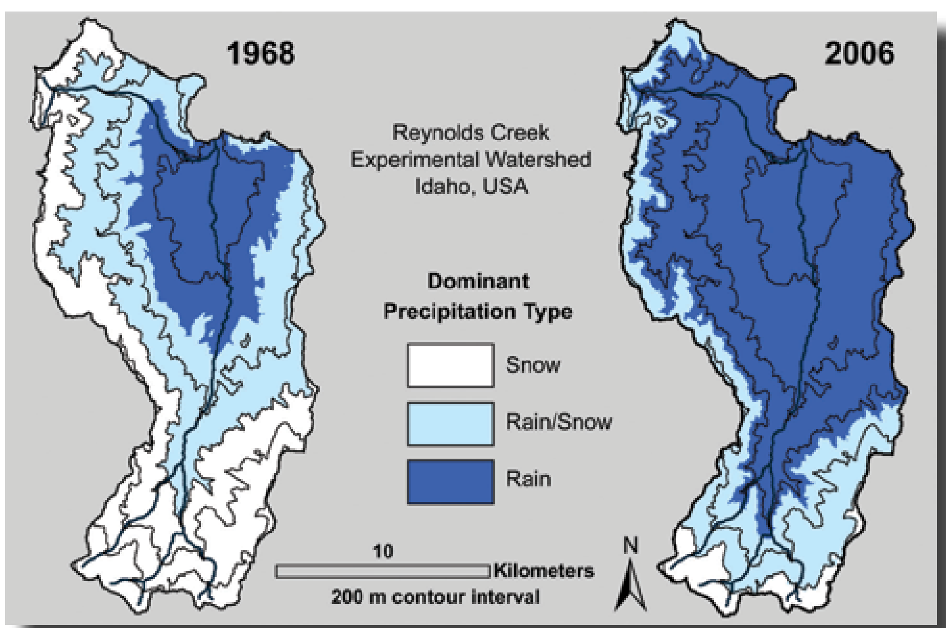
Western Mountains Sensitive to Warming

Danny Marks, a hydrologist who specializes in snow and cold-season hydrology of mountain regions, directed this analysis, working with soil scientist Mark Seyfried and other colleagues. Marks and Seyfried are at the ARS Northwest Watershed Research Center in Boise, Idaho, which manages the RCEW in Idaho's Owyhee Mountains, near Boise. The Owyhee Mountains are part of the Great Basin of the intermountain western United States. Reynolds Creek is the U.S. Department of Agriculture's premier mountain observatory.

Marks says: "Two characteristics of the RCEW, and western mountains in general, accentuate the sensitivity of the region to this shift from a snow-dominated to a rain-dominated regime, especially at low to mid elevations. First, more than 95 percent of the watershed is at mid to low elevations."

So, changes which affect those elevations are much more important from a water-resources perspective. While the ARS analysis shows that there have been changes in the high-elevation snow cover, these are relatively minor compared to the effect that warming has had on the mid and low elevations. Marks says that "this is an important consideration, because most of our snow-measurement sites across the western United States, such as the USDA Natural Resources Conservation Service's

Warming Trend Means More Rain, Less Snow in Lower Elevations



SNOTEL (Snow Telemetry) system, are high-elevation measurement sites that can't detect the strong trends shown in our analysis of the RCEW at the mid to low elevations."

Marks says the second characteristic of the region is that while it was snow dominated, most of the mid- and low-elevation land area in the watershed, and in much of the western mountains, has temperatures and storm conditions that "are not far from the rain-snow transition."

Hydrologically, the region is very sensitive to relatively small increases in temperature. This is illustrated in the long-term snow measurements at Reynolds Creek, where high-elevation sites show a modest decrease in peak snow water content, and almost no change in the date it peaks. But the mid- and low-elevation snow-measurement sites show more than a 60-percent reduction in peak snow water content. And the peak now occurs a month or more earlier.

Further, the snow season is shorter, starting later and ending sooner at all elevations. At high elevations this difference is less than a month, at mid elevations a month or two, and at lower elevations two to three months. At lower elevations, the snow cover is now ephemeral, with only a few months of continuous snow cover each year. During 8 of the first 20 years of records at the watershed, there was measureable snow on May 1 at the lowest snow-measurement site.

