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A Vaccine That Chickens Gobble Up

Story on pages 14-15

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

FORUM

Success in Controlling Some Cotton Pests Opens the Door to Others

The saying that nature abhors a vacuum could well apply to our nation's cotton fields and to the insects that infest them. While some insect pests are disappearing, others are moving into the spotlight. Efforts to eradicate the boll weevil in cotton over the years have been largely successful. The use of *Bt* cotton has also proved to be an effective way to control many caterpillars and beetles without the need for spraying broad-spectrum insecticides. Some 11.2 million acres of the 12 million acres of cotton planted in the United States in 2012 contained the *Bt* trait. These success stories have reordered the ranking of insect pests in many cotton fields, bringing to the forefront a long list of piercing-sucking insects, and at the top of that list are the lygus bugs.

While cotton growers are using less broad-spectrum insecticide these days, many of them must now spray more narrowly tailored insecticides specifically to combat lygus bugs. One type of lygus, the tarnished plant bug, *Lygus lineolaris,* is a particular problem for cotton growers in the Mid-South, while another type, the western tarnished plant bug, *L. hesperus,* is more of a threat in Arizona and California.

The stakes are high. Cotton and cottonseed had a combined farm value of $7.5 billion in 2012. Insect-related crop damage and control costs are the lowest they have been in at least 33 years, but insects still cost cotton growers $71 per acre in crop losses and control costs, according to the 2012 Cotton Insect Losses survey published by the National Cotton Council. While lygus bugs aren't the only insects included in those cost figures, they are a major concern, ranking at the top of the survey's list of 33 insect pests. They cost growers about $8 per acre to control, according to the survey conducted each year by Mississippi State University.

In this issue, you will read about efforts by Agricultural Research Service scientists to combat this onslaught. Studies done in Shafter, California, focus on the feeding habits of *L. hesperus* on the two species of cotton grown in the United States, American upland *(Gossypium hirsutum)* and American pima *(G. barbadense),* or extra-long-staple cotton. The research is designed to help growers determine when infestations have reached levels that require insecticide spraying. The article also describes research by ARS scientists in Maricopa, Arizona, focusing on two of *L. hesperus's* survival mechanisms: its production of "heat shock proteins" to cope with high temperatures and its ability to go into diapause when winter approaches and day length becomes shorter. Knowing when plant bugs enter and break diapause and being able to predict when they begin to prepare for overwintering will allow growers to apply insecticides more efficiently.

ARS researchers are also working to find environmentally friendly ways to control lygus bugs in other cotton-growing areas. In Stoneville, Mississippi, for example, teams are probing the tarnished plant bug's biochemistry and genetics, studying the timing of its diapause patterns, developing ways to manage pesticide resistance, and evaluating sterile insect control techniques. Lygus bugs also attack strawberries, alfalfa, and other crops, and the research could one day help control infestations in those crops.

The work also could translate into new methods for controlling other insect pests as well. That is critical because lygus bugs are part of a large group of piercing-sucking insects that cause tremendous damage. Beyond cotton, piercing-sucking insects are a particular concern because they vector major plant diseases, such as Pierce's disease in grapes and Huanglongbing, also known as "citrus greening," in citrus. Since just a few insects are usually enough to transmit such diseases, pest numbers have to be reduced to extremely low levels to stop disease transmission. It's a daunting challenge. But by studying the feeding habits, survival tactics, and genomes of lygus bugs, we could open doors to developing controls not only for these cotton pests, but for other crop pests as well.

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PEGGY GREB (D3085-1)

Visiting scientist Liping Kou (left) and technician Ellen Turner harvest different types of microgreens for shelf-life studies and nutrient analyses. Story begins on page 10.

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Cover: At the Animal Parasitic Diseases Laboratory in Beltsville, Maryland, support scientist Carolyn Parker prepares gelatin beads for day-old broiler chicks to eat. The beads contain *Eimeria* oocysts that will serve to vaccinate the chicks against avian coccidiosis. Story begins on page 14. Photo by Stephen Ausmus. (D3071-5)

Diverse Approaches To Tame an Emerging Pest

There is good news and bad news these days for cotton growers in the southwestern United States. Widespread use of *Bt* cotton, along with progress in eradicating the boll weevil, has reduced the need for spraying broad-spectrum insecticides. That's the good news. The bad news is that cotton still faces a serious threat from the western tarnished plant bug, *Lygus hesperus*.

A particularly hardy species of plant bug, *L. hesperus* is capable of surviving extremely dry conditions, 107˚F heat, and freezing temperatures. Along with cotton, the bug is a major pest of seed alfalfa, strawberries, and canola, and many growers rely on insecticides specifically to control it.

"We find it from Mexico to Vancouver, Canada, and that raises the question of how it can survive and thrive in these different environments," says Colin Brent, an Agricultural Research Service entomologist at the U.S. Arid-Land Agricultural Research Center in Maricopa, Arizona.

Brent and colleagues J. Joe Hull and Dale Spurgeon are searching for new ways to control *L. hesperus* by studying its feeding habits and examining two key survival mechanisms: its activation of thermal stress genes to cope with heat and its ability to go into diapause when winter approaches.

"We want to target this insect's vulnerabilities, and to do that, we need to know more about its behavior and physiology," says Hull. Information gleaned from the research could advance efforts to control other insect pests as well.

Surveying the Fields: The Importance of an Accurate Count

Before deciding whether to spray insecticides to control *L. hesperus,* cotton growers often walk through their fields waving handheld nets, count the number of lygus bugs they capture, and make a decision based on established population thresholds. Thresholds vary from one cotton region to the next. Spurgeon and ARS scientist W. Rodney Cooper, who is now with the Yakima Agricultural Research Laboratory in Wapato, Washington, have found that these "sweepnet" survey results can be easily misinterpreted. Results of their studies on the insect's feeding habits also show that factors important to the decisionmaking process are often overlooked, including the age of the insects collected in the nets, the growth stage of the cotton, and the type of cotton being cultivated.

"These all have an impact on how this insect pest feeds and on how much damage to a cotton crop it is likely to cause," says Spurgeon. He and Cooper conducted the research while at an ARS research site in Shafter, California.

In one study, Spurgeon and Cooper videotaped some *L. hesperus* feeding on cotton in the laboratory and released others to feed on caged cotton plants in the greenhouse. Their goal was to assess feeding habits and damage levels caused by two types of nymphs (3rd and 5th instars) and by young (prereproductive) adults. In another study, they examined feeding damage caused by three classes of adult insects (prereproductive; reproductive and unmated; reproductive and mated) by releasing insects on cotton plants so they could feed for up to 7 days.

