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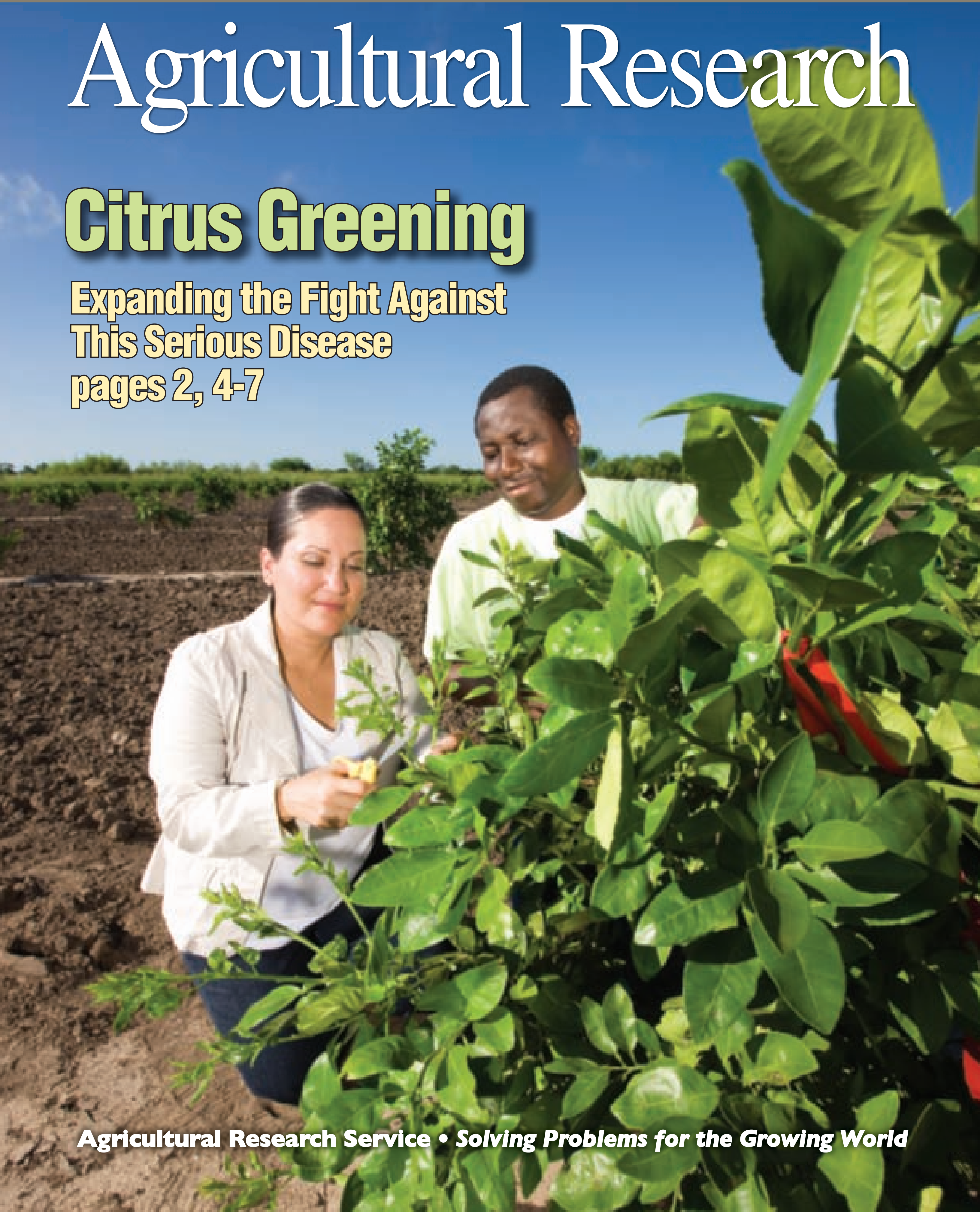
Agricultural Research

Citrus Greening

Expanding the Fight Against This Serious Disease

pages 2, 4-7



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

Working Together To Fight Citrus Greening

Of all the pests and diseases that affect the U.S. citrus industry, citrus greening, also known as "Huanglongbing" (HLB), is the most serious to date. The disease has already greatly affected the Florida citrus industry, causing millions of dollars in damage and lost revenue. The Asian citrus psyllid (ACP), the vector that spreads the disease, has recently been found in Texas and California, the other primary citrus-producing states. If HLB becomes established in these locations, the impact on the states' economies will be significant, as will the lack of domestically supplied citrus and related products. That's why ARS scientists all over the country are feverishly working to better understand and manage the disease and its vector.

Our scientists in Fort Pierce, Florida, have published the full genomic sequence of *Candidatus* Liberibacter asiaticus, the bacterium believed to cause HLB. This advance will enable researchers to identify genes responsible for the disease, enhance capabilities to culture this organism, and determine its presumed role as the causal agent of HLB. Scientists in Fort Pierce, Florida; Beltsville and Fort Detrick, Maryland; Parlier, California; and Weslaco, Texas, have developed tests and tools to improve detection of HLB and ACP. And researchers in Riverside, California, have begun testing citrus and related germplasm for tolerance or resistance to the disease and its vector.

But we know we can't take on this problem alone. In response to requests from U.S. citrus industry representatives, ARS and sister organizations the Animal and Plant Health Inspection Service (APHIS) and the National Institute of Food and Agriculture (NIFA) began a dialogue with stakeholders and state research programs to build a framework for ongoing and future HLB research. Within this framework, research projects will not continue to proceed as individual, separate entities. Instead, they will be better coordinated toward a common goal: to ensure a productive and thriving citrus industry in the face of serious diseases and pests.

In December 2009, industry and government met to establish the Citrus Health Science and Technology Coordination Group. It consists of representatives from ARS, APHIS, NIFA, the National Academy of Sciences, the Florida Citrus Production Research Advisory Council, Florida Citrus Mutual, the California Research Board, California Citrus Mutual, and Texas Citrus Mutual. At that time, three desired outcomes that correspond to each main citrus-producing state and its status with regard to HLB were identified: 1) Keep citrus groves currently affected with ACP and HLB as productive as possible (Florida); 2) Prevent or slow the spread of ACP and HLB (Texas); and 3) Keep unaffected citrus groves and trees free of ACP and HLB (California).

The Science and Technology Coordination Group also established outcome-based research groups during a 2-day workshop this past March. There, key selected researchers who are representative of and fully engaged in research related to each outcome met to develop a research framework and identify gaps for future research progress toward solutions to HLB. Representatives from ARS, APHIS, NIFA, state departments of agriculture, and citrus industry members were present to support the process and reinforce the need for a coordinated approach to planning. At this meeting, attendees also identified the need for more robust transfer and use of knowledge and products that arise from new research.

To expand on the initial planning session with the Science and Technology Coordination Group, the citrus industry hosted a research-discussion-based workshop as the first Citrus Health Research Forum this past June. Participation was expanded to include all researchers in the national citrus community—pathologists, entomologists, horticulturists, and geneticists—who were tasked with developing a strategic roadmap leading to the three desired outcomes identified last year.

Forum participants agreed that a holistic system—which includes plant nutrition, psyllid suppression, early detection and new diagnostic tools, removal of infected trees, and the potential use of various therapeutics, such as antibiotics—is necessary to maintain healthy citrus trees. To protect healthy replants, groves, and trees, participants discussed strategies for developing HLB-resistant trees and using various approaches that could forestall infection. The group also identified research gaps in better understanding ACP behavior, disease transmission, molecular methods for control, biological control, and best practices for developing an areawide control program. And because HLB affects citrus production worldwide, participants emphasized the importance of involving neighboring countries like Mexico and other international partners in the research and extension efforts.

More details from the forum, including poster abstracts and presentations, can be found on the ARS website at www.ars.usda.gov/citrusgreening/.

The forum was so well received that participants agreed to continue the dialogue and brainstorming activities with a special session at the International HLB/ACP Symposium in January 2011 in Orlando, Florida. International partners will join us there as we discuss the progress we've made and new challenges we face.

Through continued cooperative research and sharing of information, we will be better equipped to manage HLB outbreaks. With a unified strategic plan in place, we are now working with our partners to target resources toward research that will have the greatest impact on meeting short- and long-term goals for a productive, sustained citrus industry.

Gail Wisler
ARS National Program Leader
Crop Production and Protection
Beltsville, Maryland

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Solving Problems for the Growing World

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PEGGY GREB (D1989-1)

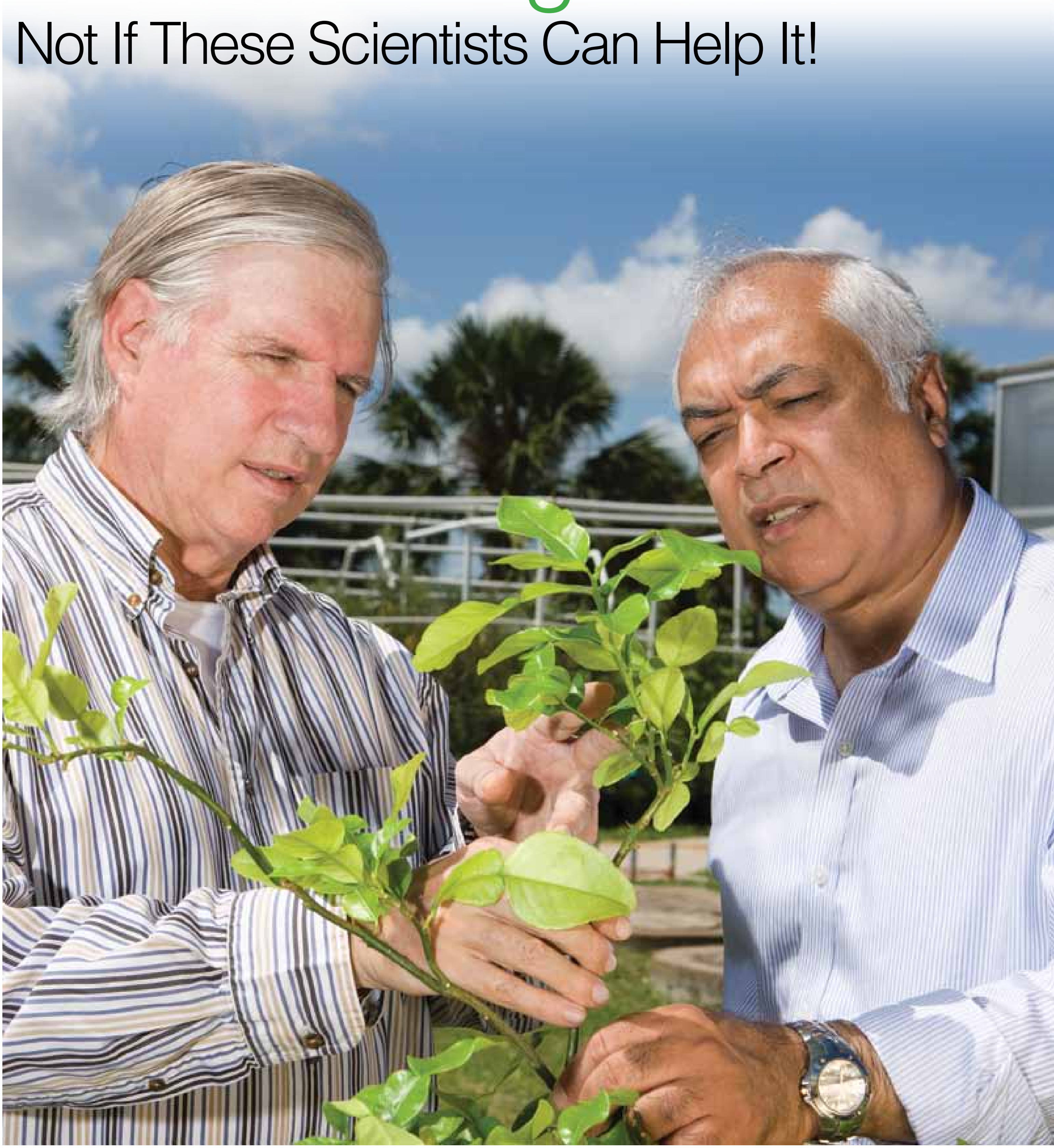
ARS is finding predators to fight the western corn rootworm (above), the most economically damaging pest of corn in North America. Story begins on page 14.

Cover: Graduate student Deanna Chapa and entomologist Mamoudou Setamou (of the Citrus Center at Texas A&M University, Weslaco, Texas) sample shoots from young citrus trees for evaluation of insecticide levels in stem phloem tissue as part of studies on controlling Asian citrus psyllid, the pest known to spread citrus greening disease. Story begins on page 4. Photo by Peggy Greb. (D2002-1)

Citrus Greening?

Not If These Scientists Can Help It!