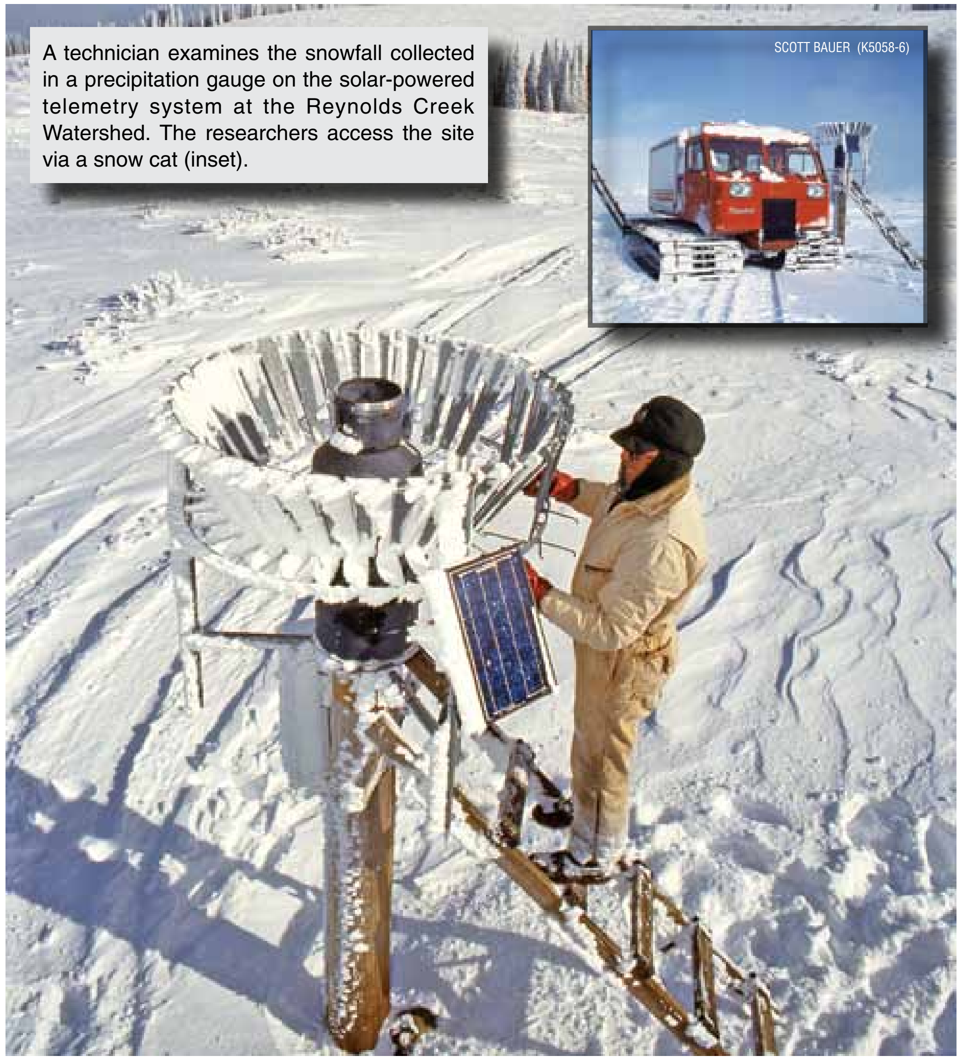
"There has been no snow at that site on May 1 since 1984," Marks says.

Nature's Reservoir in Decline

Marks also says that the effect of warming on the snow cover has been transferred directly to streamflow. "While annual total streamflow volume has not changed at the RCEW over the last 50 years, more streamflow occurs during winter and early spring and less during summer.

"At a small high-elevation area which collects water, representing just over 1 percent of annual flow from the watershed, March and April streamflow increased substantially," he says. "The streamflow was unchanged in May, and in June it decreased by more than half. From a larger mid-elevation basin, representing about 80 percent of annual flow from the watershed, March and April streamflow increased substantially, May increased slightly, while June decreased 35 percent.

SCOTT BAUER (D5061-2)


SCOTT BAUER (K5058-6)

A technician examines the snowfall collected in a precipitation gauge on the solar-powered telemetry system at the Reynolds Creek Watershed. The researchers access the site via a snow cat (inset).

"This shows that not only is streamflow occurring earlier, but more of the annual flow is concentrated in the March–June period, leaving even less for the dry summer growing season," Marks says.

West's Water Forecasting in Doubt?

