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Agricultural Research

A Glowing Review

. . . of bacteria

See story on page 16.

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

FORUM

Lifting All Boats With Organic and Conventional Research

At first glance, the divide between organic and conventional farming appears to be of Grand Canyon proportions. But when it comes to research, the distance between organic and conventional farming practices is not nearly so great.

The Agricultural Research Service has a national program that specifically carries out studies aimed at helping organic farmers to compete in the marketplace by finding ways to cost-effectively produce abundant amounts of high-quality, safe products to meet consumer demands.

Consumer demand for organically produced goods has shown double-digit growth for well over a decade, providing market incentives for U.S. farmers across a broad range of products. Organic products are now available in nearly 20,000 natural food stores and nearly 3 of 4 conventional grocery stores. Organic sales account for more than 3 percent of total U.S. food sales, according to recent industry statistics.

ARS has responded to this rising demand with an interdisciplinary program of research on the biological and physical processes of plants, invertebrates, microbes, and soils, which may naturally regulate pests and enhance soil fertility.

The agency's scientists are mainly seeking strategies to prevent the problems faced by organic growers and then, secondarily, looking for therapeutic controls that they can use.

From a practical standpoint, this whole-system approach also describes a large part of ARS's research to improve conventional agriculture. Many of the results and lessons learned from conventional ARS research can be readily applied to organic farming systems and vice versa.

For instance, ARS is researching new cover crop mixtures and strategies that can reduce losses of nitrogen to ground and surface water and reduce the need for expensive supplemental nitrogen fertilizers. This would be as much a benefit to conventional farmers as it would for organic producers, because neither group wants to pay for extra fertilizer or pollute waterways. You can read more about these new cover crop ideas on page 4.

The same dual-benefit idea applies to ARS's work on new plant varieties that are bred to be more resistant to diseases, pests, or drought. If a variety is resistant to a pest, it won't need to be treated with a pesticide, so it will be more useful to an organic farmer. But a conventional producer will also appreciate not having the expense of pesticide applications. It doesn't matter whether the breeding program was begun for the sake of organic or conventional producers; it benefits both.

More than 10 years ago, ARS began developing new and improved designs for roller-crimpers to manage and terminate cover crops while maintaining high residue cover as part of no-till agriculture. This research began as a way to reduce erosion and moisture evaporation, limit runoff, and increase infiltration and soil water-storage capability. But it now turns out that some highly effective crimpers, like those described in the story on page 6, can be of use to organic farmers as a way of controlling weeds, which are usually their largest problem.

In addition, ARS is developing specialized transition strategies for farmers who are seeking to convert from conventional to organic production systems. These strategies take into account both the economic and biological costs of conversion, so that farmers will have a complete and true picture of what their choices will mean.

There are other facets to the larger picture for society into which organic production fits. ARS scientists are conducting critical research to ensure public health and safety as well as improve organic production management. They are assessing the prevalence of pathogens that can be associated with fresh produce from organic and conventional production systems. Once they identify critical points along the production, harvest, processing, and transportation chain where pathogens could be introduced and flourish, the researchers can then develop strategies necessary to prevent that occurrence and growth.

The long-term impact of organic farming practices on the environment is another essential issue. All intensive farming—organic or conventional—has the potential for environmental impacts, so ARS researchers are determining the "environmental footprint" of organic and conventional production systems. This research may lead to guidelines that support the USDA Farm Bill Conservation Title.

ARS is uniquely situated to carry out long-term research to learn about the effects of organic farming on production and on soil, water, and air natural resources as well as on weeds and pests, all of which are part of natural ecosystems. Because ARS research is not primarily dependent on short-term grants, our scientists can plan and undertake projects that need to develop data over 5, 10, or 20 years or more in order to monitor production and environmental impacts and changes.

As research increases our understanding of crop biology and environmental interaction, we will have better answers to production management issues. What we see today as differences between conventional and organic farming systems may fade tomorrow.

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Agricultural Research

Solving Problems for the Growing World

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STEPHEN AUSMUS (D2740-4)

Don't let the bed bugs bite! In efforts to find controls for these nightmarish pests, ARS scientists are busy trying to find out what makes these insects tick. Story begins on page 14.

Cover: At Lincoln, Nebraska, microbiologist Lisa Durso examines the results of a test to quantify bacteria in cattle fecal specimens and runoff from manure-amended soils. The number of yellow and glowing wells helps determine the number of bacteria present. Story begins on page 16. Photo by Peggy Greb. (D2752-1)

ERIC BRENNAN (D2769-1)

Meeting all USDA organic certification standards, lettuce harvested from the Salinas test site is packaged in the field and delivered table ready to the store.

Robust Results From Long-Term Research on Organic Cover Cropping

"We can obtain important results from short-term field studies, but they don't always capture year-to-year variability. Long-term research often provides more reliable results that farmers need," says Agricultural Research Service horticulturist Eric Brennan.

Brennan speaks from personal experience. In 2003, he and a team of University of California-Davis collaborators began a 2-year field study that evolved into a unique long-term investigation of high-value organic cropping. The project, the Salinas Organic Cropping Systems trial, is being conducted on an ARS research farm and is now in its 10th year of certified organic production. Brennan, who works in the ARS Crop Improvement and Protection Research Unit in Salinas, California, is analyzing a huge collection of data to pinpoint findings that can benefit commercial producers of organic crops.

Production expenses for high-value organic crops like lettuce and broccoli can exceed $7,000 per acre. Producers who pay high land rent need to maximize profits with an annual two- to three-crop rotation that also includes either keeping fields fallow in the winter or planting winter cover crops.

Many farmers keep winter fields bare because of the extra work and expense of tilling cover crop residues into the soil. Planting a cover crop also requires allowing enough time for its residue to decompose before planting cash crops—and production schedules can become very tight if prolonged spring rains keep farmers from plowing under the winter cover crops.

Brennan designed a long-term investigation of several different cover cropping strategies for an annual lettuce-broccoli production system. Six of the strategies involved cover cropping every winter, and the other two involved cover cropping every fourth winter.

Brennan selected three winter cover crops often grown in the area—rye, mustard, and a legume-rye mix—and planted each at either a typical seeding rate or a rate three times higher. Seeding rates can affect a cover crop's ability to smother weeds.

All systems received the same fertilizer and irrigation inputs and pest management. Harvest and sale of the lettuce and broccoli crops—which met all U.S. Department of Agriculture organic standards—were conducted by a commercial harvester.

Only Time Will Tell

Brennan's results indicated that all three cover crops yielded more dry matter than the 2 tons of crop residue per acre often recommended for maintaining soil organic matter. The legume-rye and rye cover crops produced about 25 percent more dry matter biomass than the mustard crops. But effectively suppressing weeds with the legume-rye crops required seeding at three times the typical rate, while rye and mustard crops appeared to suppress weeds adequately with typical seeding rates.