PEGGY GREB (D1996-1)



Entomologist Robert Mangan (left) and plant physiologist Nasir Malik of ARS's Kika de la Garza Subtropical Agricultural Research Center, Weslaco, Texas, observe Asian citrus psyllid infestation on new growth, called "flush," on a Kaffir lime (also known as Kieffer lime). The tree was completely defoliated and then given 2 weeks of specific environmental conditions to induce new flush on all its branches.

In the Rio Grande Valley of southern Texas, citrus orchards cover the landscape. Texas is the third largest producer of citrus fruit in the United States, and most of it is grown in the Rio Grande Valley. Here, grapefruit is king, but orange, tangerine, tangelo, and Meyer lemon are also grown.

A couple of years ago, growers in this region started seeing a new insect on their citrus trees: the Asian citrus psyllid. This tiny pest is responsible for transmitting the deadly citrus greening disease, also known as "Huanglongbing," which devastated the Florida citrus industry in 2005.

Fearing they would have an outbreak of the same magnitude, Texas growers approached Agricultural Research Service scientists at the Kika de la Garza Subtropical Agricultural Research Center (SARC) in Weslaco, Texas, for help in preparing for an outbreak.

"We have yet to see citrus greening in our area, but the presence of the Asian citrus psyllid means it may be only a matter of time until trees become infected," says Robert Mangan, research leader of the Crop Quality and Fruit Insects Research Unit (CQFIRU) at SARC and areawide project manager. "That's why our scientists are working closely with federal and university collaborators to develop an areawide approach to control infection and spread of the disease."

Understanding Psyllid Habits

Every week, CQFIRU entomologist Donald Thomas visits citrus groves to collect samples of psyllids and potential predators. Using a small, modified hand-held vacuum and sticky traps attached to trees, Thomas has monitored psyllid populations in the area over the past year. As expected, he found that psyllid populations fluctuate depending on the amount of rain in the area. After heavy rains, psyllid numbers increase as new growth on citrus trees, called "flush," appears. That's because the psyllid relies entirely on flushing shoots for reproduction and development.

Thomas also found that psyllid populations tend to decrease during winter, when cool temperatures keep citrus trees from flushing. During this time, he found the psyllids living in other trees surrounding citrus groves, such as torchwood, persimmon, and mesquite. Thankfully, the psyllids don't seem to be reproducing on these trees, but further testing to determine this is under way. Thomas found abundant psyllids in abandoned citrus groves and urban areas where citrus trees are planted in backyards, indicating the need to develop management tools for use in those areas.

With help from colleagues in Texas and Florida, Thomas has identified at least a dozen psyllid species present in south Texas citrus groves. Some are native to the Rio Grande Valley, but others—like the Asian citrus psyllid—are not. The scientists are examining the life cycles of the species to see whether any of them can spread citrus greening. This is important in helping determine which species to target for control measures.

Thomas is also working with Mexican and U.S. federal cooperators to examine potential biological control agents for the psyllid population. A tiny wasp, *Tamarixia radiata,* has shown the most promise during observational and preliminary testing. Lacewings, beetles, spiders, and predatory mites are also candidates.

Genetically Identifying the Psyllid

In a related project that complements the areawide project, molecular biologist Jesse de León, with the Beneficial Insects Research Unit (BIRU), and colleagues from North and South America are applying molecular tools to genetically characterize the Asian citrus psyllid. This will help scientists determine where to collect preadapted natural enemies of the insect for a biological control program.

The researchers found two groups of Asian citrus psyllids in the Americas—one prevalent in South America, the other in North America. But through molecular testing, De León found the two psyllid groups to be genetically distinct. This suggests that the Asian citrus psyllid in North America did not come from South America; rather, each continent was probably invaded by insects from different countries in Asia. So, scientists may need to collect natural enemies from various Asian countries to control the psyllids in North and South America.

De León's team is also studying the *T. radiata* wasp, one of the psyllid's natural enemies, to determine whether it exists in the Americas as a single species or as genetically distinct populations. So far, the scientists have identified four groups of *T. radiata* in the Americas. The scientists will continue to apply other molecular tools and perform cross-mating studies to determine whether these groups represent a single species, very closely related species, or what's known as "cryptic species," look-alikes that are genetically different. Accurately identifying *T. radiata* will help the scientists develop a successful biological control program.

PEGGY GREB (D1997-1)

Biologist Jim Brockington (front) and plant physiologist Nasir Malik record the number and location of sprouting buds on citrus trees exposed to different environmental conditions in a growth chamber.

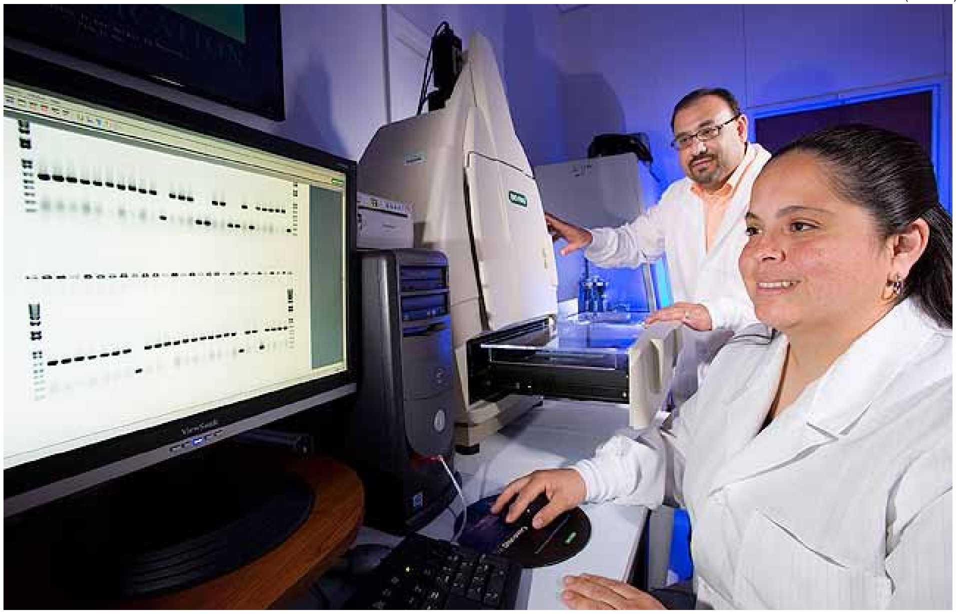
What's That Smell?

These days, entomologist Joseph Patt's laboratory smells more like a perfumery than a laboratory. That's because Patt, who is also with BIRU, is experimenting with petitgrain oils—essential oils used as perfume bases—to see which work best in mimicking the flushing shoots that attract Asian citrus psyllids. The plant volatiles emitted from these shoots may play an important role in helping the psyllids find suitable host plants on which to reproduce. A key aspect of developing a management system for psyllids is to find an attractant for baiting, trapping, and killing them.

The psyllid is commonly found on orange jessamine, a popular ornamental plant related to citrus. By analyzing the volatiles that make up orange jessamine's scent, Patt was able to formulate a synthetic mixture that proved attractive to psyllids in greenhouse testing.

The Asian citrus psyllid relies on both sight and scent to find food. Patt tested petitgrain oils on yellowish-green-colored cards, developed by Alpha Scents, Inc., to monitor

PEGGY GREB (D1999-1)



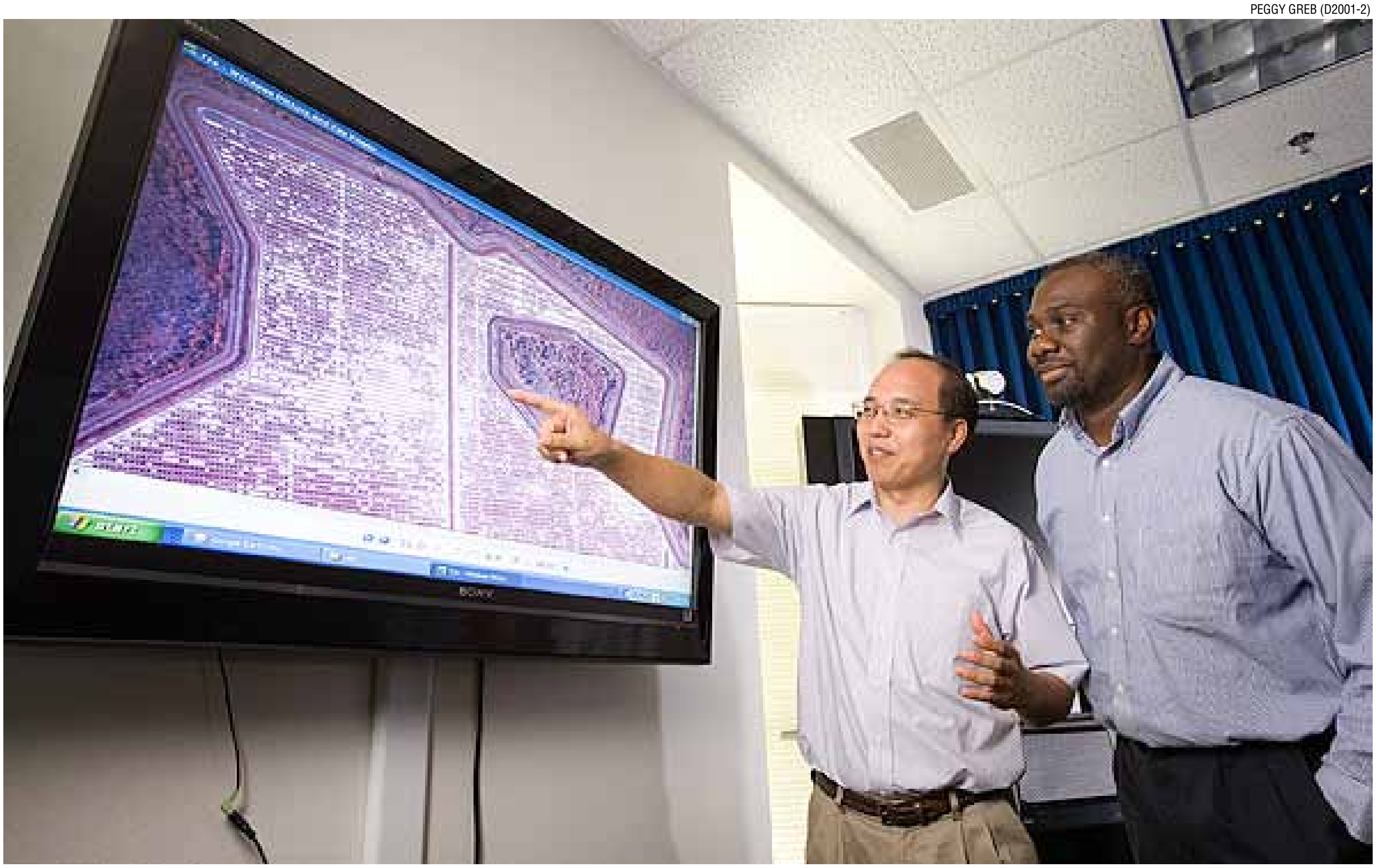
To determine where to collect natural enemies of the Asian citrus psyllid for use in a biological control program, molecular biologist Jesse de León and technician Marissa González perform a DNA analysis of psyllid populations collected in North and South America.

PEGGY GREB (D2004-2)

Adult asian citrus psyllids on a citrus stem.

Growers looking for ways to control psyllids in their citrus groves have a new

PEGGY GREB (D2001-2)



Though citrus greening is not known to be in Texas, it has wreaked havoc in Florida. ARS researchers in Weslaco, Texas, are collaborating with University of Florida researchers to fight the disease. Above, agricultural engineer Chenghai Yang (left) and soil scientist Reginald Fletcher, both with ARS at Weslaco, evaluate an aerial color-infrared image of an infected citrus grove in Florida. Trees infected with the disease have been removed from the grove as shown by the white areas and gaps along the rows. By removing infected trees, the researchers hope to prevent the disease from spreading.

PEGGY GREB (D2000-1)

ARS biological science aide Jesus Gonzalez vacuums a citrus tree to collect samples of Asian citrus psyllids for population monitoring studies.

PEGGY GREB (D1998-1)

Entomologist Joseph Patt and technician Alexandra Gomezplata examine back yard traps baited with attractants for Asian citrus psyllid.

tool: an online national database of approved pesticides for psyllid control.

the insect. Greenhouse studies showed that cards scented with petitgrain oils attracted more psyllids than unscented cards did. In the future, the scented, colored cards could be used in urban areas, where wide spraying of pesticides is forbidden, to disseminate biological control agents like fungi that will affect just the psyllid.

While Patt's results are promising, he must still confirm his findings in the field and conduct further studies to find the combination of scent components that is most attractive to these psyllids.

Inducing Flushing All Year Long

Currently, researchers seeking to better understand the Asian citrus psyllid's interaction with citrus plants must schedule their studies around when the plants flush. In the race to develop solutions to control citrus greening before it reaches southern Texas, the researchers cannot afford any lost time.