"In the mountains of the West," Marks says, "the seasonal snow cover is Nature's reservoir, storing water from winter snowstorms for release to the soil and streams during spring and early summer. Water resource management in the West is based on a reliable spring rise in streams from snowmelt. If, as we have observed in the Reynolds Creek Experimental Watershed, this system is changing, then our ability to effectively forecast water supply for the region is uncertain. Our analysis shows that climate warming is causing the rain-snow transition to move upslope. As we go to higher elevations, changes are subtle and may not show up as significant for a decade or more. But if these trends continue, as predicted, for the next 50 to 100 years, Reynolds Creek and similar areas in the West will be very different. We need to anticipate these changes and plan adjustments in our approach to managing water resources in the West."—By **Don Comis,** ARS.

This research supports the USDA priority of responding to climate change and is part of Water Availability and Watershed Management, an ARS national program (#211) described at www.nps.ars.usda.gov.

Danny Marks and Mark S. Seyfried are with the USDA-ARS Northwest Watershed Research Center, 800 Park Blvd., Boise, ID 83712; (208) 422-0721 [Marks], (208) 422-0715 [Seyfried], danny.marks@ars.usda.gov, mark.seyfried@ars.usda.gov. *

Beef Cattle

Improving Production Efficiency and Meat Quality

U.S. consumers love beef. We eat an average of about 63 pounds of it per person each year. Producing enough cattle to meet that demand requires efficiency and innovation. Agricultural Research Service scientists at the Fort Keogh Livestock and Range Research Laboratory (LARRL) in Miles City, Montana, are conducting studies designed to make cattle production more efficient and to provide better beef products for consumers.

Attaining those goals has led to strategies and technologies for reducing the cost of beef production, including more efficient nutrient use and improved reproductive performance. Reducing production costs hinges on maintaining high rates of reproductive success while reducing use of harvested feeds. A common problem that U.S. cow-calf producers face is low rebreeding performance among 2- and 3-year-old cows. This occurs when the cows' needs for additional nutrients during pregnancy and lactation have not been met. But rather than just feed young cows more, the LARRL scientists are attempting to make them more efficient so they'll need less feed.

Reducing Costs: Feed and Reproduction

Animal feed is a large part of beef producers' costs. Cereal grains—often used as a major part of heifer (young female cattle) diets—are becoming less abundant and more expensive because they are in higher demand for human food and ethanol production. Feed represents about 50 to 55 percent of total costs of developing replacement heifers.

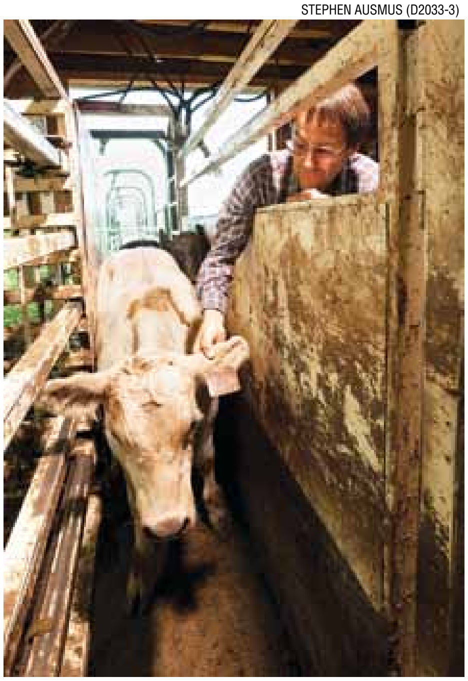
According to animal scientist Andrew Roberts and colleagues, heifers they studied developed to target weights lower than those traditionally recommended, consumed 27 percent less feed over the winter months, and gained weight more efficiently throughout the postweaning period and subsequent grazing season. "The strategy of providing less feed may reduce costs of developing each replacement heifer by more than $31 and extend their life span, with important ramifications for lifetime efficiency and profitability," says Roberts.

STEPHEN AUSMUS (D2038-39)

A cow and calf in an ARS feed-restriction study on the Upper Lignite pasture at Miles City, Montana. Feed restriction may lower the costs of developing replacement heifers and extend their lifespan.

At Miles City, Montana, animal scientist Andy Roberts identifies a calf in a study to reduce beef production cost prior to weaning.

STEPHEN AUSMUS (D2033-3)



Farm supervisor Benny Bryan (left) and geneticist Mike MacNeil load feed bins used to measure feed consumption for studies evaluating efficiency of weight gain in steers.