PEGGY GREB (D3072-1)

ARS researchers are searching for new ways to manage the western tarnished plant bug, *Lygus hesperus,* a major pest of strawberries and numerous other crops, including cotton, seed alfalfa, and canola.

The studies were designed to address whether the age of the insect plays a role in the damage levels. Results showed that nymphs and young adults, regardless of reproductive status, cause significantly more damage than older adults.

This led them to wonder how differences in feeding habits and damage levels, seen in different age groups, influence the numbers turning up in the sweepnet surveys. So, in another study, they marked adult *L. hesperus* with nail polish and periodically released them into 10-meter rows of upland

Technician Neal Hudson (formerly with ARS) sweeps cotton plants in a study to determine the efficiency of the sweepnet for capturing adult *Lygus hesperus*.

DALE SPURGEON (D3080-1)

DALE SPURGEON (D3081-1)

DALE SPURGEON (D3082-1)

Western tarnished plant bugs at various stages of development. Left to right: a newly hatched 1st instar, 3rd instar nymph, and 5th instar nymph. Although the 1st instars produce little damage to crops, their ability to survive environmental stresses is key to development of populations of older, more damaging nymphs. The 3rd instars can directly damage crops by feeding and can develop into more damaging 4th instars in as little as 2 days during summer. The 5th (and final) instar is the most injurious stage, feeding for 3-4 days before transforming into an adult.

and pima cotton. They followed standard sweepnet protocols in collecting as many of the marked insects as possible the day after each release, along with any wild *L. hesperus* attracted to the cotton.

The number of marked insects caught indicated the efficiency of the collection efforts. Releases and collections were conducted over two growing seasons. Insects caught in the nets were dissected to verify their ages.

The researchers found that in pima cotton, the more damage-inducing young adults preferred to feed on the ends of the plant branches, which are substantially shielded by foliage. That makes them harder to capture and more likely to be undercounted. This wasn't an issue in upland cotton, because it has a more open architecture. They also found that in both species of cotton, the wild populations were dominated by mature adults that cause less damage. As cotton plants grew, the sweepnet collection efforts became less efficient, because larger and leafier plants gave the insects more foliage for "hiding out."

Taken together, the results suggest that sweepnet methods for establishing thresholds for spraying may need to be reevaluated, and there may be a need for cotton-species-specific sampling methods.

DALE SPURGEON (D3084-1)

"This pest causes different levels of damage at different stages of its life cycle. It may be important to know the ages of lygus infesting your cotton, not just the total numbers," Spurgeon says.

The sweepnet study was published in July 2013 in the *Journal of Entomological Science.* The study that examined damage levels from adults in various stages of development was published in *Environmental Entomology* (April 2012), and the study comparing damage by nymphs and adults has been accepted for publication in *Environmental Entomology.*

Exploiting Stress Induced by Mother Nature

Brent and his colleagues explored the pest's overwintering biology by focusing on the ability of *L. hesperus* to protect itself by entering diapause. *L. hesperus* adults will enter diapause as the seasons change and the days grow shorter. The diapause is tied to reproductive capabilities and cold tolerance. Some adults develop mature reproductive organs and will never enter diapause, while others defer development and can apparently use the resources diverted from reproduction to survive. The researchers say that differentiating how the pests respond to their environment when in or out of diapause could reveal weaknesses that can be exploited to enhance current control strategies or to develop novel controls.

"They might become more or less susceptible to control approaches, depending on seasonally occurring changes in their behavior and development," Brent says.

The scientists induced diapause in lab-raised *L. hesperus* by exposing them to shorter day lengths. The behaviors and activity levels of the diapausing insects were compared to those of insects that never entered it. The researchers compared how much fat the insects stored, the amount of time spent feeding and at rest, and survival rates when starved and dehydrated. They put the insects in chambers designed to measure metabolic rates and even tethered them to tiny metallic arms to compare frequency of flight, flight speed, and distances traveled over 24-hour periods.

The results, published in *Entomologia Experimentalis et Applicata* (August 2013) showed that "diapausers" spent more time resting and less time feeding, had more body fat, and survived three times longer than insects that never entered diapause. Flight patterns were about the same in each group, but differed between females and males. The findings are expected to help scientists in their search for future control strategies among *L. hesperus's* survival tactics.

Adult female (left) and male western tarnished plant bugs, *Lygus hesperus,* on an alfalfa leaflet. Alfalfa is a preferred host crop that can produce large populations of *Lygus* bugs that subsequently move to other crops.

COLIN BRENT (D3083-2)

Hull and his colleagues are also working toward silencing genes that *L. hesperus* uses to survive environmental stress. To achieve that, they studied the molecular underpinnings of a specific class of proteins, known as "heat shock proteins," used by the insect to survive intense heat. Heat shock proteins have been identified as survival mechanisms in honey bees, silk moths, mosquitoes, fruit flies, and whiteflies. Identifying them in *L. hesperus* and studying how they function would shed light on the molecular mechanisms the insect uses to cope not only with intense heat, but also with other stress factors, such as drought and cold, the researchers say.

They used DNA sequencing technology to develop a "transcriptome," a snapshot of the genes expressed at a specific stage in the insect's development. Transcriptomes are less expensive than sequencing an entire genome and have been developed for other insect pests. The *L. hesperus* transcriptome focused on genes expressed in the first 5 days of adult development. Among the many genes found in the transcriptome, the researchers identified 42 that they think may be responsible for synthesizing heat shock proteins. The researchers then compared gene expression patterns between *L. hesperus* maintained at 77˚F and those exposed to 102˚F for 6 hours.

They found that the induced heat stress caused robust changes in the gene-expression pattern of two suspected heat shock proteins, moderate changes in two others, and no changes in four others.

The results, published in *PLOS One* in January 2013, provide researchers with a key to finding mechanisms that control the pest's ability to survive such intense heat.—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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Mulching and Guar Gum

Substitutes for This Familiar "Binder" Tested

COURTESY OF HYDROSTRAW, LLC (D3048-1)

Technician Nathan Hoegger of HydroStraw, LLC, applies a hydromulch containing an experimental binder to a test plot at the corporation's Manteno, Illinois, facility.

You've probably driven past highway crews that are busily spraying a green coating on newly graded slopes. They may be working with a hydraulically applied mulch, or hydromulch—a temporary, porous coating that can, for example, help protect newly sown seeds.