Plant physiologist Nasir Malik, with the Integrated Farming and Natural Resources Research Unit, is coming to their aid. His goal is to induce flushing in citrus trees to allow scientists to conduct year-round testing.

Malik has developed temperature-controlled growth chambers in which to grow citrus plants. He placed grapefruit trees in the low-temperature chambers, which were kept at 4˚–8˚C during the night and 18˚C during the day, for different lengths of time. The plants were then transferred to the greenhouse, where temperatures were warmer (26˚C at night and 29˚C during the day).

The results were outstanding. Malik was able to induce some flushing in trees that stayed in the growth chambers for as little as 2 weeks, but longer treatments induced more flushing. Trees that spent 10 weeks in the growth chambers flowered almost immediately after being transferred to the greenhouse.

Thanks to Malik, scientists can produce new flushes at will, which speeds up testing. Malik plans to expand his testing to longer intervals to see if he can achieve 100-percent flowering on the trees. He has also successfully induced branching, or new flush, in other trees, such as guava, pomegranate, and chapote *(Casimiroa greggii),* a small tree native to Mexico, thus demonstrating the utility of the technique for the nursery industry in general.

Need Something Now? Just Look Online

Growers looking for ways to control the psyllids in their citrus groves have a new tool: an online national database of approved pesticides for psyllid control.

Compiled by graduate student Deanna Chapa and university, private industry, and government cooperators, the database is the first organized collection of label information that growers and researchers can rapidly consult to determine which pesticides are effective in their area or on their crop. Users can set up queries based on criteria they select or enter. And if categories need to be added, just contact Chapa, who will continue working on this database.

With the help of ARS researchers and collaborators, citrus growers and enthusiasts in the Rio Grande Valley will be well prepared for a future citrus greening outbreak.—By **Stephanie Yao,** ARS.

This research is part of Crop Protection and Quarantine (#304) and Methyl Bromide Alternatives (#308), two ARS national programs described at www.nps.ars.usda.gov.

Robert Mangan, Donald Thomas, Jesse de León, Joseph Patt, Nasir Malik, and Deanna Chapa are with the USDA-ARS Kika de la Garza Subtropical Agricultural Research Center, 2413 East Highway 83, Weslaco, TX 78596; (956) 447-6316 [Mangan], (956) 447-6315 [Thomas], (956) 969-4856 [de León], (956) 969-4801 [Patt], (956) 969-4814 [Malik], (956) 447-6320 [Chapa], robert.mangan@ars.usda.gov, donald.thomas@ars.usda.gov, jesus.deleon@ars.usda.gov, joseph.patt@ars.usda.gov, nasir.malik@ars.usda.gov, deanna.chapa@ars.usda.gov. *

Better Ballfields and Rain Gardens Start Below the Surface

STEPHEN AUSMUS (D1976-3)

ARS hydrologist Douglas Boyer (right) and Beckley Sanitary Board operations manager Jeremiah Johnson discuss the performance of a rain garden constructed from local materials. The rain garden is being tested for its ability to reduce storm water runoff, increase infiltration, and remove excess nutrients and other pollutants from the runoff water before it gets to streams or other bodies of water.

The townspeople of Beaver and Beckley, West Virginia, soon may not have to look any farther than their neighborhood ballfield or rain garden to see the benefits of Agricultural Research Service (ARS) research.

The Appalachian Farming Systems Research Center (AFSRC) at Beaver, West Virginia, is the only ARS lab working on constructed or replacement subsoils and topsoils for growing better turfgrass on ballfields, for growing rain gardens that not only reduce rain runoff but also clean pollutants from rainwater, and for growing vegetation on former mineland.

National Turfgrass Research Initiative Works With ARS

The constructed-soil initiative is in its fourth year. ARS is doing the research in cooperation with the National Turfgrass Research Initiative, Inc., a joint turfgrass industry/ARS program created in 2007.

The initiative partners the expertise of ARS and universities. The turfgrass industry has set a high priority on improving degraded soils by constructing soils that include readily available rural, urban, and industrial byproducts that can be mixed with local soils.

Working With Everybody

ARS lead scientist Rich Zobel and the soil resource management team at AFSRC work with USDA's Natural Resources Conservation Service (NRCS); the city of Beckley, West Virginia; the Raleigh County Solid Waste Authority; West Virginia State University at Institute; West Virginia University at Morgantown; Virginia Tech at Blacksburg; the University of Ohio at Akron; and various ARS labs, including two regional research centers. Two local groups interested in water resource management and the U.S. National Park Service are also doing rain garden projects.

Zobel says these collaborations “add expertise in civil engineering, economics, landscape design, and environmental regulation.”

Zobel and research associate Amir Hass, who is with West Virginia State University and stationed at Beaver, work with Doug Boyer, a hydrologist, Charlie Feldhake, a soil scientist, and Javier Gonzalez, a soil chemist, all at AFSRC.

Constructed soil is a much more sophisticated version of the home gardener’s method of improving the soil by blending in sand, topsoil, and organic amendments such as humus, composted manure, sewage sludge, or yard and garden clippings.

A good constructed soil goes way beyond these conventional recipes. Research and chemical analyses are used to determine what is needed to ensure that soil does not become compacted and has excellent drainage, water-holding capacity, and aeration properties, plus essential nutrients for robust plant growth.

SUSAN BOYER (D1983-1)

STEPHEN AUSMUS (D1977-16)

ARS plant physiologist Rich Zobel (left) discusses the subsoil compaction plots with James Allen, director of Raleigh County Solid Waste Authority. Study results will likely shape future recommendations for athletic fields and home lawns.

Subsoil compaction plots before topsoil is added. The red/orange subsoil is a Greenbrier County (West Virginia) limestone-based soil. The lightest colored plots are Raleigh County (West Virginia) sandstone, and the darker subsoils are Raleigh County shale.

Finding the Best Soil Recipes

Zobel and colleagues are formulating recipes for constructing “designer soils” from various source materials in Ohio, Kentucky, Tennessee, West Virginia, Virginia, and southern Pennsylvania and using materials such as mine spoils, coal-combustion byproducts, poultry litter, and biochar as amendments.

So far, the most promising mixture contains quarry byproducts and composted chicken litter. It has met predetermined requirements such as the ability to transmit stormwater and sustain turfgrass or other vegetation. Chicken litter is the best organic component found so far. Chicken litter is a mixture of droppings and bedding materials—usually sawdust and wood shavings. The litter is composted in a “digester,” designed by West Virginia State University, to stabilize the mixture and remove odors and harmful microbes. Three years of testing have shown the composted poultry litter works as well as commercial fertilizer in establishing vegetation on the mined soils.

Fixing Compacted Ballfields

Zobel is initially focusing on compacted soils. He says, “Soil compaction is the first cause of problems in growing good, robust grass on ballfields and other sites. Compacted subsoil causes the grass roots to stop short, restricting the amount of soil water available. These grasses grow weakly and are easily killed by heavy use of the site.”

The scientists established experiments at the Raleigh County landfill site. They have several students from Virginia Tech working on various projects to design the specifications for bringing former landfills back into use as ballfields or residential or commercial sites.

At the landfill site, they are testing subsoils with varying levels of compaction overlain with three depths of topsoil. They will plant turfgrass and determine which combinations provide the most suitable turf. From this will emerge the best management practices for building subsoil and topsoil for playing fields. As part of the project, Virginia Tech researchers are also testing nearby football and soccer fields for compaction and nutrient content to determine recommendations for field renovation specifications.

Rain Gardens Sprouting Up Everywhere

Rain gardens are increasingly popular with homeowners and municipalities and are mandatory for many communities nationally. They are plantings in manmade depressions that catch stormwater runoff from sidewalks, parking lots, roads, and roofs. Rain gardens are constructed in various shapes and sizes, from large basins carved by front-end loaders to small streambed-like formations complete with pebbles. Not only do rain gardens slow water down to give it time to soak into the ground and be used by plants, but they also filter out sediment and chemical pollutants.

Many gardens use mainly native flowers with some grasses, but the “constructed swale” type of rain garden uses turfgrass. The only water the plants receive is the runoff from rain. Homeowners usually direct the runoff from their rain gutter downspouts with lengths of plastic corrugated drainage pipe.

“Some rain gardens, as well as ballfields, have had to be torn out and rebuilt because they weren’t built right,” says Kevin Morris, executive director of the National Turfgrass Evaluation Program. “You have to draw on scientific research to get fields and rain gardens right the first time.”

Biochar To Clean Rain Garden Water

Hass, Gonzalez, and Zobel want to take that several steps further. They are working with the ARS Southern Regional Research Center (SRRC) in New Orleans, Louisiana, and the ARS Eastern Regional Research Center in Wyndmoor, Pennsylvania, to use biochar in rain garden soils to neutralize fertilizers, pesticides, and chemicals leaked by cars. The most familiar example of biochar is the activated charcoal used in aquarium filters. Biochar is produced by subjecting materials such as coal to either gasification or pyrolysis. These are both extremely high-temperature processes that take place in the absence of oxygen.

SRRC chemists Isabel Lima and Wayne Marshall (now retired) developed an ARS-

STEPHEN AUSMUS (D1973-6)

Chemist Javier Gonzalez analyzes the composition of water-soluble organic matter from biochar. Biochar is being tested as a soil amendment to improve productivity of marginal soils.

STEPHEN AUSMUS (D1974-2)

In simulated rain garden trials, technician Gary Lambert collects leachate to identify plants best able to slow movement of water, nutrients, and fecal bacteria through soil.

patented method for turning agricultural biowaste into material similar to activated charcoal. But their materials are superior to activated charcoals.

Hass, Gonzalez, and Zobel are cooperating with colleagues at the regional research centers who are interested in biochar made from a mixture of poultry litter and peanut shells. They found that poultry litter biochar is a powerful pollutant magnet. It can attract heavy metals such as copper, cadmium, and zinc, which are ordinarily tough to snag, from wastewater.

Zobel is experimenting at two demonstration rain gardens in the Beaver area, as well as at plots at a county landfill and a mineland reclamation site.

Money for Sporty Topsoil

Morris says, “There is money for constructed-soil research for golf courses and college and professional-level playing fields, but not for sports fields at the high school, elementary school, or park levels—until now. The costs are lowered by using inexpensive byproducts available locally.”

From their past research, ARS scientists know that certain soils are unusable for ballparks and rain gardens because they tie up phosphorus, making it unavailable to plants. Therefore, ARS is working with the West Virginia Natural Resources Council and NRCS to develop a computer model that will help users choose which local soils are best for ballparks, soccer fields, rain gardens, and other uses. It will use the NRCS soil survey data, the first such use of this data.

Natural Turf vs. Artificial Turf

“Natural turf can get a bad name from overuse of poorly constructed fields,” Morris says, “but it is an inexpensive alternative to artificial turf. It can compete well when everything works. Natural turf has all the resiliency of artificial turf.”

Over the long term, Zobel envisions new turfgrass varieties, possibly perennial ryegrass and tall fescue, that will penetrate compacted soil and renovate fields without the need to till the compacted soil. “We have a lot of information from previous research on roots, soils, and microbes that we can draw on for the constructed-soil project,” Zobel says. He sees an eventual extension of the constructed-soils research and demonstration projects to homeowners and homebuilders for improving their lawns and rain gardens.

This research should one day help provide specifications for improved turfgrass fields that even the smallest of parks and schools can afford.—By **Don Comis,** ARS.

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

To reach scientists mentioned in this article, contact Don Comis, USDA-ARS Information Staff, 5601 Sunnyside Ave., Beltsville, MD 20705-5129; (301) 504-1625, donald.comis@ars.usda.gov. *

Pest-Free Christmas Trees

ROBERT HOLLINGSWORTH (D2017-1)

Washington State University plant pathologist Gary Chastagner uses a mechanical tree shaker at a commercial shipping yard in western Oregon.

When it comes to Christmas trees, Hawaii residents are just like residents on the mainland: They want the best trees they can get. But their quest to find the perfect tree could be hindered by pests, including yellowjackets, that hitch along for the ride from the Pacific Northwest to the Island State.

Although yellowjackets usually nest in the ground, mated queens who haven't yet built their nests sometimes make Christmas trees their home during the winter. So, come November, when trees are rounded up for shipment to Hawaii, these queens get rounded up right along with them.

The western yellowjacket *(Vespula pensylvanica)* is an invasive insect that has already established itself throughout the Hawaiian islands. A voracious predator, it competes with native birds for insect prey, greatly reducing native insect populations. Scientists and regulators are particularly concerned because other yellowjacket species not yet established in Hawaii could also hitchhike in Christmas trees and cause ecological damage in their new home.

In an effort to keep more unwanted insects from getting to the islands, Hawaiian officials required exporters to screen for live insects by either manually shaking 10 percent of the trees in front of mainland agricultural inspectors or mechanically shaking 100 percent of the trees with no inspector present. Most growers opt for mechanical shaking, but there is no precise specification for how long the trees should be shaken—yet that makes a big difference in whether insects remain on the trees.