STEPHEN AUSMUS (D2037-1)

"For the last 3 to 4 decades, the mantra has been 'feed them to breed them,' which means providing enough feed during the first year to ensure that young heifers reach puberty to start reproducing," he says. "But our studies indicate this doesn't seem to be optimal in the long run. Our research shows that by feeding to get all the animals bred, you are also propping up the inefficient animals—those that won't consistently produce calves when put in nutrient-limited environments later in life."

In their study, heifers (50 percent Red Angus, 25 percent Charolais, and 25 percent Tarentaise) were divided into two lifetime treatment groups: The control group was fed according to industry guidelines, and the restricted group was fed (on a body-weight basis) 80 percent of feed consumed by their control counterparts for 140 days, ending when they were 1 year old. The restricted heifers grew slower and weighed less at any point in time as a consequence of less feed. The actual amount of feed provided to restricted heifers over the entire feeding period was about 73 percent of that provided to the controls.

Final pregnancy rates were 87 percent for restricted heifers and 91 percent for the controls.

"Our results indicate that restricting feed is a matter of economics for farmers," says LARRL geneticist Michael MacNeil. "We have also found that other strategies, such as crossbreeding and providing early calving assistance, can increase rebreeding performance of young cows."

Feed Restriction Improves Efficiency

From breeding through late fall, the heifers were managed as one group. Each winter, the pregnant animals were again separated into two groups—restricted feed and control. The restricted cows were fed 20 percent less supplemental feed during the winter months than the controls. The scientists predicted that these treatments would allow nature to decide which heifers were reproductively efficient: Less efficient heifers would eventually fail to reproduce and be culled if restricted, whereas feeding more would keep them in production but result in more expense for the producer.

"Early elimination of inefficient breeders allows them to be harvested for the high-quality meat market," says Roberts.

Roberts and colleagues also found that restricting the cows at a young age might improve their efficiency throughout the rest of their life.

The restricted-feed study has been ongoing since the winter of 2001, and the researchers are now looking at the second generation—those that were born from cows on restricted diets. "An interesting thing occurred: The feed restriction seems to have made the second generation able to withstand restriction with greater efficiency," says Roberts.

In cattle, maximum production (measured by weight of calf at weaning) doesn't peak until 5 years of age. In the study, the proportion of cows that became pregnant each year and stayed in the herd until age 5 was greatest for restricted cows out of restricted dams. Restricted cows from control-fed dams had the lowest rate of survival to age 5.

The researchers found that the third-generation feed-restricted calves are lighter at birth and at weaning than those calves from cows fed at the industry standard, but the feed-restricted cows themselves are slightly fatter and heavier at the calves' weaning.

"Physiologically, the second-generation restricted cow is conserving some of the nutrients taken in for body reserves, which may result in more efficient reproduction and better survivability in the herd," says Roberts.

Getting High-Quality, Great-Tasting Beef

Improving beef quality is another priority for LARRL scientists. Marbling—the streaks of fat in lean meat—has long been an indicator of palatability, and it serves as one basis for determining the price of beef. Marbling is an inherited trait and thus amenable to genetic improvement. Marbling is measured either at slaughter or by ultrasound of the live animal. Says MacNeil, "Cattle breeders would benefit greatly from having genetic indicators of superb marbling and other sought-after traits." This is where geneticist Lee Alexander steps in.

STEPHEN AUSMUS (D2036-1)

Geneticist Lee Alexander (left) and technician Stacie Kageyama genotype calves and their parents with a DNA analyzer. Genotypes are used for research tasks such as confirming parentage, identifying locations of interest in the genome, and finding important mutations.

Alexander and his colleagues used a panel of molecular genetic markers to locate specific places in the genome that contain genes that influence traits such as marbling and fatty acid composition. They looked at the genome of a Wagyu-Limousin cross population. These breeds were chosen because Wagyu is a heavily marbled beef, and Limousin is leaner.

"Genetic markers successfully identified a region of the genome associated with the amount of marbling and relative quantities of saturated and monounsaturated fats," says Alexander. Beef with the best flavor has a higher percentage of monounsaturated fatty acids.