A typical hydromulch contains water; a green dye, so that crews can easily see where they've been; a mulch, such as wood fibers; and a binder—a compound that helps keep the mulch intact.

One binder that's commonly used for hydromulch is guar gum, an off-white powder made by grinding beans of the guar plant. When water is added, the powder quickly forms a viscous gum.

Most of the guar gum used in the United States—for hydromulching and an impressive array of other applications—is imported. A spike in guar gum prices in 2012 led Agricultural Research Service plant physiologist Steve Vaughn and colleagues to take a new look at 10 plant-derived compounds as potentially less-expensive alternatives to guar gum.

The study is part of ongoing research at ARS's National Center for Agricultural Utilization Research in Peoria, Illinois, to find new, environmentally friendly, industrial and food uses of crop plants.

For the research, Vaughn's group made experimental hydromulches, composed of water, straw as the mulch, and the candidate binder added as a dry powder in the same amount that guar gum is used in hydromulching. Each experimental hydromulch was then exposed to a series of tests that were designed to simulate the effects of warm, dry days, and of rainfall, which could loosen and carry off some of the mulch.

The dry weight of each hydromulch at the end of the tests was used, in a simple equation, to estimate the relative strength of each binder. "Essentially, less mulch loss meant a higher end-dry-weight and a stronger binder," says Vaughn, who is coordinating outdoor experiments as a followup to the lab tests.

Half a dozen binders were stronger than guar gum. They included xanthan gum, made by the bacterium *Xanthomonas campestris*; the plant-cell-wall compound lignin, to which calcium was added so that lignin could act as an adhesive; and psyllium, whose seed coat contains mucilage, a natural glue.

Others in the top six: Gums extracted from seeds of two mustard family members, camelina and lesquerella; and a starch-based material made of cornstarch loosely bound to sodium palmitate, a fat (technically, a fatty acid) found in many everyday vegetable oils. Peoria researchers George Fanta, Fred Felker, and Jim Kenar developed the environmentally sound process for creating this compound, referred to as a "high-amylose starch-lipid inclusion complex."

Vaughn says that, to the best of his knowledge, the panel of 10 compounds selected for the lab tests is unique. Although starch has been used commercially as a hydromulch binder, starch-lipid complexes made with the eco-friendly process developed at Peoria apparently had not been—until this study—lab-tested for this specific use.

Details are in a 2012 article in *Industrial Crops and Products,* written by Vaughn, Fanta, Felker, and Kenar; Steve Cermak, Bob Behle, Mark Berhow, and Roque Evangelista, also with the Peoria center; and by Ed Lee of HydroStraw, LLC.—By **Marcia Wood,** ARS.

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

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Flower Power Protects Organic Lettuce Fields

STEPHEN AUSMUS (D3049-9)

Workers in Salinas, California, harvest organic romaine lettuce produced during a field trial in which alyssum (white flowers) is planted among the lettuce for aphid control.

In the language of flowers, sweet alyssum means "worth beyond beauty." Now findings by Agricultural Research Service horticulturist Eric Brennan are highlighting some cost-effective measures to help the tiny white blossoms live up to their reputation.

"Organic lettuce growers like to use alyssum in their production because it is such an effective way to control aphids," Brennan says. "If you drive by a field in central California that has a lot of alyssum, it's almost guaranteed to be an organic lettuce field."

Lettuce growers in California's central coast plant alyssum to attract adult hoverflies that feed on the flower's pollen and nectar. After eggs laid by the well-fed females hatch, the voracious larvae prey on currant-lettuce aphids—important primary insect pests of lettuce in the region. The aphids are particularly difficult to control because they colonize the interior leaves of the lettuce plant.

Alyssum is so effective at helping to control aphid populations that it can be planted on up to 10 percent of the land used to grow organic lettuce. But agricultural rent in central California can be costly, so growers need to devote as much land as possible to cash crop production.

Brennan, who works at the ARS U.S. Agricultural Research Station in Salinas, California, wanted to figure out the most cost-effective alyssum planting patterns in lettuce fields to help organic producers maximize their profits. At a working research farm in Salinas that is certified for commercial organic production, he established experimental beds with several different planting combinations of alyssum and romaine lettuce.

Blossoms With Benefits

In five of these combinations, 2 percent to 8 percent of the lettuce plants were replaced by alyssum plants. These were called "replacement" treatments, and each replacement plot was planted at a rate equivalent to 26,440 lettuce plants per acre. In two of the other combinations, alyssum was planted in addition to the full complement of lettuce. One of these beds was planted at a rate equivalent to 27,087 lettuce plants per acre and the other at a rate equivalent to 28,598 lettuce plants per acre. These two treatments were called "additive" treatments. Brennan also varied the distribution of the flowers in the fields by either planting the alyssum in strips or interspersing it with the lettuce plants.

For two growing seasons, Brennan evaluated how alyssum biomass and flower production varied with alyssum planting density and how competition between lettuce and alyssum affected the biomass of both plants.

The scientist observed that alyssum in the additive beds produced more blossoms per gram of alyssum dry matter, possibly because the alyssum plants and lettuce plants were competing more directly for nutrients needed to support biomass

STEPHEN AUSMUS (D3051-2)

An adult hoverfly on an alyssum flowerhead. Hoverflies are a natural enemy of aphids that can infest lettuce and the flies are attracted to fields by alyssum interplanted with the lettuce.

production. This combination increased alyssum's value as an insectary plant—a flowering plant cultivated in crop fields because it attracts insect predators to feed on pests.

Brennan also noticed that in the additive treatments, alyssum and lettuce canopies began to overlap around 22 days after they had been transplanted to the field, while in the replacement beds, the canopies did not start to overlap until 32 days after transplanting. This observation provided additional evidence that competition between alyssum and lettuce in the additive treatments began earlier in the season and was more intense than competition in the replacement treatments.

Brennan thinks these findings will be useful in determining the best way to intercrop alyssum in lettuce production systems to effectively control aphids; maximize lettuce yield and profitability; and minimize the costs and complications that come with transplanting, weed management, harvesting, marketing, and postharvest tillage.

His field research indicates that additive treatments may be the most efficient intercropping approach for producing romaine lettuce hearts, where smaller lettuce plants are desired. This is because the number of lettuce plants was not reduced in additive treatments, and alyssum transplants in the additive treatments were able to produce 78 percent as many open alyssum flowers as the replacement treatments. Although lettuce from replacement beds produced more biomass, Brennan believes that this increase might not be relevant at a commercial scale once the lettuce is harvested, trimmed, and packed for market.