Entomologist Robert Hollingsworth, with the Pacific Basin Agricultural Research Center in Hilo, Hawaii, and colleagues from the Washington State University Puyallup Research and Extension Center, the University of Hawaii, and the Hawaii Department of Agriculture examined the efficacy and adequacy of the tree-shaking program. The scientists found that while mechanical shaking was significantly more effective than manual shaking, both methods failed to remove all yellowjacket queens from the trees.

Hollingsworth and colleagues tested the use of preharvest insecticide sprays as a supplement to the shaking treatment to further reduce the risk of exporting yellowjacket queens and other insects along with the Christmas trees. These results were more promising.

"We found that mist applications of an insecticide in the pyrethroid chemical class were 100-percent effective in killing yellowjacket queens and honey bee workers, which were used as surrogates for wasp pests," explains Hollingsworth. "The residues of insecticides applied 1, 3, and 6 weeks before harvest were still effective in killing insects at harvest time, despite the heavy rainfall that occurred after spraying."

Pyrethroids pose less risk to humans than many other insecticides, and products containing them are approved by the U.S. Environmental Protection Agency for use on Christmas and other ornamental trees. Some growers are already using them.

Following Hollingsworth's study, and perhaps as a result of it, Hawaii now requires that all Christmas trees destined for the islands be shaken, regardless of shaking method. Future research plans include finding out how to make the shaking treatments more effective in the absence of an insecticide treatment.—By **Stephanie Yao,** ARS.

This research is part of Crop Protection and Quarantine (#304) and Methyl Bromide Alternatives (#308), two ARS national programs described at www.nps.ars.usda.gov.

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Taking a Closer Look at Tannins

Biologist Harry Godwin collects rainwater that had run down a tree to test it for tannins or other phenolic compounds. Behind him, technician Jennifer Harrah collects soil for experiments to determine whether the compounds affect soil organic matter and nutrient cycling.

STEPHEN AUSMUS (D1975-41)

The tannins that bring us good red wine and fall colors may also be those that make soil good and livestock healthy.

Phenolic compounds, including tannins, are common in many plants. These compounds are believed to enter soil from plant roots, from decaying leaves, and possibly from "teas" formed as rain and snowmelt run down tree bark and drip off the leaves.

Javier Gonzalez, a chemist, and Jonathan Halvorson, a soil scientist, at the Agricultural Research Service's Appalachian Farming Systems Research Center (AFSRC) in Beaver, West Virginia, head a tannin project, now in its third year. Together with studies by other AFSRC scientists, their work indicates that tannins are important components of agroecosystems that can affect production and environmental quality.

Tannins and Silvopastures

The researchers became interested in tannins through their work with silvopastures, intentionally thinned wooded areas used to graze livestock. Soil processes and water quality in silvopastures may be influenced by the mix of trees or the type of compounds they release into soil.

Halvorson and Gonzalez wondered whether some species would add different chemical compounds, including tannins, to soil than others. They also wanted to know how the thinning would affect the types and amounts of tannins in soil and, in turn, the effects on soil organic matter and the entire ecosystem.

It all came down to one main question for Gonzalez: "Which trees should I cut down to make room for grazing livestock and not adversely affect soils in the long term?"

She Wrote the Book on Tannins

A call from Halvorson with a question about tannins early in the project brought in the expert who literally wrote the book on tannins, Ann Hagerman.

Hagerman, of Miami University in Oxford, Ohio, is an expert on tannin chemistry. She wrote the *Tannin Handbook,* a compilation of methods developed in her laboratory for analyzing tannins.

Hagerman says, "Tannins in the environment are not a new topic. They've been targeted as being important players in soil formation and water chemistry for many years. But our understanding of the processes has been hampered for a long time by the very use of the term 'tannin.'

"The problem is that there are probably at least 1,000 very distinct compounds that all fall into the category 'tannin.' This is where my chemistry background comes into play. When my lab does an experiment, we don't think 'tannin'—we go for a specific compound with a specific, known chemical structure."

SUSAN BOYER (D1978-1)

Sheep in a silvopasture at the Appalachian Farming Systems Research Center in Beaver, West Virginia.

Testing Tannins' Many Roles

A cooperative agreement, combining the capabilities of Hagerman's lab with those of AFSRC, was developed to identify important features of tannins that determine their interaction with soils. Results from joint studies by Miami University staff and students and ARS scientists will be used to develop recommendations for using tannins to manage soil.

Halvorson, Gonzalez, Hagerman, and colleagues added tannin solutions to forest and pasture soil samples and found that the rate and amount of binding to soil varied by the type of tannin present. With ARS scientists from Pendleton, Oregon, they also tested the influence of different soil amendments on tannin binding and compared soils from around the United States and Canada. Halvorson and Gonzalez also observed how some tannins interact with soil proteins, another indicator of the important role of tannins in soils.

STEPHEN AUSMUS (D1972-3)

ARS technician Dianne Snuffer extracts tannins from samples of rainwater collected after it ran down trees. She is using the solid phase extraction technique to investigate the effects of tannins on soil organic matter, nutrient cycling, and water quality.

ARS scientists are also learning more about how tannins interact with important metals in soil. Studies headed by Gonzalez and a postdoctoral scientist at Miami University are determining how metals are mobilized in the soil by phenolic compounds related to tannins. In other work with Hagerman, AFSRC plant physiologist Thomas Kinraide found that binding by tannins may alleviate the toxic effects of metals, such as aluminum, on root growth.

Hagerman and ARS colleagues are also beginning studies of how forest soil microbes metabolize tannins and related compounds. They are using a device that measures CO_2 emitted from microorganisms in soil. "We found the technique allowed us to accurately compare soil microbial responses to various tannin compounds," Hagerman says. "This should help us learn about the varied ecological functions performed by tannins."

Tannins May Benefit Livestock, Too

Other AFSRC scientists, including chemist Joyce Foster and animal scientist Kenneth Turner, are evaluating the nutritional and deworming potential of tannin-containing forages, like birdsfoot trefoil for goats.

With the knowledge gained from this project, scientists expect to learn how to manage agricultural soils for the benefit of production and the environment by taking advantage of natural help from certain tannins.—By **Don Comis,** ARS.

This research is part of Pasture, Forage, and Range Land Systems (#215), an ARS national program described at www.nps.ars.usda.gov.

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Predators Can Be a Farmer's Best Friend

An adult western corn rootworm *(Diabrotica virgifera virgifera),* the most economically damaging pest of corn in North America.

PEGGY GREB (D1990-2)

One of the worst pests of corn in the world, the corn rootworm, may owe its success partly to its larvae's very sticky blood.

Jonathan G. Lundgren, an entomologist at the Agricultural Research Service's North Central Agricultural Research Laboratory in Brookings, South Dakota, and colleagues discovered this recently, working with CABI researchers in Switzerland and Hungary.

CABI is an international, not-for-profit, science-based, development and information organization that researches natural ways of controlling pests, and it has been helping to lead the effort against corn rootworm's European invasion.

The work with CABI involved 2 years of laboratory and field experiments, begun in 2007, in the United States and Hungary. The experiments are the latest in Lundgren's research on corn rootworm predators, which began 5 years ago. Although rootworms have been a major pest for 100 years, this is—remarkably—the first comprehensive research program on corn rootworm predators to be conducted.

Sticky Blood Shuts Down Would-Be Rootworm Predators

In the Brookings laboratory, Lundgren and colleagues offered hungry predators (from formidable ground beetles to wolf spiders to ants) a smorgasbord of rootworm larvae and pupae. In all, they have tested 10 different predator species from Europe and North America, including beetles native to central Europe and those native to the Midwest and Great Plains of the United States.

The rootworms came from the Brookings lab's world-class rootworm-rearing facility.

The predators aggressively attacked the squirming rootworm larvae and immobile pupae at first. They wolfed down the pupae. But immediately after attacking the larvae, some of the predators quickly retreated and began vigorously cleaning their mouths, which had been glued shut by coagulated rootworm blood. The coagulation is the prey's attempt to heal its wounds from the predator's first bite—as well as to give its predator something else to chew on.

Blood Defense One Key to Rootworm Success in Corn Fields

The ability of rootworm larvae to fend off predators may be a major reason for their success in corn fields. But luckily, not all predators are easily chased off by the rootworm larvae.

While the rootworm larvae repelled all the predators under laboratory conditions, the blood had varying effects on different species.

The sticky blood was most effective against two common species of ground beetles, *Harpalus pensylvanicus* and *H. rufipes.* They did not return for a second try during the 10-minute observation period, nor did the ants or the *Carabus monilis* ground beetle.

Beetles belonging to three of the most voracious predatory beetle species returned almost immediately to have another go at their adversary. They are *Pterostichus anthracinus, Pt. melanarius,* and *Poecilus cupreus.*

Chemoreceptors Are Nice To Have

One of the beetle species that did not return has such long mandibles that the rootworm blood never even reached its mouth, indicating that chemoreceptors in the beetle's external mouthparts—rather than rootworm blood—made them decide against eating the rootworms.

An attacking ant, or the blood itself, cued her sisters in to the repellent rootworm blood such that once an ant attacked, none of her sisters would touch it.

Wolf spiders on the other hand hardly ever left their prey unattended, repeatedly biting the rootworms until the larvae were consumed.

PEGGY GREB (D1991-1)

A larva of western corn rootworm *(Diabrotica virgifera virgifera).*

PEGGY GREB (D1994-1)

In field tests, ants attack a restrained western corn rootworm larva but are soon repelled.

PEGGY GREB (D1986-1)

Entomologist Jonathan Lundgren dissects an insect predator taken from the field to determine what it has been eating.

Field Tests Confirm Predators Prefer Maggots to Rootworms

Field tests in a Hungarian corn field, over the same 2-year period, showed similar results as the lab experiments, but the repulsion and avoidance weren't as extreme.

The scientists pinned mature rootworms and fly maggots (a known favorite prey item for many predators) in place at the base of corn plants and periodically observed their condition throughout the day and night. They also looked for attacking predators. They found that substantially more predators attacked the fly maggots than attacked the rootworms.

Back in South Dakota, Lundgren and colleagues seeded a no-till corn field with rootworm eggs, at the rate of 1,000 eggs per foot of crop row, then captured the predators that came hunting. Scientists collected 1,500 predators of more than 80 species. By searching for rootworm DNA in their guts, Lundgren found that predators with sucking mouthparts (especially spiders, harvestmen, and mites) ate more rootworms than any of the other predators tested.

"This showed that sucking predators, such as wolf spiders, are better suited to eating rootworms than are chewing insects, such as beetles," Lundgren says. He adds that he now suspects the rootworm defense is the reason. "When insects suck fluids, they may be able to bypass the ability of the blood to stick and linger," he says.

The scientists also found that, besides spiders, harvestmen, and small rove beetles, two species of ground beetles *(Scarites quadriceps* and *P. chalcites)* and mites were the most common predators of rootworm larvae and eggs.

PEGGY GREB (D1987-1)

Entomologist Jonathan Lundgren and technician Janet Fergen use sweep nets to collect beneficial insects in an agricultural habitat.

Lundgren says his whole approach is "managing crop fields to encourage large and diverse predator populations. This can be done by reducing or eliminating tillage and pesticide use and diversifying vegetation."

Cover Crop Can Help Predators and Farmers Win

Armed with this information, and knowing the pest he is up against, Lundgren began to manipulate corn fields to try to encourage predators to eat more of the repellent larvae. In a separate 3-year study, Lundgren found that a winter cover crop (slender wheatgrass) increases the number of predators so dramatically that there are few corn rootworms left to eat the roots of corn seedlings when they sprout. This study is ongoing, and Lundgren is working with an economist to determine whether the cover crop can compete economically with using *Bt* corn or other pesticides to control the rootworms.

Over the long term, Lundgren is considering the idea of "knocking out" the rootworm genes responsible for their defense, to make the insects even more susceptible to predators.

"Future research will help to address how the rootworm's defenses contribute to its success as an invasive pest and to develop ways to overcome these defenses in search of sustainable, ecologically based pest management," he says.—By **Don Comis,** ARS.

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

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PEGGY GREB (D1993-1)

A harvestman *(Phalangium opilio),* one of the more important predators of corn rootworm larvae.

Rolling Rye
Guidelines Help Determine When's Best

MATTHEW RYAN (D1980-1)

STEVEN MIRSKY (D1981-1)

In a field of rolled cereal rye, ecologist Steven Mirsky evaluates ground coverage of the rye mulch and weed emergence through it.

Above: A front-mounted roller-crimper rolling over a cover crop.
Left: Front view.

WILLIAM CURRAN (D1981-2)

Cereal rye is increasingly being used as a cover crop because it prevents erosion, helps the soil retain nutrients, and reduces the need to till the soil. Organic farmers also use cereal rye to help control weeds. When used as a cover crop, rye is planted in the fall, killed in the spring, and left to decompose. Then, soybeans and other cash crops are seeded through the dried-up plant residue, which forms a surface mulch.