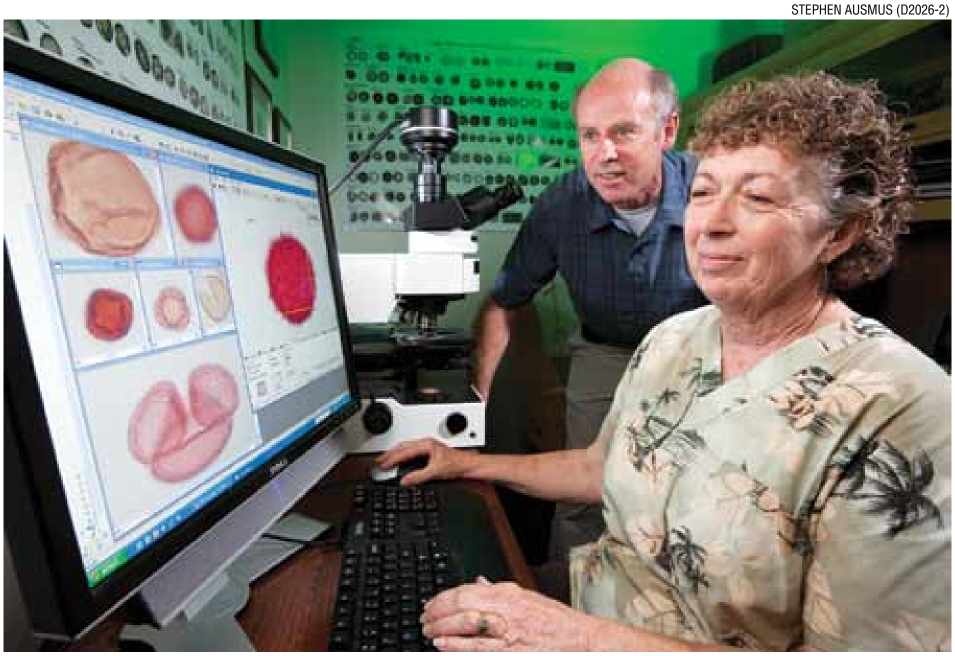
These results may lead to a better tasting and healthier product for consumers through breeding systems that lead to an improved fat profile in beef.—By **Sharon Durham,** ARS.

This research is part of Food Animal Production, an ARS national program (#101) described at www.nps.ars.usda.gov.

To reach scientists mentioned in this article, contact Sharon Durham, USDA-ARS Information Staff, 5601 Sunnyside Ave., Beltsville, MD 20705-5129; (301) 504-1611, sharon.durham@ars.usda.gov. *

Mystery Solved: Detecting the Source of a Boll Weevil Outbreak

STEPHEN AUSMUS (D2026-2)



On a monitor attached to a compound light microscope, ARS palynologist Gretchen Jones and meteorologist John Westbrook examine images of pollen grains found on boll weevils.

In late summer of 2007, cotton growers in Texas's Southern Rolling Plains had reason to be alarmed. Hundreds of boll weevils were turning up in their fields. By the end of the year, more than 6,000 weevils were found in monitoring traps spread across several counties where the insect had been eradicated since 2000.

But a team of Agricultural Research Service scientists with a variety of skills was able to track down the likely origins of the reinfestation, giving cotton growers and entomologists some guidance on how to eradicate them and shedding light on just how far boll weevils can travel under certain conditions.

Boll weevils have been a threat to cotton growers since 1892, when they entered Texas from Mexico and proceeded to invade much of the south-central and southeastern United States. Large-scale eradication efforts, begun about 30 years ago, eliminated the boll weevil from much of the southern United States, but they remain a problem in pockets of eastern and southern Texas. Successful eradication efforts drastically reduce pest-management costs and insecticide use. Reinvasion is a constant threat, even in areas where they have been eliminated. Guarding against reinfestation is coordinated by the grower-supported Texas Boll Weevil Eradication Foundation, which funds monitoring programs and insecticide applications throughout the state.

"Whenever eradication personnel capture boll weevils where they're not expected, they want to know everything possible about where the weevils may have come from. That information plays a major role in deciding the most appropriate response," says Thomas Sappington, an ARS entomologist in the Corn Insects and Crop Genetics Unit in Ames, Iowa. If the infestation is small scale, eradication personnel may need to spray only a few surrounding cotton fields. But if there is evidence of a widespread reinvasion, they may have to conduct more extensive spraying.

In this case, the eradication foundation in the Southern Rolling Plains spent $1.4 million in increased insecticide applications alone, plus outlays for increased trapping, after they discovered the infestation.