A Scattered Approach

Brennan has also concluded that an alternative to intercropping lettuce with strips of alyssum in specific rows would be to randomly intersperse alyssum plants throughout all lettuce rows, an approach that is already being used by some lettuce producers. Dispersing the alyssum throughout the field could minimize competition between lettuce and alyssum. It may also encourage adult hoverflies to forage for pollen and nectar more evenly throughout the field.

This alternative would also eliminate concentrated strips of alyssum that are difficult to hand weed—an important consideration in organic cropping because hand weeding is expensive and weeds that survive can produce seed that infest future crops. Scattering alyssum throughout a field could also minimize postharvest tillage requirements, because alyssum shoot residue can be difficult to incorporate into the soil, especially when it is concentrated in strips.

"This was a fun and colorful trial," concludes Brennan, who published results from his work in *Biological Control*. "I'm getting international requests for the paper, and now I'm working with a large-scale organic lettuce producer on different ways of planting alyssum and lettuce."—By **Ann Perry,** ARS.

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

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Various patterns of intercropping alyssum with organic romaine lettuce for aphid control were assessed at ARS fields in Salinas, California.

STEPHEN AUSMUS (D3050-3)

Specialty Greens Pack a Nutritional Punch

"Microgreens" is a marketing term used to describe tiny, tender, edible greens that germinate in soil or a soil substitute from the seeds of vegetables and herbs. Smaller than "baby greens," and harvested later than "sprouts," microgreens can provide a variety of leaf flavors, such as sweet and spicy. They are also known for their various colors and textures. Among upscale markets, they are now considered a specialty genre of greens that are good for garnishing salads, soups, plates, and sandwiches.

Microgreens began showing up on chefs' menus as early as the 1980s, in San Francisco, California, according to a local industry source. Today, the U.S. microgreens industry consists of a variety of seed companies and growers.

A microgreen has a single central stem, which has been cut just above the soil during harvesting—in fact, home gardeners often snip them with scissors. The seedlings are well suited for local growers because microgreens are harvested just 7 to 14 days after germination when the cotyledons (seed leaves) have fully developed and before the true leaves have expanded.

PEGGY GREB (D3089-2)

ARS scientists analyzed key nutrients in 25 different varieties of microgreens and found that red cabbage microgreens (shown here) had the highest concentrations of vitamin C. These nutritious microgreens are ready to harvest just 10 days after planting.

Now, a team of Agricultural Research Service scientists and colleagues has published several studies that shed light not only on microgreens' nutritional benefits, but also on their complex shelf-life requirements.

Tiny But Mighty Produce

Microgreens are usually harvested at 1 to 3 inches tall and, depending on the species, are sold with the stem attached to the cotyledons. Plants with two cotyledons are called "dicots," and those are the leaves that the scientists studied. Plants with a single cotyledon are "monocots."

Crops that germinate easily and grow quickly are good candidates for growing as microgreens. ARS plant physiologist Gene Lester led a team of scientists who analyzed the key nutrients in different varieties of vegetable microgreens. The study results could be used as a reference in estimating the amounts and adequacies of specific vitamins that are gained when consumers eat microgreens, according to the authors.

The researchers determined the concentration of essential vitamins and carotenoids in 25 commercially available varieties of microgreens. Key nutrients measured were ascorbic acid (vitamin C), tocopherols (vitamin E), phylloquinone (vitamin K), and beta-

PEGGY GREB (D3087-1)

At the Food Quality Laboratory in Beltsville, Maryland, food technologist Yaguang Luo takes samples of swiss chard microgreens for microbial studies. She is collecting information on different plant species grown as microgreens in order to ultimately assist growers, grocers, and chefs.

carotene (a vitamin A precursor), plus other related carotenoids in the cotyledons.

The team showed that different microgreens contained widely differing amounts of vitamins and carotenoids. Total vitamin C content ranged from 20 to 147 milligrams (mg) per 100 grams of cotyledon fresh weight, depending on which plant species was being tested. The amounts of the carotenoids beta-carotene, lutein/zeaxanthin, and violaxanthin ranged from about 0.6 mg to 12.1 mg per 100 grams of fresh weight. For comparison, an average apple weighs 100-150 grams.

Among the 25 microgreens tested, red cabbage, cilantro, garnet amaranth, and green daikon radish had the highest concentrations of vitamin C, carotenoids, vitamin K, and vitamin E, respectively. In general, microgreens contained considerably higher levels of vitamins and carotenoids—about five times greater—than their mature plant counterparts, an indication that microgreens may be worth the trouble of delivering them fresh during their short lives.

Growing, harvesting, and handling conditions may have a considerable effect on nutrient content. Additional studies are needed to evaluate the effect of these agricultural practices on nutrient retention. The study was published in 2012 in the *Journal of Agricultural and Food Chemistry*.

Buckwheat Is Not Just for Pancakes

Buckwheat "seeds" look like cereal grains, but they are actually dry, hard-covered fruits called "achenes." Each achene contains one small seed. During germination, the seed bears cotyledons, thus accounting for buckwheat's candidacy as a microgreen.

Gram for gram, buckwheat has almost the same amount of protein as oats, according to the ARS National Nutrient Database for Standard Reference. It's also gluten free.

In a study headed by ARS food technologist Yaguang Luo with the Food Quality Laboratory in Beltsville, Maryland, the researchers focused on buckwheat microgreens, which, in addition to high protein, are considered high in antioxidants, flavonoids, carotenoids, and alpha-tocopherol. Like all microgreens, buckwheat microgreens typically have only a few days of shelf life. The team found that storage temperature and atmospheric composition are key variables when it comes to fresh-cut buckwheat microgreens. They extended shelf life by storing the greens at a relatively low 5˚C and by elevating CO_2 and reducing O_2.

The researchers also fine-tuned packages to provide the optimal atmospheric composition required to extend the shelf life of buckwheat microgreens. Commercial microgreens are most often stored in plastic clamshell containers, which do not provide the right balance of O_2 and CO_2 for live greens to "breathe." Among package materials called "films," differences in permeability are referred to as "oxygen transmission rate."

The researchers found that buckwheat microgreens packaged in films with an oxygen transmission rate of 225 cubic centimeters per square inch per day had a fresher appearance and better cell membrane integrity than those packaged in other films tested. Following these steps, the team maintained acceptable buckwheat microgreen quality for more than 14 days—a significant extension, according to authors. The study was published in *LWT-Food Science and Technology,* in 2013.