In some cases, farmers are opting to use a tool known as a "roller/crimper," which can flatten and kill an actively growing field of rye in a single pass. There are several designs, but most involve some type of rolling, paddle-wheel-like cylinder that attaches to a tractor and barrels over a field, tamping and crimping the rye into a smooth mat to kill it. The technology has been used for at least 15 years in small farms in South America and is slowly catching on in the United States, according to Steven Mirsky, an ecologist in the Sustainable Agricultural Systems Laboratory in Beltsville, Maryland.

Mirsky estimates that rolling a field of rye uses 10 times less energy than mowing. Rolling is also faster and only needs to be done once a season. And while mowing leaves bits of rye in the field that decompose quickly, rolling leaves rye residue intact, forming a thick mat that provides better weed suppression.

Rollers present their own challenges. If you roll the rye too early in the spring, it won't die off, but will grow back and take up moisture from the soil, essentially competing with the cash crop and diminishing yields.

But Mirsky and other ARS researchers hope to encourage more U.S. farmers to adopt the technology because of the potential benefits to soil quality and the reduced energy and production costs. They are studying ways to maximize the benefits of a roller, and with the help of a computer model, they are developing guidelines for predicting the best times for growers to use rollers to kill their rye. The guidelines are based on regional weather patterns over the course of a growing season, and their hope is that the guidelines will be used nationwide.

Mirsky planted two common types of rye, Aroostook and Wheeler, in Pennsylvania test plots, at six 10-day intervals, in two successive falls. He used a 1.5-ton steel crimper, constructed by colleagues at Pennsylvania State University, to flatten it out at 10-day intervals each spring. He then visually rated the rye's regrowth on a 0-to-100 scale, 6 weeks after each plot was flattened.

The results show that the best time to roll the rye is when it reaches 50 to 75 percent of its flowering state, because that is when rolling consistently kills the cover crop. The results, published in *Agronomy Journal,* were consistent for both varieties of rye, and it didn't matter exactly when in the fall the rye was planted or when in the spring it was rolled, as long as the plants had reached 50 to 75 percent of their flowering state.

The computer simulations developed as part of the project also adequately predicted the best dates for growers to roll their rye, with those dates timed around regional heating and cooling patterns and how early or late in the fall the rye is planted.

They are working toward a Web-based tool that growers around the country will be able to use, possibly by typing in ZIP Codes or other information that identifies the locations of their fields.—By **Dennis O'Brien,** ARS.

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

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Ridding the Texas-Mexico Border of Cattle Fever Ticks

AL SIEBENAIER (D2013-1)

Above: Entomologist Mat Pound (left) and technician Wayne Ryan load corn, used as bait to attract deer, into the hopper of an automatic deer-collaring device.

Right: Infrared image of a doe in position to be collared at night.

Bovine babesiosis, commonly known as "Texas cattle fever," is a deadly disease of cattle caused by single-celled organisms that are transmitted by cattle fever ticks. Texas cattle fever greatly harmed the cattle industry in the United States until the beginning of the 20th century.

Thanks to highly effective and collaborative control efforts established through the Cattle Fever Tick Eradication Program (CFTEP) in 1906 between producers and state and federal agencies, cattle fever ticks were largely eradicated from this country by 1943. As a result, the United States became free of Texas cattle fever, but cattle fever ticks still thrive in Mexico. CFTEP established a permanent quarantine or "buffer" zone along the Texas-Mexico border to keep the ticks out of the United States. But these potentially disease-carrying ticks—*Rhipicephalus (Boophilus) microplus* and *R. annulatus*—are now reinfesting Texas outside of the quarantine zone, thus increasing the risk of outbreaks of Texas cattle fever. Its reemergence could cause devastating monetary losses for U.S. beef and dairy producers.

Agricultural Research Service scientists in Kerrville, Texas, are developing and testing new interventions to eliminate cattle fever ticks within our borders and mitigate the impact on the U.S. livestock industry.

Once Gone, but They're Back!

"Keeping cattle fever ticks eradicated from the United States, and thus keeping the national cattle herd free of cattle fever, is a current and critical agricultural biosecurity issue of national importance. Bovine babesiosis is listed by the World Organization for Animal Health as a notifiable disease," says Adalberto Pérez de León, director of the Knipling-Bushland U.S. Livestock Insects Research Laboratory in Kerrville. "CFTEP personnel are the only ones who can officially detect and report cattle fever tick infestations, which triggers statutory procedures for eradication. It is estimated that the livestock industry currently saves at least $3 billion annually as a result of the eradication of Texas cattle fever and cattle fever ticks.

"The number of cattle fever tick outbreaks inside and outside of the permanent quarantine zone fluctuates over time. It took 6 years to accomplish reeradication after a significant incursion by ticks into the United States in the 1970s. During the last 5 years, the level of cattle fever tick activity in the United States has again increased to alarming levels."

The increased spread of infestation is likely due, at least in part, to the growing populations of white-tailed deer and other wild ungulates along the Texas-Mexico border. ARS entomologist J. Mathews Pound and his colleagues at the Kerrville laboratory have intensively studied the impact white-tailed deer have on the cattle fever tick population and developed interventions to eradicate the ticks from the country.

Deer Complicate Eradication Efforts

Early in the fever-tick eradication program, white-tailed deer weren't a concern because they were rare in the southern United States. Cattle were dipped in insecticides to kill the ticks, but the ticks eventually found another host as the deer population grew.

"Native and nonnative species of wild ungulates figure prominently among the likely causes for the apparent reinvasion of the United States by cattle fever ticks," says Pound. "White-tailed deer are regarded as the major complicating factor in current eradication efforts since they are suitable hosts for cattle fever ticks. Removing cattle from infested pastures was a viable eradication method in past years, but now it's unlikely to be effective as long as white-tailed deer remain within an area."

To control disease-carrying ticks on deer, Pound and his colleagues developed a device called the "4-Poster Deer Treatment Bait Station" (see *Agricultural Research,* June 2006, pp. 8-9). It lures deer into a feeding apparatus that uses rollers to apply insecticide to the animal's head, ears, and neck. As the deer grooms, it transfers insecticide to other parts of its body, killing most of the ticks on the animal. The current research project Pound leads at the Kerrville laboratory focuses on the development of technologies to eradicate ticks on cattle and wild deer.

Microspheres and Collars Do the Job

Currently, producers with infested pastures must round up and dip their cattle in insecticide every 2 weeks for 9 months until no ticks remain in the pasture. A less labor-intensive and cheaper way to rid the pasture of ticks is needed.

In efforts to help cattle producers, Pound and his colleagues reformulated a broad-spectrum antiparasitic medication—doramectin—into an injectable microsphere treatment.

"A single injection of microspheres—akin to time-release capsules in human drugs—greatly reduces the number of treatments needed and protects cattle for up to 4 months, killing parasites and saving cattle ranchers considerable expense. The technology shows great potential for helping to maintain the eradication of fever ticks from the United States," says Pound.

"The treatment has been tested with excellent results on the island of St. Croix against the tropical bont tick, which transmits a disease called 'heartwater' to cattle. We are working to extend the effective period to 6 months, which would be most useful in treating U.S. cattle for ticks."

Partners for commercializing this technology are being sought.

Heartwater disease causes an acute high fever, loss of appetite, and respiratory distress. It progresses to include excessive chewing motion, eyelid twitching, galloping movements, and eyes rolling back. Death can occur in less than 1 week.

To provide an alternative to the 4-Poster device for treating white-tailed deer, Pound and co-inventor Craig LeMeilleur created a new system that can automatically apply pesticide-impregnated neckbands to wild deer in a bait station apparatus much like the 4-Poster. Instead of rollers, the device has an assembly for holding a collar in an open position and a trigger that applies the collar when a deer places its neck over the trigger support.

DEER'S ROLE

The increased spread of Texas cattle fever is likely due, at least in part, to the increasing populations of white-tailed deer and other wild ungulates that are spreading cattle fever ticks along the Texas-Mexico border.

J. MATHEWS POUND (K2015-1)

Allen Miller (retired ARS agricultural engineer) prepares to inject doramectin-impregnated microspheres developed by ARS into a bull to protect it from tropical bont ticks, known to transmit the potentially fatal heartwater disease on the island of St. Croix, U.S. Virgin Islands.

SCOTT BAUER (K5437-3)

A population explosion of white-tailed deer in parts of the United States has increased the risk of transmission of some diseases.

The automatic collaring device was patented by ARS in 1999, and is now in its fifth generation of development. The collars can be detached remotely after the insecticide's effectiveness wears off.

A population explosion of white-tailed deer throughout the eastern United States has increased the risk of diseases transmitted to humans by different species of ticks. Technologies developed for control of cattle fever ticks on deer will also help control other deer-associated ticks, which transmit diseases such as Lyme disease, human babesiosis, and two kinds of ehrlichiosis.—By **Sharon Durham,** ARS.

This research is part of Animal Health (#103) and Veterinary, Medical, and Urban Entomology (#104), two ARS national programs described at www.nps.ars.usda.gov.

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Saving America's **Hemlocks**

PEGGY GREB (D2008-1)

Horticulturist Susan Bentz and geneticist Richard Olsen examine bagged branches of hybrid hemlocks inoculated with hemlock woolly adelgid.

Hemlocks are popular, graceful trees well known to gardeners and landscapers. Unlike the poisonous herb made famous by Shakespeare and Socrates that goes by the same name, hemlocks are nonpoisonous, shade-tolerant, evergreen trees widely planted in backyards, landscapes, and gardens.

Tsuga canadensis, commonly known as the "eastern hemlock" and nicknamed the "Queen of Conifers," and *Tsuga caroliniana,* commonly known as the "Carolina hemlock," are the most commonly encountered hemlocks in the eastern United States. The eastern hemlock grows naturally in the Appalachian Mountains and as far west as Minnesota, while the Carolina hemlock is restricted to the Blue Ridge Mountains in the Carolinas and Virginia. Both play an important role in their native habitats, helping to form stable communities integral to the health of Appalachian forests.

Until recently, hemlocks have been very popular for landscape planting. But over the last few decades, the eastern and Carolina hemlocks have been under attack by a small sucking insect called the hemlock woolly adelgid *(Adelges tsugae),* or HWA. The insect, which is related to aphids, was accidently introduced from Asia and first observed in Virginia in 1951. It has since spread to forests and backyards in 17 eastern states, killing hemlock trees and devastating natural ecosystems.

"The hemlock trees are the HWA's food source," explains ARS plant geneticist Richard Olsen. "The insect virtually sucks the life out of the tree as it feeds. Unfortunately, our hemlocks in North America aren't resistant to HWA, and no natural enemies of HWA exist here, so we often see a tree killed within just a few years of being infested."

Faced with the extinction of our native hemlocks, the U.S. National Arboretum began a breeding program in the 1990s to determine the potential for crossing native hemlocks with those thought to be resistant to HWA. The work began under the direction of ARS plant geneticist Denny Townsend (now retired) and ARS horticulturist Susan Bentz. Olsen now oversees the breeding program since Townsend's retirement.

Searching for More Resistant Genetic Material

In its native range of Asia, the HWA causes little damage to the hemlock trees it feeds on. This is because hemlocks in that area have evolved with the insect, developing resistance to its attacks. North American hemlocks evolved independently from HWA, which explains their lack of resistance.

At the beginning of the breeding project, ARS scientists obtained exotic species of hemlock from gardens throughout the country. But only two or three hemlock trees from China were available. While these trees were used to produce tolerant hybrids, the scientists needed a more diverse representation of resistant germplasm to optimize long-term success of the hybridization program.

In the early 1990s, ARS and public gardens throughout North America joined forces to create the North American China Plant Exploration Consortium. Together, these institutions made several collecting expeditions to Asia to find resistant hemlocks. The scientists mainly focused their efforts on collecting material from China, searching forests throughout the country. Each institution in the consortium brought back accessions for their respective collections.

"At the end of the expeditions, ARS brought back accessions of three *Tsuga* species: *T. chinensis*, *T. dumosa*, and *T. forrestii*," says ARS horticulturist Kevin Conrad, who co-led the first expedition in 1996 to collect seeds of these hemlocks. "During one of these expeditions, we also found a species related to hemlock called '*Nothotsuga longibracteata*.' This is a novel conifer species gardeners might be interested in."

The ARS accessions are housed in the Woody Landscape Plant Germplasm Repository, which Conrad manages. There, Conrad and his colleagues maintain living plants in the field to provide seed and cuttings for scientific studies. Because hemlock seed have a short storage life, scientists must regularly replenish seed to maintain stock.

The hemlock accessions are just a few of the more than 1,400 accessions maintained in the genebank. Conrad and his staff also maintain more than 2,800 plants at field sites in Beltsville, Maryland, and Washington, D.C. While not all accessions in the collection are available for distribution, many of them are used in research projects by scientists all over the world.