"In earlier short-term studies, we didn't observe any difference in dry matter production among the three types of cover crops. We also thought that higher seeding rates were always needed," Brennan says. "But in the 8-year study, we clearly saw that mustard did not produce as much biomass as the other two crops and that the legume-rye mix required seeding at the higher rate for effective weed suppression."

The long-term study also provided Brennan with more data about year-to-year yield

JIM LEAP (D2765-1)

In a field planted with a cover crop mixture, horticulturalist Eric Brennan counts the number of plants of each species in the mixture to determine plant density. This field contains a mix of rye, vetch, peas, and faba bean.

variations in the legume-rye mix, including why legumes, which make up most of the seed costs, are not consistently abundant. "We think cooler early-season weather helps legumes compete with the rye," Brennan says. "So if farmers are expecting a hot and dry fall, they might not want to spend the money for a legume cover crop; they might decide to just use rye instead."

Notes on Nitrogen

One benefit of many cover crops is that they take up nitrogen from the soil, which helps reduce nitrate losses into ground water and nearby streams and waterways. When farmers prepare fields for planting by disking cover crop residues into the soil, the nitrogen accumulated by the cover crop gets recycled back into the soil and can be taken up by cash crops that follow.

Brennan's data revealed that the long-term average nitrogen uptake by rye and mustard did not differ significantly (98 and 102 pounds of nitrogen per acre, respectively). But the legume-rye mix accumulated 135 pounds of nitrogen per acre, possibly due to nitrogen fixing by the legumes.

Farmers often rate the quality of cover crop residue by its carbon/nitrogen ratio. "High-quality" residues have lower carbon/nitrogen ratios, so the residue decomposes more quickly and may provide nitrogen to the cash crops that follow. But during wet spring periods, nitrogen that has been rapidly released by these residues may also leach below the root zone more easily. On the other hand, "low-quality" residues with high carbon/nitrogen ratios may temporarily deplete soil nitrogen, as organisms use it to break down the residue.

Brennan determined that cover crop carbon/nitrogen ratios increased through the winter and were always higher in rye than in the other two cover crops. Slower decomposition of low-quality rye residues could use more soil nitrogen than the decomposition of the other two cover crops and might reduce the amount of nitrogen available to the cash crops.

The scientist also used his data on carbon/nitrogen crop ratios and cover crop dry matter production to calculate that cover crop contributions to soil carbon levels would be around 30 percent greater for rye and the legume-rye mixture—1.4 tons per acre—than for mustard, which only added 1.1 ton of carbon per acre.

The Price To Pay

Brennan concluded that rye and mustard planted at typical seeding rates were the most cost-effective cover crops for maximizing dry matter production and weed control. Planting 80 pounds of rye seed would cost $28 per acre, while planting 10 pounds of mustard seed would cost $30 per acre. Seed costs for planting a legume-rye mix at the three times the typical rate—125 pounds per acre, the level needed for good winter weed suppression—was nearly10 times more, at $275 per acre.

But on the plus side, the legumes can help fix nitrogen in the soil. "Farmers have to decide if it's worth the extra cost to plant a legume-rye cover crop, which might contribute more nitrogen for the next crop, or if it makes sense to use more pelleted organic fertilizer made from chicken litter and protein meals instead," Brennan explains.

"These findings can help farmers in high-value vegetable production systems figure out where to spend their money," he concludes. "And as in conventional cropping systems, cover crops can help organic producers improve their environmental footprint with sustainable practices that help reduce soil nitrogen losses—an issue that is becoming increasingly important for agricultural producers."

Some of these findings have appeared in *Agronomy Journal* and *Applied Soil Ecology*.—By **Ann Perry,** ARS.

This research is part of Agricultural System Competitiveness and Sustainability (#216) and Climate Change, Soils, and Emissions (#212), two ARS national programs described at www.nps.ars.usda.gov.

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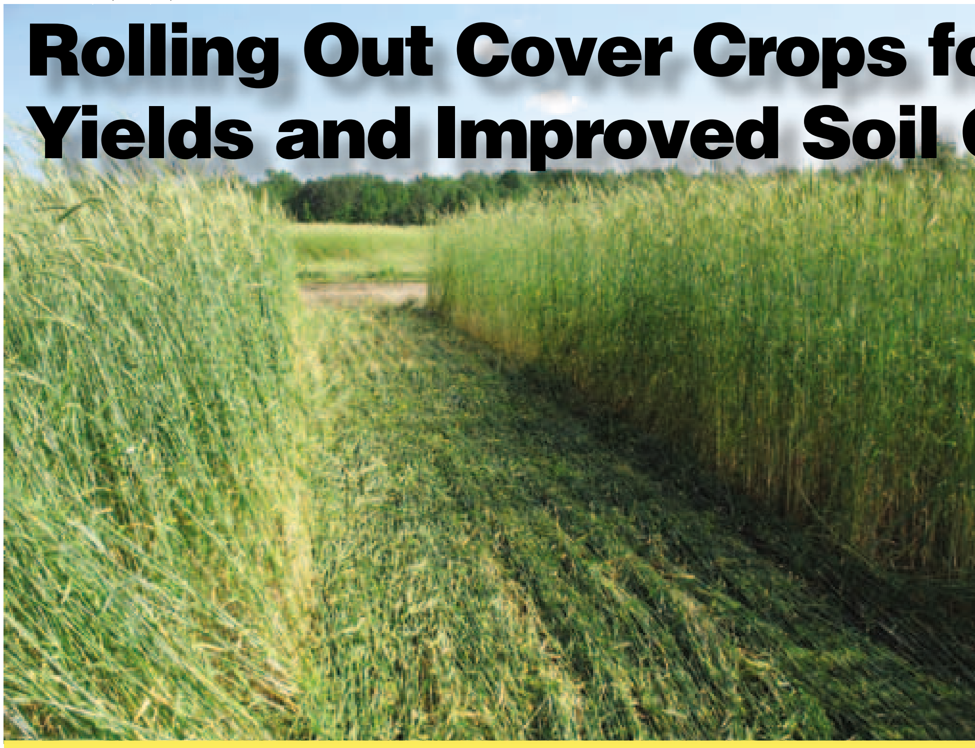
A field test site in Salinas, California, showing a checkerboard of different cover crop treatments being tested, including mustard (yellow flowers), rye, and fallow.

ERIC BRENNAN (D2764-1)

Rolling Out Cover Crops for Higher Yields and Improved Soil Quality



TED KORNECKI (D2762-1)

A rye cover crop just after it was flattened with an ARS-patented two-stage roller/crimper.