"Due to their short shelf life and growing requirements, bringing safe, high-quality microgreens to market can be relatively complex and labor-intensive," says Luo. "More studies are needed to understand their postharvest processing requirements." Studies on individual plant species grown and harvested as microgreens are helping to fill the dearth of information on this budding industry, which will ultimately assist growers, grocers, and chefs.—By **Rosalie Marion Bliss,** ARS.

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

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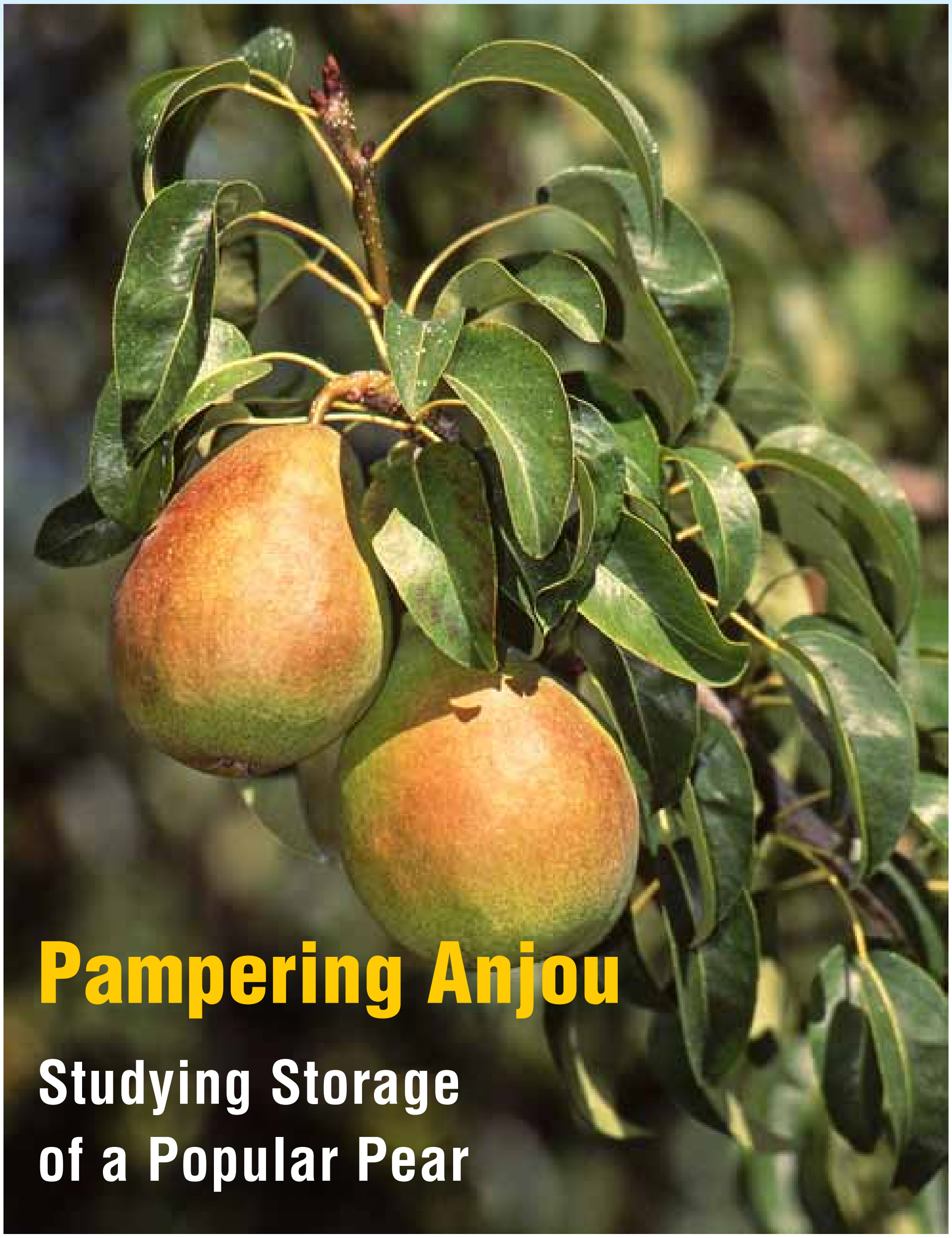
Plant physiologist Gene Lester and research assistant Zhenlei Xiao extract human health nutrients from microgreens.

PEGGY GREB (D3088-1)

DOUG WILSON (K4315-13)



Anjou pears like these don't ripen on the tree. Commercially grown Anjou pears are ripened and stored indoors with the use of science-based technologies.

Pampering Anjou

Studying Storage of a Popular Pear

No wonder the Anjou is one of America's favorite pears. When properly ripened, this chubby fruit is sweet and juicy, with a pleasing, buttery-smooth texture.

In the United States, Anjou (also called d'Anjou) pears are grown commercially in Washington and Oregon, where they are harvested in late summer. Thanks to storage technologies that postpone ripening, the pears are usually available through the following spring at the produce section of your local supermarket.

Long-term storage of this highly perishable fruit typically involves keeping it in sealed storerooms in which the chilled air is low in oxygen. This refrigerated, "controlled atmosphere" environment slows the fruit's respiration and helps keep some storage-associated diseases at arm's length.

But the Anjou is not always an easy pear to store. The pears can develop storage-related diseases, or other problems, such as internal or external browning. Or, once taken out of storage, they can sometimes be annoyingly slow to ripen.

In Washington's Wenatchee Valley, one of the nation's premier pear-growing regions, Agricultural Research Service plant physiologist Jim Mattheis and colleagues conduct studies that are designed to help growers and packers sidestep these and other storage-associated problems.

I-MCP and the Anjou

In a series of Anjou-focused studies that began in 1998, Mattheis and colleagues investigated the use of a compound, 1-MCP (methylcyclopropene). Their intent? To see how well this compound could delay ripening. Ideally, delayed ripening prolongs storage life—without lessening the pears' ability to ripen on demand later on.

1-MCP has been used in this country since 2002 to extend storage of apples. Mattheis's team, however, is the first to document, in a peer-reviewed scientific journal, its use on Anjou pears kept in refrigerated storage for up to 8 months.

The team's article, published in the *Journal of Agricultural and Food Chemistry* in 2003, indicated that results were mixed. On the positive side, the team found that applying 1-MCP, as a gas, at 100 parts per billion—a level approved as safe for food use—prolonged refrigerated storage of the pears as compared to that of untreated, refrigerated pears.

What's more, the 1-MCP treatment resulted in significantly less superficial scald and pithy brown core. Superficial scald is characterized by a dark-brown discoloration of the skin, which makes the pear unmarketable. Pithy brown core, as its name implies, discolors the pear near its core and changes the pear's texture from delectable to unpleasantly fibrous.