Collecting the Evidence

Pinpointing the origins of the 2007 infestation took diverse skills. Sappington is an expert at using DNA to identify insect populations. Boll weevils travel on the wind, and John Westbrook, a meteorologist with the Southern Plains Agricultural Research Center (SPARC) in College Station, Texas, uses modeling techniques and weather data to analyze the effects of wind patterns on insect movement. Gretchen Jones, also based at SPARC, is a palynologist, or pollen expert, who can often identify an insect's itinerary by the type of pollen grains it picks up.

All flowering plants release pollen, and the pollen grains have distinctive shapes. Cotton pollen has a structure that makes it a particularly good forensic tool. It clings to the face, legs, and body of a boll weevil and will remain in the insect's gut for about 24 hours.

There are limitations to all three tracking techniques, the researchers say. Pollen analysis, for instance, involves matching grains released by plants in one location with pollen taken

STEPHEN AUSMUS (D2032-21)

Entomologist Tom Sappington and technician Lisa Fraser examine DNA sequences of boll weevil microsatellite markers to be used in population assignment analysis.

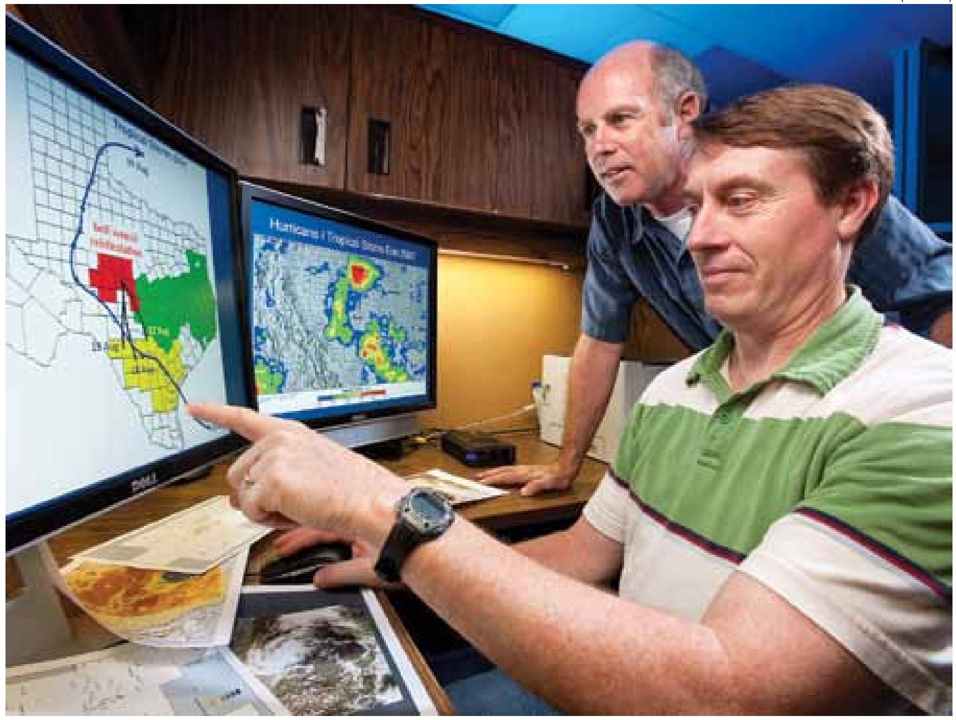
from mobile insects, so it can be difficult to tell at what point along an insect's journey the pollen was picked up. Winds blow in all directions, so you have to know when weevils arrived in a specific area to say whether wind patterns from a specific storm brought them there. In population genetics, finding distinctions in DNA that set one group apart from another depends on how isolated they are from each other. When insect groups breed with each other, the gene flow can make them hard to tell apart.

The researchers had one clue. Tropical Storm Erin swept through South Texas in August 2007, passing 112 miles to the south near Uvalde in an area known as the "Winter Garden District." Between Uvalde and the infested region is the Texas Hill Country, an arid region of scrubby vegetation with plants that produce types of pollen not found among the captured weevils.

Weevils frequently disperse in late summer and early fall when cotton is fully grown and fields are being harvested. "In those scenarios, it's easier to detect them with traps," says Westbrook. Some trapping studies have shown that weevils are capable of flying hundreds of miles. There were no studies that focused on exactly how far they can travel if propelled by a storm.