Growers who use cover crops are increasingly turning to a tool that can flatten out their actively growing fields, usually in a single pass. Known as a "roller/crimper," the technology can help reduce and sometimes eliminate the need for herbicides and is ideal for organic farmers and growers interested in reducing herbicide use.

Cover crops can improve soil quality; and in organic operations, they play a major role in keeping weeds in check. Crimpers boost those benefits. They have been used for years in South America and are beginning to catch on in the United States, says Ted Kornecki, an agricultural engineer at the Agricultural Research Service's National Soil Dynamics Laboratory in Auburn, Alabama. He has conducted a study evaluating several crimpers to give guidance to growers and has patented three crimper designs.

There are several types of crimpers. Most involve some type of rolling, paddle-wheel-like cylinder that attaches to a tractor and barrels over a field, tamping down and crimping the cover crop into a smooth mat to kill it. About 3 weeks later, a planter, running parallel to the roller's path, can plant seeds directly into the ground without significantly disturbing the biomass mat. The technology has shown promise in early trials and demonstrations.

"It definitely works," says Frank Randle, who helped evaluate a crimper similar to one designed by Kornecki as part of a 4-year demonstration project on his farm near Auburn. Randle used cereal rye and crimson clover as cover crops before planting organic watermelon, squash, okra, and tomatoes. The clover was difficult to kill with the crimper, but the device terminated the rye effectively.

After the 4th year, Randle did have to till the plots to control some perennial weeds, but the crimper could be used again continuously for years after that.

"Yields actually increased a bit over time with the rye because we were adding carbon to the soil, and these are sandy soils that really need some help," Randle said.

The project was a cooperative effort between ARS and USDA's Natural Resources Conservation Service (NRCS). Funding was provided by an NRCS Conservation Innovation Grant.

"Termination Rates" Are Key

Cereal rye is a fairly common choice of cover crop among growers, Kornecki says. Rye is typically planted in the fall, killed in the spring, and left to decompose before a cash crop, such as corn, is seeded through it. The effectiveness of crimping a cover crop largely depends on its "termination rate," or the percentage of it that dies after crimping. Studies show that termination rates of about 90 percent are optimal to ensure that enough residue remains on the soil surface to form a dry, brittle mat that will be easy to penetrate with seeding equipment.

"The more plant biomass you have on the soil surface, the more benefits you see," Kornecki says.

The problem is that vegetable growers need to plant their vegetables at recommended times in the spring for sufficient yields. It can be difficult to hit that "sweet spot" when the time is right for spring planting and the cover crop has reached the optimal stage for termination. With rye, the time is just after flowering.

"If you roll it too early, it's very difficult to kill. The root system is strong and it will compete with the cash crop for moisture and nutrients, and that can reduce the yield," Kornecki says.

Conventional growers can use herbicides to kill their cover crops, but organic growers don't have that option. They are at the mercy of their planting schedules and must sometimes roll cover crops before they are at the right stage for termination.

Assessing Impacts on Sweet Corn

Several different crimpers have been developed, but none has been evaluated for no-till conventional and organic vegetable operations. To get some answers, Kornecki and his colleagues assessed the effects of three experimental roller-crimper systems on soil moisture, yield, and rye termination rates over three growing seasons in a northern Alabama sweet corn field. Each year, they planted the rye in October and crimped it the

TED KORNECKI (D2778-1)

In an experiment at Randle Farms near Auburn, Alabama, technician Corey Kichler operates a two-stage roller/crimper that has a primary drum and a spring-loaded secondary drum with attached equally spaced crimping bars.

following April. They planted sweet corn 3 weeks after crimping the rye—seeding it directly into the rye residue with a no-till planter—and harvested it in August. They passed the crimpers over the rye at two different speeds (3.2 and 6.4 kilometers per hour) to assess the effects of speed on rye termination rates and soil moisture.

The rollers evaluated were an original straight bar, similar to technology developed in South America; a smooth roller with a crimping bar; and a two-stage roller that has both a smooth drum and a spring-loaded crimping bar. The latter two rollers were designed and patented by Kornecki. They compared the rollers to a control treatment where glyphosate was applied to kill the rye and a smooth drum roller was used to flatten it.

They found that roller type and operating speed did not affect soil moisture. At either speed, the rollers produced higher yields than the control treatment in the first year of the study, when rainfall was plentiful, and in the second year, when drought occurred. None of the roller designs was as effective at killing the rye as the glyphosate control treatment, however, with overall termination rates of about 50 percent, well below the recommended 90 percent. But that was because the researchers did what most growers do: They planted the cash crop at the recommended planting dates, which meant rolling the rye earlier in its growth cycle than when it ideally should have been rolled. The researchers believe that with improved timing, the rollers could produce optimal termination rates.

The results, published in 2012 in *HortScience*, give guidance to organic vegetable growers who cannot use herbicides. The researchers recommend that growers in Alabama minimize the risk of low termination rates by planting rye by late September instead of mid-October so that it can be rolled 2 weeks earlier in the spring. They also recommend making multiple passes with the roller to increase termination rates.

Kornecki is seeking commercial partners to develop the two larger, patented rollers evaluated in the study as well as a new one he has developed and patented. Intended for smaller operations, it can be guided like a lawn mower and uses a 2-wheel walk-behind small tractor as a power source.—By **Dennis O'Brien,** 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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Agricultural engineer Ted Kornecki adjusts the crimping force of his patented smooth roller with oscillating crimping bar.

TED KORNECKI (D2758-1)

PEGGY GREB (D2744-1)

ARS plant pathologist Carolee Bull examines the bacterium that causes bacterial blight of cruciferous crops such as arugula, broccoli, Brussels sprouts, and cauliflower.

Emerging Enemy of Veggies Unmasked

Chic, trendy veggies like arugula and baby broccoli, and familiar stand-bys like Brussels sprouts and cauliflower, are vulnerable to attack by a once-puzzling pathogen. Agricultural Research Service plant pathologist Carolee T. Bull and colleagues have, in laboratory, greenhouse, and field research, detected, identified, renamed, and classified the plant-killing microbe that's now officially known as *Pseudomonas cannabina* pv. *alisalensis*. "Pv." stands for "pathovar" and indicates that the microbe is a specific pathogenic form, or strain, of a species.

Their work has sorted out some of the taxonomic confusion surrounding classification of the large, complex group of harmful bacteria in the genus *Pseudomonas*, to which *P. cannabina* pv. *alisalensis* properly belongs.

In so doing, the team has helped growers, vegetable processors, fellow scientists, and anyone who enjoys eating good-for-you cruciferous veggies.