New Hybrids Show Promise

At a field site in Beltsville, Maryland, rows of native, Asian, and hybrid hemlocks bask in the sun, each possessing a varying degree of tolerance to HWA. Curiously, the scientists could produce hybrids of Carolina hemlock but not of eastern hemlock. Carolina hemlock is a relic species, surviving in a small area and, surprisingly, is more closely related to the Asian hemlock species than to eastern hemlock. The threatened extinction of natural stands of Carolina hemlock by HWA and its genetic compatibility with its overseas cousins underscores the importance of germplasm preservation.

Since the start of the breeding program, the scientists have developed 140 *Tsuga* hybrids, 108 of which are suitable for testing. In 2006, Olsen and Bentz, along with USDA Forest Service entomologist Michael Montgomery, began a multiyear field trial to test each hybrid's degree of tolerance to HWA. They artificially infested the hybrids by attaching bundles of HWA-infested branches to the hybrids' lower branches and secured them to the trees with mesh bags to prevent the insects from escaping. The scientists tested more than 170 trees over the course of the trial.

"We found *T. chinensis* and its hybrids to be the most tolerant to HWA," says Olsen. "Two types of resistance were observed: fewer adelgids settling on the trees, called 'antixenosis,' and slower growth and lower survival of the settled adelgids, called 'antibiosis.'"

Olsen explains that the current hybrids are very appealing, not only due to their tolerance to HWA, but also because of their good vigor and shape. Even so, the scientists still have several years of testing to complete before they can release them to the public.

Olsen is preparing to send out the hybrids for long-term evaluation at several research sites in the eastern United States. He and Bentz are also completing their second 2-year study evaluating the hybrids' HWA tolerance, this time in container-grown trees. They will continue their breeding efforts, hopefully finding a way to incorporate the aesthetic traits of eastern hemlock and the HWA resistance of exotic hemlocks into successful hybrids.

Thanks to scientists at the U.S. National Arboretum, we're one step closer to seeing hemlock trees that can stand up to this pesky pest.—By **Stephanie Yao,** ARS.

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

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PEGGY GREB (D2006-1)

Woody landscape plant germplasm curator Kevin Conrad harvests cuttings of Chinese hemlock, *Tsuga chinensis,* grown from seed collected in China.

In Peoria, a Brisk Business in **Biodiesel Research**

It's now clear that biofuels need to play a bigger part in the U.S. fuel supply. So Agricultural Research Service scientists are continuing their search for low-cost, low-maintenance plants that can be used to produce biodiesel—and ways to use biodiesel coproducts to improve the fuel's performance.

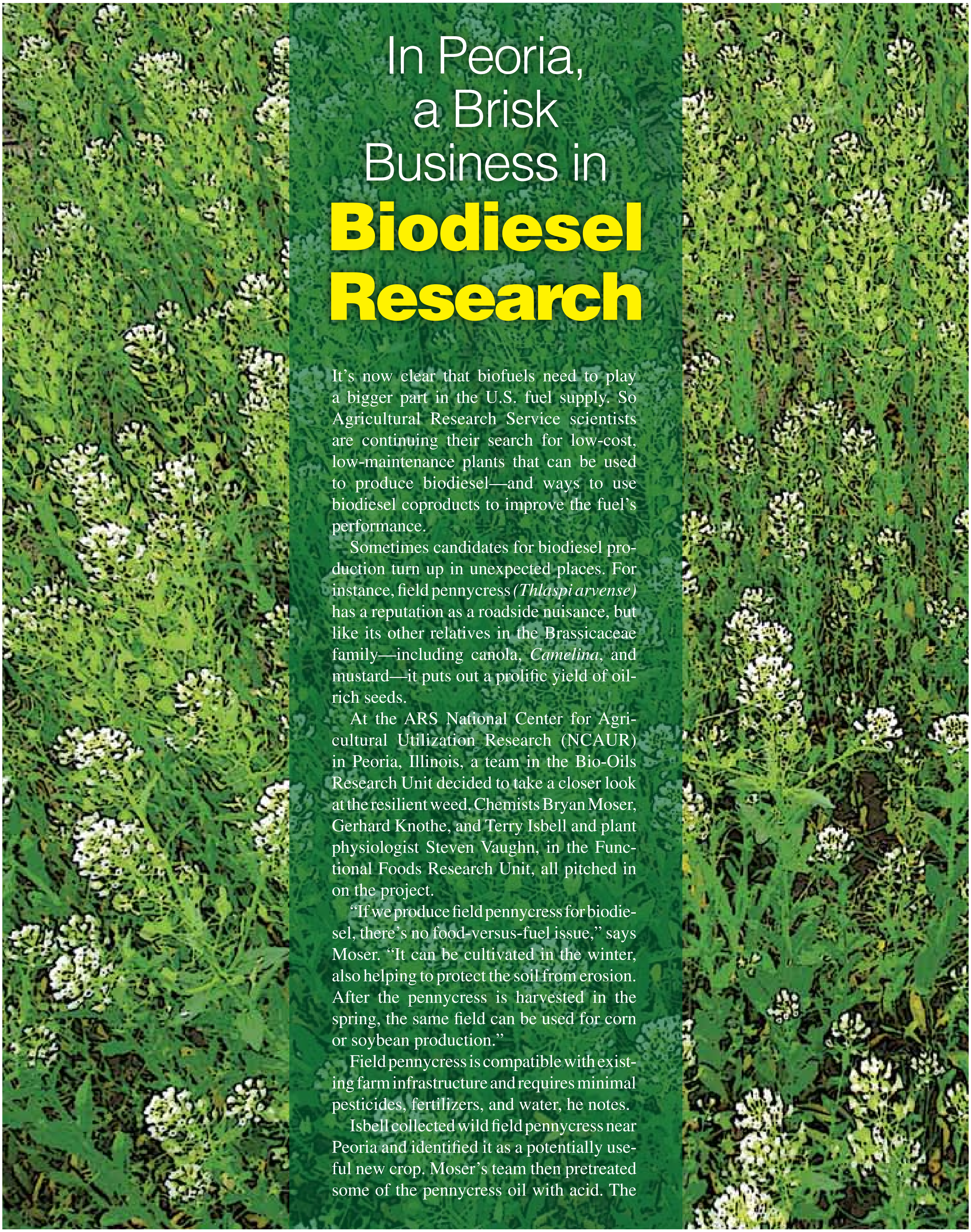
Sometimes candidates for biodiesel production turn up in unexpected places. For instance, field pennycress *(Thlaspi arvense)* has a reputation as a roadside nuisance, but like its other relatives in the Brassicaceae family—including canola, *Camelina*, and mustard—it puts out a prolific yield of oil-rich seeds.

At the ARS National Center for Agricultural Utilization Research (NCAUR) in Peoria, Illinois, a team in the Bio-Oils Research Unit decided to take a closer look at the resilient weed. Chemists Bryan Moser, Gerhard Knothe, and Terry Isbell and plant physiologist Steven Vaughn, in the Functional Foods Research Unit, all pitched in on the project.

"If we produce field pennycress for biodiesel, there's no food-versus-fuel issue," says Moser. "It can be cultivated in the winter, also helping to protect the soil from erosion. After the pennycress is harvested in the spring, the same field can be used for corn or soybean production."

Field pennycress is compatible with existing farm infrastructure and requires minimal pesticides, fertilizers, and water, he notes.

Isbell collected wild field pennycress near Peoria and identified it as a potentially useful new crop. Moser's team then pretreated some of the pennycress oil with acid. The



Field pennycress *(Thlaspi arvense)*.

scientists then reacted a type of alcohol called "methanol" with pretreated and untreated oils to produce biodiesel and glycerol from both. Pennycress oil was unusual among crude oils in that it yielded high-quality biodiesel without pretreatment.

These processes generated methyl esters—in other words, biodiesel. After some additional refining, the finished biodiesel was tested to see if it met the biodiesel fuel standard established by the American Society for Testing and Materials (ASTM). A similar standard has been developed in Europe by the Committee for Standardization (CEN). The results suggest that with some work, the problematic pennycress could become a commercial commodity.

A Cold Start

All diesel-based oils start to gel at some point as temperatures drop. So the cloud point—the temperature at which crystals become visible in the fuel—is a crucial factor in both biodiesel and petrodiesel production. Another important property is the pour point, which is the temperature at which the fuel fails to pour because it has sufficiently solidified. The average cloud and pour points for field pennycress biodiesel were -10ºC and -18ºC—well below the cloud and pour points of soybean oil-based biodiesel. This result suggests that field pennycress biodiesel is better suited to cold climates than most other biodiesel fuels.

Another important factor is a fuel's cetane number, which indicates how quickly the fuel combusts once it's injected into the combustion chamber of a diesel engine—the higher the number, the better the performance. The cetane number of field pennycress methyl esters was 59.8, which exceeded the minimum cetane number limits specified by ASTM (47) and CEN (51).

Missed It By That Much

On one measure, however, field pennycress fell short of the mark. Oxidative stability is another critically important quality of biodiesel. A higher oxidative stability is desired and means that the fuel is more stable to unwanted oxidative degradation. The oxidative stability of methyl esters generated from field pennycress oil fell below the minimum value specified in the CEN standard but exceeded the ASTM standard. This indicates that biodiesels derived from field pennycress would need antioxidant additives to meet some industry standards for commercial fuels.

STEPHEN AUSMUS (D1782-6)

Former ARS chemist Shailesh Shah (left) and technician Erin Walter purify glycerol, a biodiesel coproduct that can be used to produce value-added materials.

In addition, field pennycress methyl esters didn't meet the CEN standard for kinematic viscosity (a measure of thickness), but were within the range specified in the ASTM standard. Blending with less-viscous fuels could be a strategy to overcome the relatively high kinematic viscosity of field pennycress biodiesel.

Moser thinks these may be only small setbacks in the search for a new biodiesel feedstock, since essentially all biodiesel fuels require antioxidant additives, and blending is becoming more common. "We're looking for biofuel crops that can be grown in the Midwest," he says. "Our results show that field pennycress could well be one of those crops."

A Coproduct Comes Full Circle

When producers generate 9 gallons of biodiesel, they also generate 1 gallon of glycerol. Making use of the glycerol on a 10-gallon scale isn't too tough—but U.S. biodiesel production reached 700 million gallons in 2008. Even though purified glycerol can be used in cosmetics and other products, there's currently a glut in the global glycerol market because of biodiesel production.

So chemist Shailesh Shah, formerly in the Bio-Oils Research Unit, wrestled with the question of how to use all that glycerol. He took 23 different types of vegetable oils and diluted them with glycerol derivatives to see if this could improve their pour points and cloud points.

Shah found the glycerol-based diluents had the most impact on the pour point of a type of chemically processed soybean oil called "epoxidized" soybean oil. When he added glycerol at 10 percent volume to this oil, the pour point dropped from -6.3ºC to -11.7ºC, a 54-percent decrease. When he added glycerol at 5 percent volume to camelina oil, the pour point dropped from -17.7ºC to -20.3ºC. The pour points of palm oil, mid-oleic sunflower oil, and sunflower oil also dropped significantly when diluted with glycerol derivatives.

Shah also found that the effect of glycerol derivatives on pour point could vary depending on whether the fatty-acid chain in the glycerol is a straight chain or a branched chain. Branched chains had more of an effect on the pour points of vegetable oils with higher levels of saturated fats, such as palm oil. Straight chains were more effective at lowering the pour points of vegetable oils with higher levels of unsaturated fatty acids, such as camelina oil.

"Glycerol utilization is a very important part of biodiesel research," Shah says. "It's a challenge for producers, but this research shows we might be able to use glycerol, which is a coproduct of biodiesel, to improve low-temperature properties of vegetable oils and, potentially, biodiesel."—By **Ann Perry,** ARS.

This research supports the U.S. Department of Agriculture priority of developing new sources of bioenergy and is part of Bioenergy (#213) and Quality and Utilization of Agricultural Products (#306), two ARS national programs described at www.nps.ars.usda.gov.

To reach the scientists mentioned in this story, contact Ann Perry, USDA-ARS Information Staff, 5601 Sunnyside Ave., Beltsville, MD 20705; (301) 504-1628, ann.perry@ars.usda.gov. ✻

STEPHEN AUSMUS (D1780-2)

ARS chemist Bryan Moser uses a reflux condenser to produce field pennycress biodiesel.

Public Gardens and ARS Working Together in Plant Preservation

KEITH WELLER (K7474-20)

Tall, stately oaks form the backdrop for the U.S. National Arboretum's dogwood collection.

Have you ever wondered what you would do if your favorite landscape plant was threatened to near extinction by a pest or disease? Well, it has happened several times throughout history and continues today. The recent and potentially catastrophic infestation of the emerald ash borer *(Agrilus planipennis)* has threatened to destroy native ash trees. Currently, sudden oak death *(Phytophthora ramorum)* is attacking oak trees in the West and threatens to spread to the rest of the country.