Sappington focused on identifying patterns in the boll weevils' genetic makeup, comparing the DNA of 20 weevils captured in the reinfested Southern Rolling Plains zone with the DNA of dozens of weevils from 24 other sites in Texas and northern Mexico. Jones examined pollen grains found clinging to body parts and inside the guts of another 16 captured weevils, comparing them under a light microscope with pollen from plants blooming in Uvalde and in Cameron, an area east of the infested region where some officials suspected the weevils originated. Westbrook studied wind patterns and analyzed possible migration paths with help from HYSPLIT, a computer model originally designed by the National Oceanic and Atmospheric Administration for federal studies that track the movements of smoke, particulate matter, and other airborne pollution.

STEPHEN AUSMUS (D2027-1)



Using weather data maps that track storm paths and precipitation amounts, meteorologists John Westbrook (left) and Ritchie Eyster estimate boll weevil dispersal pathways.

The researchers concluded that the weevils likely came from the Winter Garden District. Westbrook found that Tropical Storm Erin skirted the southern and western sides of the reinfested region as it passed through, generating winds for 7 days that could have brought weevils up from the Winter Garden District. Jones found pollen on the invading weevils from nine plants common in Uvalde, including ragweed. She also found that the weevils lacked pollen from any of the numerous plants abundant in Cameron samples. Sappington reached the same conclusions by studying differences in DNA stretches known as "microsatellite loci," which can show collective patterns unique to each weevil population.

The results, published in the *Journal of the Royal Society Interface,* prompted growers to target the Winter Garden District for stepped-up eradication efforts. The work could also lead to better control measures. One way to prevent reinfestations is to harvest and destroy stalks early. The results of the study provide guidance on when approaching storms and hurricanes may warrant an early harvest. The multipronged approach also could be used as a model for resolving future questions about whether unexpected population spikes in fruit flies, aphids, or other pests are being caused by populations of insects from other areas.—By **Dennis O'Brien,** ARS.

STEPHEN AUSMUS (D2025-1)

A female boll weevil on a cotton boll.

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

To reach scientists mentioned in this article, contact Dennis O'Brien, USDA-ARS Information Staff, 5601 Sunnyside Ave., Beltsville MD 20705-5129; (301) 504-1624, dennis.obrien@ars.usda.gov. *

VICKI BRADLEY (D2050-1)

Managing Grass Germplasm and Its Microbial Tenants

The Western Regional Plant Introduction Station (WRPIS) in Pullman, Washington—one of four plant introduction stations in ARS's National Plant Germplasm System—boasts one of the world's largest collections of cool-season forage and turfgrasses, totaling nearly 21,000 accessions.

It's also a treasure trove of microbial germplasm in the form of endophytes, a specialized group of *Neotyphodium* fungi that live symbiotically within certain grasses.

The endophtyes' presence in grasses can be a mixed blessing, however. On one hand, they produce metabolites that naturally repel and reduce survival of important insect pests while also bolstering the grasses' stress tolerance, especially to drought. On the other hand, some of the metabolites, notably ergot alkaloids, produced by the endophyte in grasses like tall fescue are the primary cause of fescue toxicosis in grazing livestock.

Fortunately, intensive research over the past several years has identified new endophyte strains that don't cause fescue toxicosis but still confer desirable benefits. A notable example is the discovery by New Zealand scientists of a non-ergot-producing endophyte strain during their examination of a fescue accession from WRPIS that originated in Morocco. These scientists inserted this nontoxic strain into tall fescue varieties, now marketed under the brand name MaxQ. Some consider MaxQ fescues to be promising replacements for the widely used Kentucky-31 tall fescue variety in the United States, which contains a toxic endophyte.

According to ARS entomologist Steve Clement, public and private research organizations in the United States and abroad are increasingly mining the WRPIS grass collection to identify more nontoxic strains. Such interest underscores the importance of considering grass endophytes as important components of biodiversity that, like grass accessions, must be conserved for research and development. In this regard, research by Clement and collaborating scientists at ARS's Forage Seed and Cereal Research Unit in Corvallis, Oregon, showed that current seed-regeneration practices at WRPIS are suitable for maintaining viable grass endophytes for storage in the genebank.

Clement is also conducting studies to better characterize the diverse grass-endophyte associations that exist in the collection and the anti-insect properties of these associations. That information, in turn, should help scientists better understand the potential for using endophytes to endow cereal crops like wheat and barley with resistance to insects and abiotic stresses.

Of particular interest are the protective effects of grass endophytes and their specific metabolites against globally important insect pests of forage and cereal grasses. In recent research, Clement observed a decline in the survival of cereal leaf beetles—new invasive pests of Pacific Northwest seed nurseries—that fed on endophyte-infected grasses. Very few cereal leaf beetles survived after eating leaves from endophyte-infected wild tall fescue *(Lolium arundinaceum)* accessions originating from North Africa and stored at WRPIS.