Bull is in the ARS Crop Improvement and Protection Research Unit in Salinas, California. The lab is located in the Salinas Valley, a region nicknamed "The Salad Bowl of the World" because of the quality, quantity, and diversity of vegetables grown there.

The pathovar was named for the Alisal district of Salinas, a neighborhood that's home to many ag workers. The district is also near the site where, in 1995, the microbe was collected—for laboratory analysis—from a commercial crop of broccoli raab, a leafy veggie with an intense, mustardy flavor.

Bull's detective work with the microbe dates back to 1998, when colleagues Steve Koike, a University of California Cooperative Extension farm advisor; and Ron Bunch and the late Jim Mannasero, both with D'Arrigo Brothers Company of California, a major developer and producer of fresh vegetables, came to her for her input.

With funding from the company and ARS, Bull and coworkers began investigating what was, at the time, an unidentified pathogen affecting broccoli raab.

In the years that followed, the microbe was detected not just on that vegetable, but also on several other cruciferous crops in the region, including arugula, broccoli, Brussels sprouts, cauliflower, and rutabaga.

In addition to showing up in California vegetable fields, the pathogen has, to date, been found on crops in Nevada, New Jersey, Oklahoma, South Carolina, and Wisconsin, and overseas in Australia, France, Germany, and Greece.

Unsightly Spots Ruin Crops

P. cannabina pv. *alisalensis* causes a disease known as "bacterial blight of crucifers." Symptoms on afflicted leaves include water-soaked spots that develop a bright-yellow border. Eventually, the spots coalesce and turn brown, giving the leaves a burnt, unattractive appearance that makes the harvest from the plants unmarketable.

Besides ruining a harvest, bacterial blight can rack up other costs. For instance, it can cause delays in planting of previously infested fields. A study by Bull and colleagues showed that tilling, after harvest of broccoli raab plants infected with the pathogen, contaminated the soil and led to infection of the next crop.

"Allowing more time to elapse after tilling reduces incidence of the disease," she says. But such delays can disrupt growers' tight schedules for rotating fields from one crop to the next. Losses attributable to the bacterial blight pathogen affect consumers, too. Shortages of a popular specialty veggie at the supermarket or local produce stand, for example, can drive up prices.

STEPHEN AUSMUS (D832-10)

Brussels sprouts, a cruciferous crop that is vulnerable to bacterial blight.

Sorting Out Who's Who Among *Pseudomonas* Species

The blight pathogen is among a number of pseudomonads that fluoresce when, in the lab, scientists expose them to ultraviolet (UV) light. This trait, and others, may have contributed to confusion surrounding *P. cannabina* pv. *alisalensis* and another microbe, *Pseudomonas syringae* pv. *maculicola*, best known as the culprit behind pepper spot disease—first known as "pepper spot disease of cauliflower."

Not only do both pathogens fluoresce under UV light, but they also perform similarly in several physiological tests typically administered if microbes glow in the UV assay. Also, the host ranges of these two microbes overlap to some extent. An example: broccoli, cabbage, and cauliflower are hosts to both of the pathogens.

"In the past, these shared traits contributed to the inability to separate these pathogens from one another," Bull says. "For decades, any fluorescent pseudomonad causing disease on a crucifer was almost automatically identified as *Pseudomonas syringae* pv. *maculicola*, regardless of the severity of the symptoms.

"Pepper spot disease is mild compared to bacterial blight. Bacterial blight can lead to complete crop loss, while pepper spot disease does not."

Why is it so important to know precisely "who's who" when identifying a plant pathogen?

"Correct identification and classification is the first step toward controlling plant diseases," explains Bull, whose research includes finding environmentally friendly, affordable ways to undermine costly plant pathogens.

Carolee Bull and University of California Cooperative Extension farm advisor Steve Koike scout for bacterial blight in a field of broccoli raab.

PEGGY GREB (D2746-1)

There are other compelling reasons for precise identification of microbes that make plants ill. Growers who are correctly informed about the cause of a disease can make better decisions about which crops to plant—or not plant.

"We showed that the host range of the blight pathogen is broader than the host range of the pepper spot pathovar," says Bull. "Our host-range data helps growers plan strategic crop rotations, such as planting lettuce, a nonhost of the blight pathogen, in blighted fields."

Correct identification is also essential to plant scientists worldwide who detect, diagnose, report, and track outbreaks of plant diseases. These reports provide vital profiles of the behavior of disease organisms in various climates and soils and help delineate the pathogens' host ranges.

Modern Tactics Used for Reliable Identification

Bull's group used what she describes as "a suite of assays" to quickly and reliably differentiate the blight and pepper spot pathogens from each other—as well as from other microbial species and strains.

Some of the tests, such as rep-PCR (repetitive polymerase chain reaction), focus on the microbes' genetic makeup.

Another approach relies on a virus, PBS1. Referred to as a "bacteriophage" in its bacteria-killing role, the virus is "a pathogen that kills a pathogen," says Bull.

Her team isolated PBS1 from cells of *P. cannabina* pv. *alisalensis* found in soil in a broccoli raab field. Tests in her lab showed that although PBS1 is capable of killing the blight pathogen, it does not kill the pepper spot pathovar, meaning that it can be used to differentiate one from the other.

"This bacteriophage-based assay is a fast, reliable laboratory tool for preliminary diagnosis," she says. "We've shown that the bacteriophage can be modified to detect and quantify *P. cannabina* pv. *alisalensis* infection of plants that have yet to develop any external symptoms."

Online Database Speeds Identification

In related work, Bull and other researchers have contributed key information about the genetic makeup of *P. cannabina* pv. *alisalensis*, *P. syringae* pv. *maculicola*, and more than 100 other *P. syringae* pathovars, to a new database called "PAMDB" (Plant-Associated and Environmental Microbes Data Base).

Intended for use by plant disease specialists at universities, plant health labs, and elsewhere, this online compendium, publicly available at www.PAMDB.org, displays the sequence, or makeup, of DNA fragments of genes common to bacteria.

"These 'housekeeping genes,'" Bull notes, "are essential to the survival of all known bacteria. The sequences of these DNA fragments are used in sequence analyses to get a quick, tentative identification of a microbe and to help determine taxonomic relationships among microbes.

"Labs can use ordinary protocols to amplify and sequence DNA from the microbe they're trying to identify and then compare those sequences to ones posted at PAMDB to find a match."

The database includes sequences from not only *Pseudomonas* species and strains, but also from other kinds of plant-dwelling microbes ("from A to X—*Acidovorax* to *Xanthomonas*," Bull says), and encompasses pathogenic and nonpathogenic bacteria alike.

Plant Doctors of Tomorrow?