Since the beginning of the 20th century, the Agricultural Research Service and public and private cooperators have been working hard to ensure that we are better prepared to conserve these key components of our native forests, parks, gardens, and yards. In 1990, Congress reaffirmed its commitment to ornamental plant conservation by charging ARS with the enormous task of preserving highly valuable woody ornamental plant germplasm. It became apparent to ARS leaders early on that successful plant collection and preservation could not be accomplished without cooperation between the agency and public and private institutions.

One such partnership exists between the U.S. National Arboretum, part of ARS, and the American Public Gardens Association's (APGA) North American Plant Collections Consortium (NAPCC). The NAPCC is a network of arboretums and botanical gardens focused on collecting, maintaining, and preserving plant germplasm from all over the world. This year marks the 15th anniversary of the partnership between NAPCC and ARS. Built on a mutual interest in conserving plant genetic resources, it works well because both ARS and APGA are committed to setting a high professional standard for managing garden collections for the purpose of plant conservation.

The plant collections of botanical gardens and arboretums in North America represent a significant sample of the entire plant diversity found throughout the world. Including more than 100,000 acres and half a million diverse collections, the nearly 500 institutional members of APGA can be a major force in landscape plant conservation. Pam Allenstein, manager of NAPCC, explains: "Our members follow a high curatorial standard and have a long-term commitment to plant preservation to ensure that germplasm is available for future use." Coupled with ARS's National Plant Germplasm System, which manages working collections of more than 530,000 plant accessions, it has become a formidable partnership for success.

The National Arboretum's Woody Landscape Plant Germplasm Repository, which is curated by horticulturist Kevin Conrad, helps facilitate this critical collaboration between these two organizations.

"Our role is to work closely with NAPCC and Pam to help bridge the gap between the responsibilities of federal government and APGA and combine the resources each can contribute to woody landscape plant conservation," says Conrad. "We also focus on plants not covered by NAPCC's program or which might not be a high priority for them."

Thanks to this continued cooperation, we can rest assured the plants we enjoy and rely on will be here for years to come.—By **Stephanie Yao,** ARS.

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

Kevin Conrad is in the USDA-ARS Floral and Nursery Plants Research Unit, U.S. National Arboretum, 10300 Baltimore Ave., Beltsville, MD 20705-2350; (301) 504-5196, kevin.conrad@ars.usda.gov. *

A Green Light for Postfire Grazing

Two Agricultural Research Service scientists have found that the Wyoming big sagebrush steppe plant community can accommodate grazing cattle and still make a successful comeback after a fire, as long as producers carefully monitor their livestock.

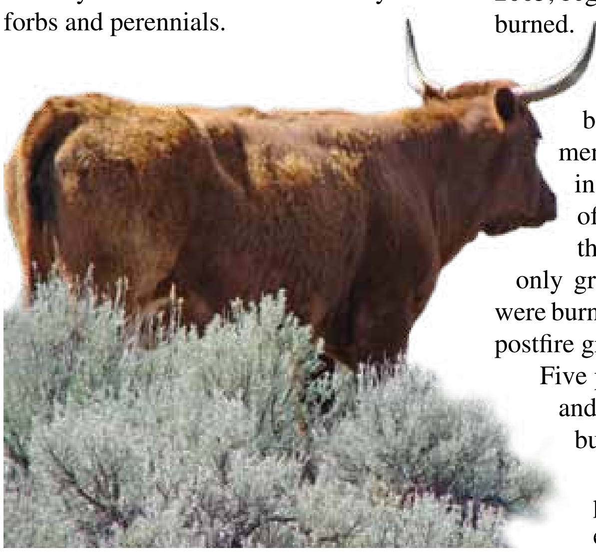
"Managers have typically kept grazing cows out of the burned area for two seasons after a fire," says rangeland scientist Jon Bates, who works at the Eastern Oregon Agricultural Research Center (EOARC) in Burns, Oregon. "But our research shows that, at least for rangeland that's in good condition, there's no real difference in plant recovery when grazing begins soon after a fire."

In the big sagebrush steppe, periodic wildfires are part of the vegetation community's historical disturbance regime, and land managers mimic those dynamics with managed fires. Bates teamed with EOARC rangeland scientist Kirk Davies to see how vegetation recovery differed between plant communities where grazing was permitted after a fire and where it was not allowed.

The scientists set up thirty 5-acre trial plots in the Northern Great Basin Experimental Range in eastern Oregon, which is dominated by Wyoming big sagebrush and perennial bunchgrasses. They burned most of the plots in the fall of 2002, which killed almost all of the Wyoming sagebrush, and then studied how different grazing management systems affected the recovery of the forbs and perennials.

KIRK DAVIES (D2018-1)

Cattle grazing on sagebrush in southeastern Oregon near Little Juniper Mountain.



Five "summer" plots were grazed in 2003 and 2004, beginning the first summer after the fire and when grasses and forbs had already completed their growth for the season and were mostly dormant. Five other summer plots were grazed in the summers of 2004 and 2005, beginning 2 years after the plots were burned.

"Spring" plots were grazed in early to mid-May, before grasses began their reproductive development. Five of these plots were grazed in 2004 and 2005 at the beginning of the second growing season after the 2002 fire. Another five were only grazed in 2005, 3 years after they were burned, which replicates many current postfire grazing strategies.

Five plots were burned but not grazed, and the remaining five plots were not burned or grazed.

Cattle that grazed in the burned plots were removed after they had eaten 40 to 50 percent of the available forage. This grazing level is considered to be moderate or slightly higher than moderate for big sagebrush steppe.

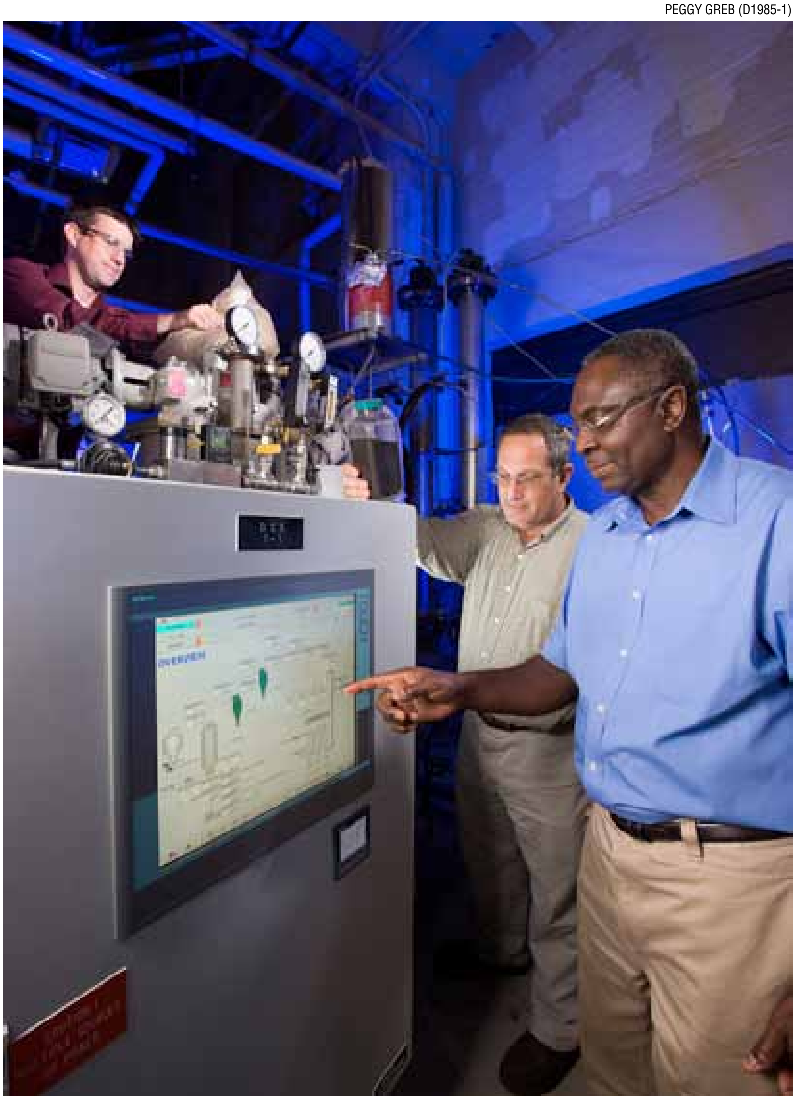
The scientists gathered data on herbaceous canopy cover, density, annual yield, and perennial grass seed yield on all 30 plots. They found that postfire plant recovery did not differ significantly between grazed and ungrazed plots. In addition, all the burned plots had more herb cover, herb standing crop, annual yield, and grass seed yield by the second or third year after the fire than the unburned control plots.—By **Ann Perry,** ARS.

This research is part of Pasture, Forage, and Range Land Systems (#215), an ARS national program described at www.nps.ars.usda.gov.

Jonathan D. Bates and Kirk W. Davies are with the USDA-ARS Eastern Oregon Agricultural Research Center, 67826-A Hwy. 205, Burns, OR 97720; (541) 573-8932 [Bates], (541) 573-4074 [Davies], jon.bates@ars.usda.gov, kirk.davies@ars.usda.gov. *

New Fuels From an Ancient Crop

PEGGY GREB (D1985-1)



Chemical engineer Akwasi Boateng (right) and mechanical engineer Neil Goldberg (center) adjust pyrolysis process conditions while chemist Charles Mullen (left) loads the reactor with bioenergy feedstock.

Barley has been cultivated for thousands of years, yet it doesn't always make the list when energy experts discuss potential biofuel crops. And bio-oil—a liquid fuel generated when heat breaks down plant matter—is still a low-profile energy alternative. But research by Agricultural Research Service scientists at the Eastern Regional Research Center (ERRC) in Wyndmoor, Pennsylvania, could give a big boost to producing bio-oil from barley feedstocks.

As a renewable transportation fuel, bio-oil made from barley byproducts—or any other biofeedstock—has several advantages. The fuel can potentially be refined, stored, and distributed with the infrastructure already in place for the petroleum fuel industry. And perhaps in the future, consumers will be able to fill up their cars with gasoline or diesel derived from bio-oil without needing special adaptations in their fuel systems.

ERRC lead scientist and chemical engineer Akwasi Boateng worked with chemist Charles Mullen, mechanical engineer Neil Goldberg, chemist Robert Moreau, and research leader Kevin Hicks on studies that evaluated the yields of bio-oil from barley straw, hulls, and dried distillers grains (DDGS). All three feedstocks are byproducts of fermentation of barley grain for ethanol—a biofuel option that is slowly gaining ground in the Middle Atlantic States and the Southeast, where farmers could cash in on the production of winter barley cover crops while continuing to raise corn and other food crops in the summer.

The researchers produced bio-oil from all three barley byproducts via "fast pyrolysis," an intense burst of heat delivered in the absence of oxygen. In the lab, a kilogram of barley straw and hulls yielded about half a kilogram of bio-oil with an energy content about half that of No. 2 fuel oil.

The energy content of bio-oil from barley DDGS—including DDGS contaminated with mycotoxins, which can't be used to supplement livestock feed—was even higher, about two-thirds that of No. 2 diesel fuel oil. But it was more viscous and had a shorter shelf life than the bio-oils produced from straw or hulls.

The fast-pyrolysis process also creates a solid byproduct called "biochar," which might improve the water-holding capacity and nutrient content of soils. Amending soils with biochar can sequester carbon in the soil for thousands of years.

Based on these studies, the scientists suggest that colocating fast-pyrolysis units in commercial barley grain ethanol plants could be a win-win proposition for farmers in the Middle Atlantic States. Barley grain could be used to produce ethanol, and the byproducts could be used to produce bio-oil either for transportation fuels or for producing heat and power needed for the grain-to-ethanol conversion.

Growing winter barley would also help reduce soil erosion and nitrogen leaching, both major concerns for farmers in the Chesapeake Bay Watershed. Adding biochar as a soil amendment could further benefit food crop production and carbon sequestration efforts—all of which shows that even venerable old crops can be used to help solve current energy challenges.—By **Ann Perry,** ARS.

This research supports the U.S. Department of Agriculture priority of developing new sources of bioenergy and is part of Bioenergy (#213), an ARS national program described at www.nps.ars.usda.gov.

To reach scientists mentioned in this story, contact Ann Perry, USDA-ARS Information Staff, 5601 Sunnyside Ave., Beltsville, MD 20705-5129; (301) 504-1628, ann.perry@ars.usda.gov. ✻

CO_2 in Soil Easily Measured

Lucretia Sherrod is a biological science technician who believes that if the right lab equipment or procedure doesn't exist, you just make it yourself.

Her latest creation is a modified carbon dioxide (CO_2) analyzer. With this, she and her colleagues have developed a rapid and cost-effective way to estimate carbon decomposition rates by monitoring soil respiration. Traditional methods of measuring CO_2 levels are labor intensive and time consuming.