In addition, the research suggested that the treatment might provide an alternative to ethoxyquin, which is used today to control scald on Anjou. An ethoxyquin alternative would be useful for exports if the European Union decides, at some point, to ban the compound.

Another plus: applying 1-MCP may have the potential to enable packers to store Anjou pears in regular air (the air we breathe), with refrigeration, instead of in controlled atmospheres. "Pears stored in air in our 1-MCP study had delayed ripening and no scald or pithy brown core," says Mattheis. Cutting controlled atmosphere out of the storage picture, by using air instead, might reduce storage costs and perhaps have environmental benefits, as well.

But the study also showed that when the 1-MCP-treated pears were taken out of cold storage and exposed to ethylene—a natural compound that triggers ripening—the warmth and the ethylene did not consistently or predictably initiate ripening. The resulting delays in ripening can be frustrating, disruptive, and costly for packers and buyers alike.

The 2003 study has paved the way for follow-up experiments, in which the Wenatchee scientists are examining various combinations of 1-MCP application rate; storage duration, temperature, and regimen (controlled atmosphere or air); and post-storage temperature. This work "has shown promise in promoting consistent Anjou ripening after storage," Mattheis says. In the meantime, about 5 to 10 percent of Pacific Northwest-grown pears are being treated with 1-MCP.

Monitoring Chlorophyll Fluorescence: Not Well-Suited to Anjou?

Standard controlled atmosphere provides pears with 1.5 to 2 percent oxygen. Storing scald-sensitive fruit, such as Anjou pears, in very-low-oxygen concentrations—that is, below 1 percent—tends to prevent scald, says Mattheis. However, these very low concentrations may present other risks to the pear's quality, such as development of off-flavors or black speck—an unwanted speckling of the pear's skin.

With these risks and benefits in mind, the team explored the potential of using chlorophyll fluorescence monitoring as an "early warning system" of increased risk of low-oxygen-associated problems in stored Anjou pears. With stored apples, an increase in fluorescence levels of the chlorophyll in the peel apparently correlates well with increased risk of low-oxygen-linked problems. So, when apple chlorophyll fluorescence levels go up during very-low-oxygen storage, storehouse managers raise the oxygen level slightly to prevent damage to the fruit.

Use of the technology for keeping an eye on stored apples and pears is still relatively new, and very little research data about its applicability to Anjou has been published. Mattheis and co-workers have helped fill in the knowledge gap by conducting preliminary tests of Anjou pears stored at the conventional 1.5 percent oxygen or at extremely low levels of 0.5 or 0.4 percent.

With Anjou, fluorescence levels did not warn of the development of disorders. For instance, black speck and pithy brown core developed with no detectable changes in the pears' chlorophyll fluorescence levels.

These and other findings are documented in articles published in 2010 and 2013 in *Postharvest Biology and Technology.*

"For now," says Mattheis, "we advise packers to use caution before relying on chlorophyll fluorescence for monitoring stored Anjou pears in very-low-oxygen conditions."

Mattheis collaborated in the research with Wenatchee plant physiologist David Rudell; former postdoctoral research associates Xuetong Fan (now with ARS in Wyndmoor, Pennsylvania), Luiz Argenta, and David Felicetti; industry colleague Nate Reed; and former Oregon State University researcher Jin Bai, today with ARS at Fort Pierce, Florida.—By **Marcia Wood,** ARS.

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

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Fresh pears add flavor, fiber, vitamin C, and more to a hearty oatmeal.

IMAGE COURTESY OF PEAR BUREAU NORTHWEST (D3096-1)

Chickens Open Wide for Gelatin Bead Vaccine

Making sure all newborn chicks are vaccinated right out of the hatchery isn't always easy. Some birds may be missed by standard poultry vaccination methods and, consequently, left with little defense against intestinal diseases. Developed by scientists at the Agricultural Research Service, a new vaccine delivery system to prevent diseases like coccidiosis may be more appetizing to birds than traditional methods.

Coccidiosis, a common and costly poultry disease, is caused by tiny, single-celled parasites. These parasites, which belong to the genus *Eimeria,* live and multiply in the intestinal tract and cause tissue damage that hinders the bird's ability to digest feed and absorb nutrients. Infected birds shed oocysts—the egglike stage of the parasite—in their feces, and the oocysts transform into infectious forms once in litter, soil, feed, or water. As chickens peck around in the litter, they can ingest the oocysts and become infected. The results are slower weight gain and growth and sometimes death.

The disease costs an estimated $350 million in the United States and more than $3 billion worldwide each year. Until recently, coccidiosis outbreaks were mainly controlled by medicating feed with anticcocidial drugs, but the parasite's increasing resistance to drugs prompted the development of vaccines.

Scientists at ARS's Henry A. Wallace Beltsville Agricultural Research Center (BARC) in Beltsville, Maryland, are developing better vaccines and methods of protection against coccidiosis and other poultry diseases. Collaborating with researchers at the nonprofit Southwest Research Institute (SwRI) in San Antonio, Texas, they have developed an effective vaccine delivery system by putting low doses of live *Eimeria* oocysts inside gelatin beads that chickens readily gobble up.

STEPHEN AUSMUS (D3070-9)

At the Animal Parasitic Diseases Laboratory in Beltsville, Maryland, newly hatched chicks ingest gelatin beads, a new vaccine delivery system, to protect them from the disease called "coccidiosis." Inside the beads are *Eimeria* oocysts.

Beads Are Better

Microbiologist Mark Jenkins and zoologist Ray Fetterer, in BARC's Animal Parasitic Diseases Laboratory, are attempting to increase vaccine uptake by studying alternative delivery methods. With the standard industry vaccination method, about 100 chicks at a time are placed into a tray that's detected by a light sensor as it moves, activating the release of vaccine spray above the heads of the chicks. A harmless red dye in the spray is used to identify birds that have been vaccinated. Chicks inhale or ingest the vaccine, which induces protection against disease.

Jenkins used a hand-held sprayer to deliver a vaccine formulation to newly hatched chicks, and then he measured vaccine uptake.

STEPHEN AUSMUS (D3069-12)

Biologist Mark Jenkins (left) and zoologist Ray Fetterer observe 1-day-old broiler chicks ingesting gelatin beads containing the vaccine for coccidiosis.

STEPHEN AUSMUS (D3071-5)

Support scientist Carolyn Parker prepares gelatin beads for vaccination studies. Broiler chicks readily gobble up the beads—a new delivery system to ensure chicks get enough of the vaccine.