In unmasking the blight pathogen, Bull and colleagues worked with more than two dozen undergraduate students funded by grants to the nearby Hartnell College and by California State University-Monterey Bay. Bull became the university's first "Mentor of the Year" in May 2012.

"Our students use their education in authentic research experiences," she says. "Many of our alums are in demand as 'budding' plant pathologists in M.S. and Ph.D. programs. Two of our students have won 3-year, $90,000 fellowships from the National Science Foundation for graduate work in plant pathology."

The scientists—and many of the students—have documented their pepper spot and bacterial blight findings in peer-reviewed scientific articles in *Phytopathology*, *Plant Disease*, and *Systematic and Applied Microbiology.*

Cruciferous vegetables are a $1.9 billion crop in the United States. Many are an excellent source of vitamin C and also provide dietary fiber, calcium, and iron.—By **Marcia Wood,** ARS.

This research is part of Methyl Bromide Alternatives (#308) and Plant Diseases (#303), two ARS national programs described at www.nps.ars.usda.gov.

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PEGGY GREB (D2745-1)

Technician Polly Goldman (left) observes bacterial characteristics while biological science aide Teresa Jardini isolates DNA from bacteria for pathogen identification.

Travel Patterns of Wind-Blown Soil Microbes

A dust storm near Lubbock, Texas, in June 2010. When strong winds carry away soil, microbes in the soil can act like hitchhikers and go along for the ride. ARS scientists are studying the soil microbes carried in the wind and shedding light on wind erosion effects on soil quality.

When strong winds carry away soil, microbes in the soil can act like hitchhikers and go along for the ride. With help from a wind tunnel and the latest DNA technology, Agricultural Research Service scientists are shedding light on the travel patterns of these soilborne hitchhikers. The work has implications for soil health and could lead to management practices that minimize the damage to soils caused by wind erosion.

Scientists have been studying wind-eroded soils since the 1930s, but few studies have focused on wind's effect on soil microbes. Microbes drive a number of critical processes, such as releasing enzymes that spur turnover of organic matter, sequestering carbon in the soil, and making nutrients available to plants.

Soil microbes include bacteria, fungi, and protozoa. Veronica Acosta-Martinez and Terrence Gardner in the ARS Wind Erosion and Water Conservation Unit in Lubbock, Texas, focused on bacterial populations that could be classified by DNA sequencing. Gardner is a visiting scientist in Acosta-Martinez's laboratory, and his position is funded by Alabama A&M University.

The researchers characterized the bacterial diversity in eroded sediments and their soil sources, focusing on the types of bacteria associated with coarse particles and fine dust particles. They classified the bacteria found in each type of soil by phylum, class, and genus using pyrosequencing, a process that allowed them to identify up to 100 times more DNA in each sample than what they would have detected with traditional soil-sequencing methods.

The project was a collaborative effort. Scott Van Pelt, Ted Zobeck, and Matt Baddock, also in the Lubbock unit, collected airborne dust and samples from organic-rich soils susceptible to wind erosion from fields in Michigan where potatoes, beets, and onions had grown a few years earlier. To simulate wind, they used a portable field wind tunnel that they had previously developed for other studies.

The results, published online in the *Journal of Environmental Quality*, showed that certain types of bacteria, in the phylum Bacteroidetes, were more predominant in the fine dust while other types, in the phylum Proteobacteria, were more predominant in the coarse sediments. As part of the study, Francisco Calderon, an ARS scientist in Akron, Colorado, used infrared spectroscopy to assess the effects of wind on the soil's carbon composition, a critical factor in soil health. Those results showed that wind had the same effect on the soil's carbon composition as it had on the bacteria, "fractionating" it so that certain types of soil carbon were more abundant in the dust, while other types were more common in the coarse sediments.

Acosta-Martinez says the fact that Bacteroidetes were associated with fine dust may be significant, because studies have shown that they resist desiccation and have survival mechanisms that make them able to cope with extreme conditions and explore new habitats when carried long distances. On the other hand, the fact that Proteobacteria were associated with coarse eroded sediments, which travel shorter distances, may explain how soils can retain important qualities despite damaging winds. Proteobacteria are critical for carbon and nitrogen cycling in the soil, and the fact that they are more likely to stay close to home during dust storms is good news for soil health, Acosta-Martinez says.—By **Dennis O'Brien,** ARS.

This research is part of Climate Change, Soils, and Emissions, an ARS national program (#212) described at www.nps.ars.usda.gov.

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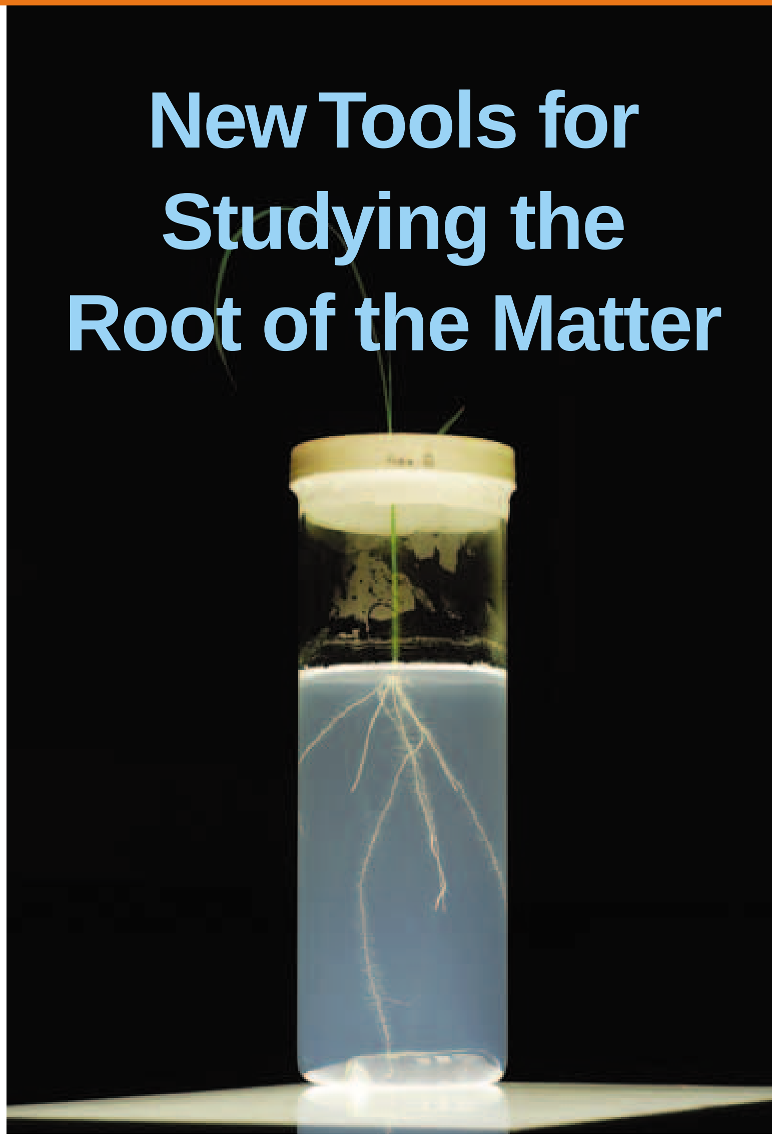
New Tools for Studying the Root of the Matter



RANDY CLARK (D2771-1)

Azucena rice, an upland cultivated variety, growing in a growth cylinder system used to capture the dynamic growth of root systems in three dimensions.