Soil microbes decompose plant material and release carbon for possible storage in soil. But microbes also "breathe out" carbon as CO_2. The best scenario is when more carbon is stored in soil than is lost through microbial respiration.

Sherrod transformed a single-cell infrared gas analyzer meant for monitoring CO_2 levels in greenhouses into an easy and environmentally sound way to measure soil respiration. She can run 90 samples an hour, instead of 10 to 24 per hour using traditional techniques.

After a soil sample is incubated in a chamber for 3 days, Sherrod inserts a needle through the chamber's seal and collects a sample of the atmosphere for analysis. Her system easily makes room for add-ons such as an oxygen analyzer.

Her test can measure the CO_2 produced and oxygen consumed when microorganisms eat soil organic matter. She has publicized this test with an abstract at the 2009 annual meeting of the American Society of Agronomy, the Crop Science Society of America, and the Soil Science Society of America. She is also writing a paper on the procedures. Any lab can easily build the test equipment for immediate use.—By **Don Comis,** ARS.

Lucretia A. Sherrod is in the USDA-ARS Agricultural Systems Research Unit, 2150 Centre Ave., Fort Collins, CO 80526; (970) 492-7352,lucretia.sherrod@ars.usda.gov. ✻

Infrared Sheds Light on Beneficial Microbes

Infrared spectroscopy can quickly spot beneficial fungi on roots in soil, according to Francisco Calderon, a soil scientist at the Agricultural Research Service Central Great Plains Resources Management Research Unit in Akron, Colorado.

This type of spectroscopy has become established practice for quick and reliable analysis of grain and forage quality, as well as other agricultural uses, thanks in part to ARS scientists. But Calderon's idea to use it for detecting fungal-root associations in soils was never explored before. The ability to quickly analyze field soils for these beneficial fungi, called "mycorrhizae," would allow scientists to judge which crop rotations or other farming practices increase the fungi. This is important nationwide for improving crop yields and is especially critical for semiarid areas like those found in the Central Great Plains.

Mycorrhizal fungi live in a symbiotic relationship with plants. The fungi help plants by taking up soil nutrients and water that would otherwise be difficult for plant roots to reach. In exchange, the fungi feed on the carbon sources that plants provide.

Calderon says the test could simplify, accelerate, and improve the objectivity of measurements of mycorrhizae in root samples, compared to the standard method of visual scoring through a microscope. It may also be more accurate than the newer technique of analyzing fatty acids in mycorrhizae on roots. Also, he says, "Since there is no destruction of the samples, researchers can perform other analyses on the same samples after this test is done."

Calderon developed the technique with soil scientists Veronica Acosta-Martinez at Lubbock, Texas, and Merle Vigil at Akron. Other ARS colleagues in this study include microbiologist David Douds at Wyndmoor, Pennsylvania, and chemist James Reeves at Beltsville, Maryland.

They measured the reflectance of infrared light from dried, powdered carrot root samples. They found that the cell wall chitin and fatty acids in mycorrhizal fungi have distinct spectral signatures, absorbing infrared light at different wavelengths than standard chitin, fatty acids, and nonmycorrhizal roots. The researchers accomplished this using both mid-infrared and near-infrared spectroscopy.

Next, they plan to study the spectral properties of other crop fungal species to see whether there are universal spectral signatures for this important group of organisms.—By **Don Comis,** ARS.

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Defatted Corn Protein Produces Palatable Gluten-Free Bread

A good, crusty roll with dinner is a pleasure most people take for granted. But for millions of Americans, this simple, basic pleasure is off limits because they cannot tolerate proteins found naturally in grains like wheat, barley, and rye that are used in flours.

Agricultural Research Service chemists Scott Bean and Tilman Schober, in the Grain Quality and Structure Research Unit in Manhattan, Kansas, had some success developing gluten-free pan bread from other grains, but they couldn't make free-standing rolls because they spread out too much. "The bread was considered lower in quality than comparable wheat bread," says Bean. Gluten-free grains include corn, sorghum, and rice.

Now Bean and Schober have found a way to make rolls from corn that are more than just gluten-free: they also rise more and resemble wheat rolls.

In previous studies, Bean and Schober found that a corn protein called "zein"—a readily available byproduct from corn wet milling and fuel-ethanol production—could be used to make a more wheatlike dough. The dough still didn't meet their standards, though, because it lacked strength, and the rolls produced from it were too flat. They used a commercially available zein in that study.

But more recently, Bean and Schober found that by removing additional fat from zein, they were able to produce a dough more similar to wheat dough and free-standing hearth-type rolls that resemble wheat rolls. "We found that removing more of the fat from the protein's surface allows the proteins to stick to each other much like wheat proteins do—leading to the elastic nature of wheat dough," says Bean.

Even better than corn for baked products, according to Bean, is sorghum—a gluten-free grain of choice as a wheat substitute. But since corn and sorghum are similar, they used the former as a research model.

"Corn protein, in our view, is an intermediate step to achieving the Holy Grail of gluten-free breads—forming a wheatlike dough using nonwheat proteins, resulting in products with a fluffy, light texture," says Bean.

This research may prove useful for the 2-3 million Americans affected by celiac disease, a condition in which the human immune system erroneously attacks the intestine when gluten is ingested, causing severe diarrhea and inability to absorb nutrients. Gluten-free palatable rolls from corn, rice, and sorghum would be a welcome addition to their diet.

A paper on this work was accepted by the *Journal of Cereal Sciences*.—By **Sharon Durham,** ARS.

Scott Bean is in the USDA-ARS Grain Quality and Structure Research Unit, 1515 College Ave., Manhattan, KS 66502; (785) 776-2725, scott.bean@ars.usda.gov. ✻

New Soybeans With Seed Rot Resistance Identified

The fungus *Phomopsis longicolla* is largely to blame for a disease called *Phomopsis* seed decay (PSD) that has claimed more than 5 million bushels of U.S. soybeans each of the past 5 years. The seed disease is most problematic in midwestern and southern states.

Control strategies used by farmers have been inconsistent. These include rotating soybeans with corn or wheat (nonlegume crops on which the fungus can't grow), treating seed with fungicides, and tilling the soil to disrupt spore dissemination. The ideal defense is to plant resistant varieties.

To that end, Agricultural Research Service plant pathologist Shuxian Li is coordinating a 3-year project out of Stoneville, Mississippi, to screen for PSD resistance in hundreds of soybean germplasm accessions, breeding lines, and commercial cultivars collected from around the world. "Resistant varieties can provide protection for soybean producers at no additional cost beyond the price of planting the seed," notes Li, in ARS's Crop Genetics Research Unit.

Her efforts to identify resistant sources kicked into high gear in April 2009 following a grant from the United Soybean Board (USB). Li's collaborators on the project are Pengyin Chen and John Rupe, professors at the University of Arkansas in Fayetteville, and Allen Wrather, a professor at the University of Missouri in Portageville.

The USB grant expands on prior field trials the team had conducted since May 2007 in Mississippi and Arkansas that identified several promising PSD-resistant soybean lines from commercial varieties provided by Mississippi State University collaborators and plant introductions from the USDA Germplasm Collection. Typically, the resistant lines identified from this research showed little or no incidence of PSD and had a high germination rate with strong vigor. Additional screening using local strains of *P. longicolla* will also be conducted on soybeans from other sources (including 28 countries).

To expedite their research, Li is developing new and fast screening methods to identify sources of PSD resistance and map resistance genes. The team is also identifying DNA markers associated with the expression of these resistance genes in mapping populations of offspring plants derived from cross-breeding.

Once the markers have been validated, the team will make them publicly available for use in marker-assisted selection, an approach that will save soybean breeders considerable time and expense in developing elite commercial cultivars for growers.—By **Jan Suszkiw,** ARS.

Shuxian Li is in the USDA-ARS Crop Genetics Research Unit, 141 Experiment Station Rd., Stoneville, MS 38776; (662) 686-3061, shuxian.li@ars.usda.gov. ✻

ARS Overseas Lab Sets the Stage for Reuniting a Weed and Its Enemies

The search is on for insects, mites, microbes, or nematodes that could nibble on, gnaw through, or sicken silverleaf nightshade in a biologically based approach to controlling this noxious weed, which hails from the Americas but has spread to southern Europe, Africa, India, Australia, and elsewhere.

Among other harm it causes, the purple-flowered perennial weed *Solanum elaeagnifolium* outcompetes native plants, reduces crop yields, and diminishes pasture productivity. Its toxin-containing orange berries can also poison livestock. Chemical and mechanical controls like mowing sometimes work against the weed. But severe infestations can render such controls too costly, impractical, or environmentally harmful to use repeatedly.

Biocontrol is considered sustainable because it involves releasing select natural enemies of the weed that will feed or develop on it exclusively and continue doing so until their host is reduced in numbers toward the natural balance that existed in its native range.

"Typically, these biological control agents are insects that severely damage or kill the weed, leave useful plants alone, and restore the ecological balance between the weed and its environment," explains Walker Jones, who, in April 2010, completed a 5-year assignment as director of the Agricultural Research Service's European Biological Control Laboratory (EBCL) near Montpellier, France.

Before returning stateside to lead ARS's National Biological Control Laboratory in Stoneville, Mississippi, Jones and ARS national program leader Daniel Strickman established a cooperative project with the Benaki Phytopathological Institute in Athens, Greece, to explore the feasibility of starting continental Europe's first-ever classical weed-biocontrol program.

Normally, EBCL serves as a sort of way station, where promising biocontrol agents collected from Europe, Asia, or Africa are screened for potential release into the United States to manage invasive species. But in this instance, the lab will switch gears—serving as a receiving point for candidate organisms from North America that could open the door to biologically controlling infestations of the weed in Greece and other countries in the Mediterranean basin, where effective native natural enemies have yet to be tested.

The first stages have already begun. At Montpellier, EBCL molecular biologist Marie-Claude Bon is using DNA-based methods to analyze the genetic diversity of silverleaf nightshade populations collected from sites in the southwestern United States, Argentina, Greece, France, and Australia. Her analysis will help to determine the weed's center of origin and trace the route of its world invasion.

"This, in turn, will pinpoint where to locate co-evolved natural enemies," says Jones. Once identified, they'll be sent to Montpellier and rigorously tested under quarantine to ensure their host specificity and safety as biocontrol agents intended for release.

First, however, a survey of Greece's weed populations must be completed. "After processing these data, we're hoping to use satellite photos to have a precise map of silverleaf nightshade populations and densities in various areas of Greece," says Javid Kashefi, an EBCL entomologist stationed at the American Farm School in Thessaloniki.

"This weed very likely came from the southwestern United States and northern Mexico," says Jones. "We wanted to use EBCL's unique experience, location, and facilities to establish a biocontrol project that would benefit Europe—sort of as thanks for the biocontrol agents we've acquired and sent to the United States for the past 90 years." There's also keen interest for similar projects in North African countries where silverleaf nightshade is the top weed pest, he adds.—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.

Walker Jones is with the USDA-ARS National Biological Control Laboratory, 59 Lee Rd., P.O. Box 67, Stoneville, MS 38776; (662) 686-5487, walker.jones@ars.usda.gov. ✻

Silverleaf nightshade *(Solanum elaeagnifolium).*

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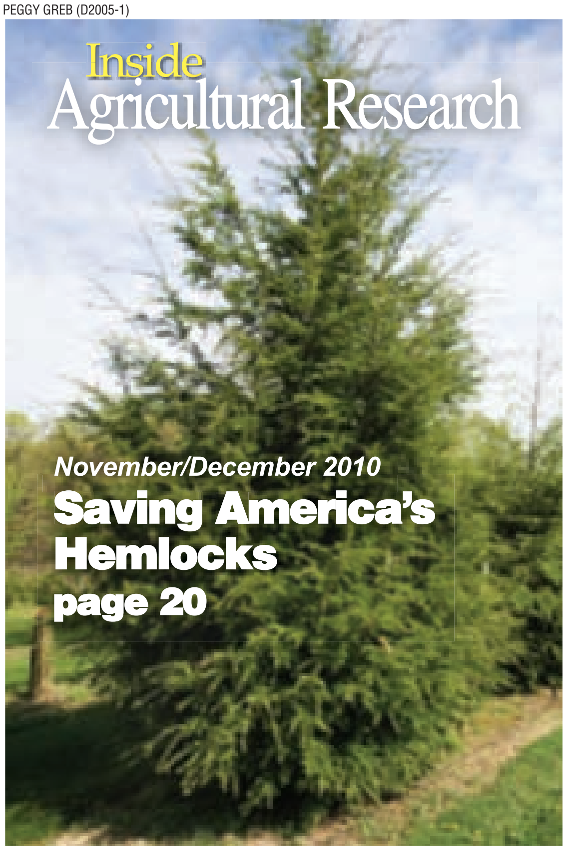

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