"With spraying, some chicks were getting a lot of the vaccine and some weren't getting any," Jenkins says, leaving them vulnerable to acute coccidiosis and associated necrotic enteritis.

Scientists looked at gelatin beads as an alternative vaccination method. They experimented with different formulations, sizes, and colors. The beads they settled on were red or green and about 2 millimeters in diameter, similar to the size of feed grains fed to young chicks.

"Our primary goal was to develop a formulation that would prevent the gelatin beads from drying out when they're put into poultry houses, where temperatures can exceed 90°F," says Joseph Persyn, SwRI manager of microencapsulation and nanomaterials. "The beads needed to retain moisture to keep the *Eimeria* oocysts active and to remain pliable so the chicks would eat them." When beads dry out, they become as hard as pebbles, Persyn adds.

Beads for Broilers

After seeing positive results in chicks of egg-layer hens, Jenkins and Fetterer evaluated the effectiveness of the gelatin bead vaccine in broilers, which are raised for meat.

Vaccine uptake and protection against *Eimeria* challenge infection was compared between day-old chicks fed gelatin beads, those immunized with a hand-held sprayer, and a control group. Chicks fed gelatin beads had a vaccine uptake 10- to 100-fold greater than the spray-vaccine group. Also, birds that consumed vaccine beads displayed higher and more uniform protection against coccidiosis than spray-vaccinated birds.

"Response was amazing in broilers," Jenkins says. "You put them in a cage and they run over and start eating right away. Within an hour, those beads were all gone."

Scientists also examined the efficacy of vaccine beads in chickens raised similarly to those in a poultry house. Newly hatched chicks were vaccinated by either a spray method or gelatin beads. Chicks were then raised in floor-pen cages in direct contact with litter. At 4 weeks of age, all chicks received an experimental challenge dose of *Eimeria* oocysts. Chicks immunized with beads displayed significantly greater weight gain than an unvaccinated control group. Their ability to convert feed into body mass also was greatly enhanced.

"Gelatin beads may effectively improve vaccine delivery to chickens in the field and thereby reduce the incidence of coccidiosis," Jenkins says. "This would ultimately improve performance in broilers as well as egg-layers."

The next step is to test the gelatin beads in a commercial broiler house, Jenkins says. The scientists are looking for a poultry industry partner to take the next step in evaluating the beads.

The collaboration between ARS and SwRI will continue. Research will focus on investigating methods to improve the gelatin bead formulation and the possibility of developing a vaccine delivery device that can be used in commercial poultry houses.

A patent application has been filed by ARS and SwRI scientists for their technology of incorporating *Eimeria* oocysts into gelatin beads.—By **Sandra Avant**, ARS.

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

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National Inventory Takes Stock of Crops' Wild Relatives

An estimated one of every five plant species worldwide is endangered by habitat loss, climate change, invasive species, and other threats. In the United States alone, roughly 30 percent of native plant species are similarly threatened, and a surprising number may be closely related to crop plants we use every day. Losing these and other crop wild relatives (CWR) could be detrimental to agriculture both domestically and abroad, researchers say. That's because these wild relatives provide critical sources of genetic diversity that can be tapped for an array of economically important traits—such as resistance to emerging pests and diseases, increased yield, and better drought tolerance or adaptability.

Over the past few years, Agricultural Research Service and collaborating scientists have worked to create a first-of-its-kind inventory for U.S. wild and weedy crop relatives that prioritizes the species by their importance to breeding new, improved varieties. In doing this, the researchers looked at how closely related the wild species are to crops—especially those grown for food—which determines the ease or difficulty in transferring desirable traits to their cultivated cousins. Another consideration was their availability in gene banks or protected habitat areas, explains ARS plant geneticist Stephanie Greene.

KEITH WELLER (K4414-2)

Cranberry and some other crops have few native wild relatives represented in U.S. gene banks. ARS scientists hope to expand representation of those wild relatives to preserve genetic diversity for future crop breeding.

Greene has been collaborating on the inventory project with John Wiersema, a botanist with the ARS National Germplasm Resources Laboratory in Beltsville, Maryland, and scientists from the International Center for Tropical Agriculture (CIAT) in Colombia, Wageningen University (WU) in The Netherlands, and the University of Birmingham (UB) in the United Kingdom. Additional support was provided by the Global Crop Diversity Trust, which spearheads a worldwide project to collect CWR that haven't been conserved in gene banks and to breed useful CWR traits into crops.

Such efforts "also provide a good opportunity for international collaboration, as no single country is self-sufficient in CWR diversity," comments Nigel Maxted, a senior UB lecturer on plant genetics conservation. "Each country grows a mix of crops that originate outside its borders, so cross-border collaboration is key if all breeders are to retain access to the CWR diversity they need to sustain food security in the face of climate change."

All told, the U.S. national inventory covers 4,596 taxa from 985 genera and 194 plant families that are either indigenous to the United States or have become "naturalized"—established of their own accord following human introduction. Among CWR of major crops, the genus *Helianthus* (sunflower) is the most abundant, numbering 73 total species, including *H. annuus*

PEGGY GREB (D3093-1)

Wild relatives of the genus *Helianthus* (sunflower) are abundant in U.S. gene banks, because sunflower is one of the few crops native to North America. Shown is a wild annual sunflower, *H. annuus,* one of 73 total species represented.

STEPHEN AUSMUS (K10716-1)

Many fruits, such as these blackberries, have crop wild relatives that are native to the United States. These wild relatives provide sources of genetic diversity for important traits such as resistance to pests and disease, better yield, and drought tolerance.

(domesticated as the sunflower). Other important CWRs include species closely related to strawberry; stone fruits such as cherries and plums; blackberries; raspberries; blueberries; and grapes. The inventory also contains entries for traditional or iconic crops unique to the United States, such as pecan, American chestnut, and wild rice, as well as so-called "wild-utilized species," with direct use for medicinal, ornamental, landscaping, environmental restoration, or industrial purposes.

"We have identified approximately 250 species of CWR that are considered high priority because they have a lot of potential in plant breeding," says Greene, who works in ARS's Plant Germplasm Introduction and Testing Research Unit worksite in Prosser, Washington. "We also identified species that are vulnerable because they are rare, endangered, or have not been collected and placed in gene banks." Crop wild relatives with limited representation in U.S. gene banks include species related to cotton *(Gossypium),* lettuce *(Lactuca),* plums *(Prunus),* gooseberry *(Ribes),* sugarcane *(Saccharum),* cranberry *(Vaccinium),* and wild rice *(Zizania),* she adds.