Like any structure, a root has its own architecture. Some roots burrow straight down into the soil. Others scatter weblike tentacles. However they turn out, root systems play a critical role in the health and survival of today's crops. That's why Agricultural Research Service scientists in Ithaca, New York, have developed a new tool for studying root architecture, one that brings them out in three-dimensional color. It's a major step forward in a challenging field.

Studying root-system architecture can be extremely difficult because the opaque nature of soils makes it hard to capture and visualize the root structures. Also, field conditions, such as soil chemistry and soil moisture, can vary from one site to the next, and changing conditions dramatically affect the shape, size, and health of a root system. But root structure, health, and formation have become important traits of interest in recent years as scientists search for varieties of crops with roots that equip them to adapt to drier habitats and changing climates.

Scientists have grown plants hydroponically in pots and on plates and have used tools ranging from magnetic resonance imaging to computer-assisted tomography to study root architecture. The latest technology includes transparent gels that allow them to observe roots at different stages of development as the root and plant grow together in a kind of suspended animation.

Randy Clark, doctoral student at Cornell University, images rice root systems for subsequent digital reconstruction of the root systems in three dimensions.

With computers and digital photography, researchers have even developed two- and three-dimensional imaging systems to record roots as they develop in the transparent gels.

Leon V. Kochian, director of the ARS Robert W. Holley Center for Agriculture and Health in Ithaca, and Randy Clark, a Cornell University doctoral student working with him, have developed a three-dimensional imaging system and software package that allows scientists to collect data on root systems faster than ever before and study root architecture in unprecedented detail. Called "RootReader3D," the system gives scientists the ability to analyze root structures and growth patterns, compare one root system with another, and genetically map and explore traits that give plant roots the capacity to reach into the soil and collect water, phosphorus, and other nutrients, Kochian says.

Testing the System on Rice

To test their system, the researchers grew two varieties of rice in transparent gels and tracked root growth by imaging the plants and their roots for 10 days. "We put the plants on a computer-controlled rotational stage and took digital images of the root system at every 9 degrees of rotation, so there were 40 images of each plant. The entire imaging process takes about 5 minutes per plant," Kochian says.

They chose rice for the analysis because rice root systems are extremely complex, and the way rice adapts to environmental changes can vary widely from one type of rice to the next. Cultivated rice ranges from highly managed, irrigated systems to types grown in unmanaged fields. The researchers intentionally selected two very different types of rice, Azucena and

IR64, for analysis. IR64 is a lowland rice grown in flooded rice paddies, while Azucena is an upland rice adapted to nonirrigated fields.

In more recent experiments, the researchers have imaged the entire root systems of 400 different rice lines in a rice-diversity panel; this has involved phenotyping the root systems of 2,000 rice plants and then developing three-dimensional images of each root system. They scored the root systems for 27 traits that describe both the individual root types, such as the primary root, crown roots, and lateral roots, as well as the architecture of the whole root system. They noted when each root component first appeared and tracked its growth. The ability to isolate and track such root components will help scientists make comparisons among crops and characterize the developmental and genetic differences in root systems, with the ultimate goal of identifying genes underlying control of root system architecture, Kochian says.

Scientists elsewhere had previously taken two- and three-dimensional images of root systems of plants grown in gels. But those systems required up to an hour to collect enough data for a single three-dimensional image. The RootReader3D system offers more detailed results and is much faster. With it, scientists can image more than 100 root systems a day, providing the information needed to conduct genetic-mapping experiments of root traits.

The researchers found that the RootReader3D system was able to delineate, in greater detail than ever before, significant differences between two rice root systems. The results showed that Azucena rice had deeper roots than its irrigated cousin IR64 and that the two root systems were significantly different in terms of their "bushiness," how their root volume was distributed, and the vertical position of the center mass of the root system.

Results were published in and featured on the cover of the June 2011 issue of *Plant Physiology,* with Clark as lead author and Kochian as senior author. The work is part of a long-term collaboration between Kochian's research unit and Susan McCouch, a professor of plant breeding at Cornell. The research was partially funded by the National Science Foundation. The RootReader3D software is available at www.plantmineralnutrition.net/rootreader.htm.

Leon Kochian, director of the ARS Robert W. Holley Center for Agriculture and Health in Ithaca, New York, examines a rice plant growing in a growth cylinder system.

The researchers hope to use the data they collect using RootReader3D in future studies that identify genes controlling important root developmental traits. The ultimate goal is to be able to help plant breeders develop varieties of rice—and other crops—with roots that make them better equipped to handle drought, heat, poor soil quality, and other stresses in a changing world.

"It will be an enormous help, not only in comparing different root systems, but also in exploring the molecular basis of root formation and root architecture to gain an understanding of the genetic mechanisms that give plants the ability to form the kind of roots they need to survive and thrive," Kochian says.—By **Dennis O'Brien,** ARS.

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

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RANDY CLARK, JON SHAFF, BRITTANY GREEN (D2770-1)

A sorghum plant growing in a newly devised hydroponics growth cylinder system used for analyzing root system architecture of large cereal crops under an array of nutrient and stress regimes.

STEPHEN AUSMUS (D2739-8)

Above: Immature bed bugs molt and shed their skins only after blood feeding. ARS entomologist Mark Feldlaufer collects blood-fed, immature bed bugs that he will isolate in vials to collect individual shed skins for chemical analysis.

Identifying Compounds To Help Control Bed Bugs

When planning an attack on bothersome pests, it's a good idea to know your enemies, especially if they happen to be bed bugs. These small, flat, blood-feeding insects—an uncommon problem since the 1950s—have once again become a menace in homes, apartments, hotels, shelters, and even places of work in the last 10 years.

Despite extensive efforts to determine whether bed bugs transmit disease-causing pathogens, no evidence indicates that they are a problem other than their biting, blood feeding, and dirty habits. However, some people have severe reactions to bed bug bites, and getting rid of the insects can be costly. Problems often go unnoticed until bug populations increase substantially.