"As the science of endophyte discovery and conservation develops, we'll see expanded opportunities for using these unique fungi in agriculture," says Clement.—By **Jan Suszkiw,** ARS.

This research supports the USDA priority of promoting international food security and is part of Plant Genetic Resources, Genomics, and Genetic Improvement, an ARS national program (#301) described at www.nps.ars.usda.gov.

Stephen Clement is in the USDA-ARS Plant Germplasm Introduction and Testing Research Unit, Western Regional Plant Introduction Station, 59 Johnson Hall, Pullman, WA 99164; (509) 335-3572, stephen.clement@ars.usda.gov. *

Photo caption: Forage and turfgrass seed-regeneration nursery at the Western Regional Plant Introduction Station, Pullman, Washington.

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

Locations Featured in This Magazine Issue

Corvallis, Oregon
3 research units ■ 152 employees

Western Regional Research Center, Albany, California
8 research units ■ 250 employees

Davis, California
3 research units ■ 111 employees

Pullman, Washington
6 research units ■ 144 employees

Northwest Watershed Research Center, Boise, Idaho
1 research unit ■ 21 employees

Northern Plains Agricultural Research Laboratory, Sidney, Montana
2 research units ■ 66 employees

Fort Keogh Livestock and Range Research Laboratory, Miles City, Montana
1 research unit ■ 27 employees

Central Great Plains Research Station, Akron, Colorado
1 research unit ■ 28 employees

Southern Plains Agricultural Research Center, College Station, Texas
4 research units ■ 138 employees

Ames, Iowa
8 research units ■ 501 employees

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

Oxford, Mississippi
3 research units ■ 102 employees

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

Center for Medical, Agricultural, and Veterinary Entomology, Gainesville, Florida
4 research units ■ 144 employees

U.S. Department of Agriculture
Agricultural Research Magazine
5601 Sunnyside Ave.
Beltsville, MD 20705-5130

Official Business

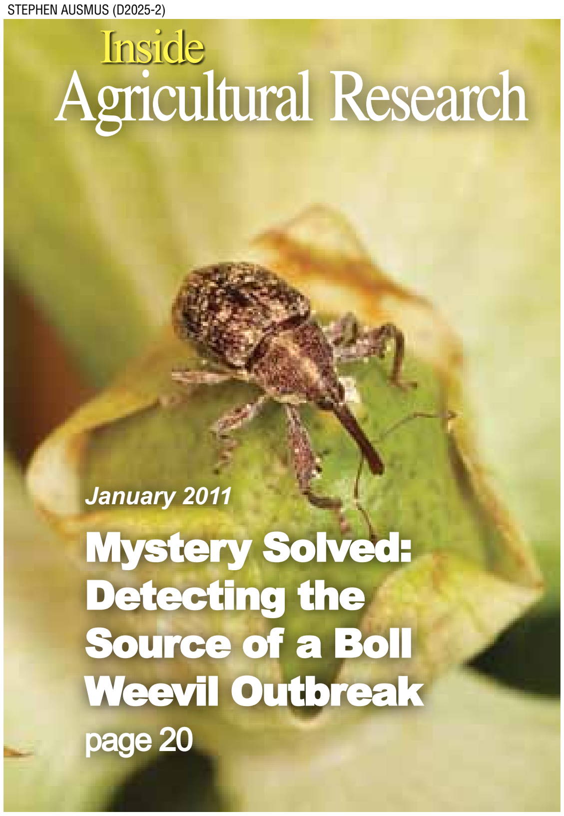
PRST STD
Postage and Fees Paid
U.S. Department of Agriculture
Permit No. G-95

Please return the mailing label
from this magazine.

To stop mailing ☐

To change your address ☐

STEPHEN AUSMUS (D2025-2)



Subscribe

for $60.00 per year
($84.00 foreign
addresses)
Visa and Mastercard
accepted.
Prices subject to change.
Order By:
Fax: (202) 512-2104
Phone: (202) 512-1800
Mail:
New Orders
Superintendent of
Documents
P.O. Box 371954
Pittsburgh,
PA 15250-7954

bookstore.gpo.gov.

PEGGY GREB (D2076-1)

Visit us on the Web at ars.usda.gov/ar