National CWR inventories have already been assembled in Europe and other parts of the world. Despite its 20,000 species of native or naturalized plants, North America (excluding Mexico) has not been considered a major center of crop genetic diversity, as Central and South America, the Middle East, and other regions have been. This is because few major crop progenitors other than sunflower are native to North America, note Greene, Wiersema, Maxted, and coauthors Colin Khoury (CIAT, WU), Andy Jarvis (CIAT), and Paul Struik (WU) in the July 2013 issue of *Crop Science.*

Nonetheless, there are many examples of native wild species that have played key roles in ensuring the continued health and productivity of crops grown worldwide. The inventory itself lists 17 major crops that have benefitted from traits associated with 55 native CWR. For example, in the late 1800s, North American wild grape rootstocks provided a key source of *Phylloxera* resistance necessary to develop hardier European grape varieties. Today, these rootstocks remain foundation sources of protection against the sap-sucking insect. More recently, cultivated sunflowers worldwide have benefitted from their wild North American relatives in the form of resistance to rust, *Sclerotinia,* downy mildew, and other diseases and pests.

In addition to the value to plant breeding, other considerations in prioritizing the U.S. national inventory included the CWR's conservation status, an effort aided by accessing sources such as NatureServe, a nonprofit information clearinghouse for rare or endangered species and threatened ecosystems. The team also compiled a list of major world crops, using sources such as the United Nation's Food and Agriculture Organization, and queried the ARS Germplasm Resources Information Network (GRIN) maintained in Beltsville, Maryland, to determine which CWRs occurring in the United States were genetically related to the crops.

Future plans are to fully integrate the inventory's information into GRIN to take advantage of the existing data there and facilitate searches by taxonomy, threat status, and geographic distribution. Wiersema will play a lead role on that effort.

Meanwhile, Khoury, in pursuit of his Ph.D., is performing a "gap analysis." This involves examining the distribution of CWRs and identifying areas where species are already protected, such as in national parks, or in need of protection. Of particular interest are regions where CWR species overlap and the benefits of establishing a reserve to safeguard them could be especially high. Khoury's analysis also looks at gaps that could be filled in the germplasm collections of U.S. gene banks, including the National Plant Germplasm System. The results, coupled with the inventory, "will be used by ARS to direct future domestic collecting efforts and to help develop a national CWR conservation strategy that involves land management agencies, such as the U.S. Forest Service," says Greene.—By **Jan Suszkiw,** ARS.

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

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New Rice Naturally Fends Off Weeds

Rice is a staple food for more than half the world's population, with Africa and Asia leading consumption of this nutritious, energy-rich grain. No matter where it's grown, though, rice faces stiff competition from weeds, which can outcompete the crop for sunlight and nutrients.

But not all rice is so easily bullied. Certain indica rice types from Asia, in fact, produce root secretions called "allelochemicals" that, along with other competitive traits, naturally keep weeds at bay. Despite their prospects for reducing herbicide use, weed-suppressive varieties haven't caught on with U.S. growers, largely because of their poor grain quality.

Now, a Stuttgart, Arkansas, team of Agricultural Research Service and University of Arkansas Division of Agriculture (UADA) researchers has worked to tackle the problem by crossing the indica rices with southern U.S. long-grain cultivars. In 2013, in the journal *Plant and Soil,* the team reported its most recent success, STG06L-35-061, a newly developed rice line that combines the best of both worlds. This line has the agronomic traits sought by the U.S. rice industry as well as weed suppression (allelopathy).

According to David Gealy, a plant physiologist at ARS's Dale Bumpers National Rice Research Center in Stuttgart, the new line is the top pick of some 50 total rice lines that were selectively developed for such traits as high grain yield and quality, early maturity, stem strength, pest and disease resistance, and allelopathy to barnyardgrass and other weeds. Gealy conducted the evaluations as part of a cooperative rice breeding-and-selection program together with UADA rice breeder Karen Moldenhauer and ARS plant geneticist Melissa Jia.

STG06L-35-061 owes its winning combination of agronomic and allelopathic traits to the commercial cultivars Katy and Drew—both tropical japonica rice types—and PI 312777, an indica line. The team's trials included multiyear tests of the new cultivar's yield, height, and flowering time in both weed-infested and weed-free plots, with barnyardgrass as the dominant species. Several commercial cultivars, including Katy, Drew, and Lemont, along with several indica lines, were also tested for comparison.

DAVID GEALEY (D3090-1)

STG06L-35-061 is a new rice line that combines desirable traits like high grain quality with a natural ability to suppress costly weeds like barnyardgrass.

In preliminary yield trials, conducted in 2008 and 2009, weed-suppression ratings for the new cultivar were 41 percent higher than Katy, 68 percent higher than Lemont, and about equal to PI 312777. In weed-free plots, the new rice averaged about 5,000 pounds of grain per acre versus 5,400 for Drew; 4,000 for Katy; and 4,300 for Lemont.

Although tall growing, the new cultivar's sturdy, upright stems kept it from lodging (falling over). Its kernels also scored well on industry tests for cooking and milling properties, with quality similar to Francis and other long-grain rices that were evaluated. Marker-based analysis of the new rice also revealed its inheritance of genes for resistance to rice blast, a devastating fungal disease of rice worldwide.

"These traits should enhance its inherent competitiveness against weeds and its suitability for use in organic or other low-input systems," the team notes in the *Plant and Soil* article.—By **Jan Suszkiw,** ARS.

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

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

Locations Featured in This Magazine Issue

Locations listed west to east.

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

U.S. Agricultural Research Station, Salinas, California
1 research unit ■ 45 employees

Yakima Agricultural Research Laboratory, Wapato, Washington
1 research unit ■ 47 employees

Physiology and Pathology of Tree Fruits Research Unit, Wenatchee, Washington
1 research unit ■ 25 employees

Vegetable and Forage Crops Research Unit, Prosser, Washington
1 research unit ■ 41 employees

U.S. Arid-Land Agricultural Research Center, Maricopa, Arizona
3 research units ■ 61 employees

Stuttgart, Arkansas
2 research units ■ 57 employees

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

Jamie Whitten Delta States Research Center, Stoneville, Mississippi
7 research units ■ 260 employees

U.S. Horticultural Research Laboratory, Fort Pierce, Florida
4 research units ■ 133 employees

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

Eastern Regional Research Center, Wyndmoor, Pennsylvania
6 research units ■ 190 employees