As part of a strategic plan to control bed bugs, scientists at the ARS Henry A. Wallace Beltsville [Maryland] Agricultural Research Center are learning more about bed bug behavior and stepping up their efforts to develop substances that can be used to control them and other biting arthropods and urban pests.

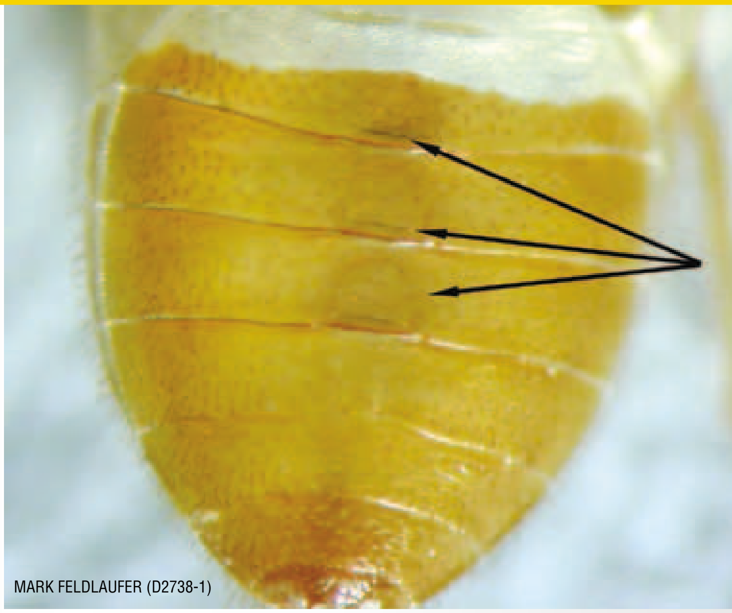
While the main thrust of the research is to discover and develop new chemicals for bed bug control, entomologist Mark Feldlaufer, chemist Kamlesh Chauhan, and retired ARS entomologist Jeffrey Aldrich also focused on compounds that affect bed bug behavior.

Alarming Behavior

"Both adult and immature bed bugs produce defensive compounds referred to as 'alarm pheromones,'" says Feldlaufer, who works at the Invasive Insect Biocontrol and Behavior Laboratory in Beltsville. "When released, alarm pheromones cause aggregated bed bugs to disperse."

STEPHEN AUSMUS (D2742-15)

Both adult and immature bed bugs produce defensive compounds referred to as "alarm pheromones." ARS scientists identified two new alarm pheromones from bed bugs.



MARK FELDLAUFER (D2738-1)

A shed skin from a bed bug. ARS scientists collected these skins and isolated chemicals involved in bed bug behavior, including aggregation. The arrows point to the bug's dorsal abdominal glands, which secrete the chemicals.

In one study, scientists identified two new alarm pheromones—4-oxo-hexenal and 4-oxo-octenal—from immature bed bugs. A Swedish research group has since identified these compounds from a related species, the tropical bed bug, and shown that the compounds are biologically active, Feldlaufer says.

"Alarm pheromones, in general, may have utility in bed bug management programs by causing these otherwise cryptic insects to disperse and thereby increasing the likelihood of contact with an insecticide or other control agent," Feldlaufer says.

For these identifications, scientists collected the cast skins, which retain chemicals from the bed bug's scent glands, at specific stages. They then used gas chromatography and mass spectrometry technology to analyze and identify the compounds in the glands.

Mass spectrometry also was used in recent research by Feldlaufer, in collaboration with biochemistry professor Gary Blomquist at the University of Nevada-Reno, to identify 17 compounds associated with the outer protective layer of the cuticle, referred to as "cuticular hydrocarbons." These compounds may play an important role in the bed bug behavior of aggregation, Feldlaufer says.

Sniffing Out Bugs

Scientists are also interested in what role these cuticular compounds—along with the alarm pheromones—play in canine detection of bed bugs.

"Using dogs trained to detect bed bug infestations is an emerging industry, and a better understanding of the chemical basis of canine detection will hopefully contribute to a harmonization of training and detection methods," Feldlaufer says. "This research will ultimately benefit the public by providing a straightforward and reliable means of early detection of bed bug infestations."—By **Sandra Avant,** ARS.

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

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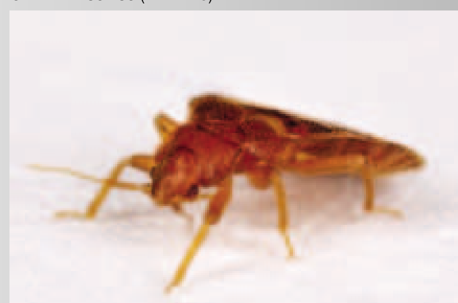
STEPHEN AUSMUS (D2741-23)



Above: An adult bed bug, *Cimex lectularius*, is roughly 4 mm in length. Immature and adult bugs are very flat, making it easy for them to hide in cracks and crevices.

STEPHEN AUSMUS (D2740-4)

A penny provides a sense of scale to the size of bed bug skins collected for analysis by ARS scientists in Beltsville, Maryland.

PEGGY GREB (D2751-1)

Studying Manure in Cows, in Feedlots, and in Fields

In Lincoln, Nebraska, technicians Jaime LaBrie (left) and Jennifer McGhee process samples for enumerating manure-associated bacteria.

Agricultural Research Service scientists at the Agroecosystems Management Research Unit in Lincoln, Nebraska, have been conducting some very thorough investigations on the microbes that dwell in cattle manure—what they are, where they thrive, where they struggle, and where they may end up.

"When we look at potential pathogens that can cause foodborne illness, we need to look at the whole bacterial ecosystem," says ARS microbiologist Lisa Durso. "For instance, some people used to think all cattle have the same bacteria in their gastrointestinal [GI] tracts. But we've found some big differences; so if we say, 'Oh, it's just manure,' we could miss important factors in pathogen control."

That's why Durso headed up a study that provided the first-ever "gold standard" accounting of the fecal bacterial types associated with beef cattle.

The researcher used pyrosequencing, a relatively new method of rapidly analyzing bacterial DNA markers, to classify the bacteria into different taxonomic groups. "People hadn't looked at doing this type of bacterial census before, because some bacteria could be cultured, but other types didn't grow well," says Durso, who conducted this investigation while she was working at the ARS U.S. Meat Animal Research Center in Clay Center, Nebraska. "Pyrosequencing let us give every bacterium a name tag ID."

Using fecal samples from six beef cattle, Durso identified a core set of bovine GI bacterial groups common to both beef and dairy cattle. But she also determined that *Prevotella* was the most common bacterial genus in the cattle she studied—occurring in 24 percent of the total number of DNA sequences she analyzed. Another published study had identified *Prevotella* in only 5.5 percent of the bacterial genes sequenced from 20 dairy cattle. And while another survey had identified *Clostridium* in 19 percent of the bacterial DNA sequenced from dairy cattle, Durso detected the genus in only 1.5 percent of the DNA sequences in her study.

Durso observed bacteria in the beef cattle that had not been reported in dairy cows. She also identified a diverse assortment of bacteria from the six individual beef cows, even though all six animals consumed the same diet and were the same breed, gender, and age. Given her results, Durso believes much more high-resolution community sequencing will be needed to identify "core" members of the bovine bacterial community.

Durso also compared her results to a survey of bacterial types she collected from beef cattle feedlot surfaces. Of a total of 139 different bacterial genera from both groups, 25 were detected in both fecal samples and feedlot floor samples, 21 were found only in the fecal samples, and 93 were found only in samples from the feedlot surfaces. She attributes the distribution differences to selection pressures bacteria face in feedlot pens that aren't present in the oxygen-free, dark, moist cattle GI tract.

The implications of these findings? "The focus on food safety is fecal contamination, and preharvest pathogen control has often been animal-centric—for instance, how to 'fix' the problem of *E. coli* in a cow's GI tract," Durso says. "But a bacterium has a different pathway once it's outside of the gut. So we need to start thinking strategically about how to control pathogens when they are at their weakest—outside the animal, rather than inside it."

Durso also partnered with Lincoln agricultural engineer John Gilley and others to study how livestock diet affected pathogen transport in field runoff from manure-amended soils. "Manure applications can help a farmer meet soil nutrient requirements, but it's more expensive to apply it every year because of the costs of labor, equipment, and fuel," Gilley says. "A farmer can reduce costs by applying enough manure to meet 2-year or 4-year soil nutrient requirements, but we need to understand more about

PEGGY GREB (D2750-3)

PEGGY GREB (D2749-1)

Left: Beef cattle in a feedlot at Clay Center, Nebraska. Right: Technician Sandy Fryda-Bradley (left) and microbiologist Lisa Durso collect fecal pats from a feedlot pen. Samples are taken back to the lab to be cultured for bacteria, including *E. coli.*

how these larger applications might be affecting the environment."

Gilley's team amended conventional-till and no-till fields at 1-, 2-, or 4-year application rates of manure from livestock that had consumed either corn or feed containing 40 percent wet distillers grains. After a series of simulated rain events, they analyzed runoff samples from the fields. They found that neither diet nor tillage management significantly affected transport of fecal indicator bacteria, but that diet did affect transport of bacteriophages—viruses that invade bacteria—in the runoff.

Gilley also conducted an investigation into how wheat residues affected water quality in runoff from plots amended with 1-, 2-, or 4-year application rates of manure. Some of the plots were covered with postharvest wheat residue, and others were bare.

The scientists found that runoff loads of dissolved phosphorus, total phosphorus, nitrate nitrogen, and total nitrogen were much higher from plots with residue cover. In addition, they observed that runoff from fields amended with 4-year application rates of manure had significantly higher levels of dissolved phosphorus and total phosphorus than fields amended with 1-year or 2-year manure rates.

"Our study—which is one of the first studies on this question—indicates there is a significant difference in how manure application rates affect runoff loads," Gilley says. "And even though crop residues can be effective in controlling soil erosion, the residues also slow the movement of water across fields. So there's more time for water to pick up nutrients from the soil."

In a follow-up study, Gilley's team found that narrow grass hedges planted at the edge of manure-amended plots reduced mean runoff loads of dissolved phosphorus from 0.69 to 0.08 kilogram per hectare and total phosphorus from 1.05 to 0.13 kilogram per hectare—similar to levels from plots that had not been amended with manure.

"This study shows that if you have hedges you can substantially reduce nutrient loads in runoff," Gilley says. "Planting grass hedges is a practice that isn't expensive and can have a substantial impact."

Results from these studies have been published in *Foodborne Pathogens and Disease*, *Applied and Environmental Microbiology*, and *Transactions of the ASABE*.—By **Ann Perry,** ARS.

This research is part of Food Safety (#108), Climate Change, Soils, and Emissions (#212), and Agricultural and Industrial Byproducts (#214), three ARS national programs described at www.nps.ars.usda.gov.

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New Club Wheat Holding Its Own Against Fungal Disease

Pacific Northwest wheat growers now have added insurance against outbreaks of yield-robbing fungi, thanks to Cara, a soft, white, winter club wheat cultivar developed by the Agricultural Research Service that boasts high levels of disease resistance and outstanding flour quality.

Washington State accounts for 77 percent, or 230,000 acres, of the entire U.S. club wheat crop, with the remainder produced in Oregon and Idaho. Much of the acreage in Washington and Oregon is planted with three cultivars: Bruehl, Cara, and Chukar. Spring-sown club wheat cultivars JD and Eden are grown on another 11,000 acres. Although several other winter club wheats are available, there's been a push to broaden the specialty crop's genetic diversity, especially amid the latest fungal disease flareup.

"Recently, we've had severe epidemics of stripe rust, which is able to mutate rapidly and overcome the resistance that's present in the current crop," says Kim Garland-Campbell, an ARS geneticist who coordinates the club wheat breeding program. "We're correcting former deficiencies, like poor disease resistance, in today's club wheat and put together a crop production package that's better than what was available."

Cara, the program's latest offering, combined high grain yields and flour quality with resistance to multiple fungal diseases, most notably stripe rust, which has inflicted yield losses of up to 40 percent. The release and subsequent sale of Cara, in 2009, "coincided with a stripe-rust epidemic and has driven a lot of the popularity," notes Garland-Campbell, with the ARS Wheat Genetics, Quality, Physiology, and Disease Research Unit in Pullman, Washington.

In Washington, Cara was planted on 17,900 acres in 2011, primarily in the Palouse, a fertile agricultural region encompassing part of southeastern Washington and north-central Idaho. Growers in Oregon and Idaho planted 1,500 and 1,000 acres, respectively, of the cultivar.

YUE JIN (D517-1)

Stripe rust can cause significant yield losses to wheat growers.

Like that from other club wheats, flour milled from Cara has low viscosity and protein content, coupled with high "break flour" and "weak gluten"—characteristics ideal for making air-leavened cakes, sugar snap cookies, biscuits, pastries, and other soft, fluffy-textured baked goods. Weakened gluten is important because it means "you can whip the batter up to give it height, whereas with a regular flour, this would give it a tough consistency," explains Garland-Campbell. "Break flour is associated with softness and gives the flour its fine texture."

Club wheat flour is mainly exported to Pacific Rim countries, including Japan, Taiwan, and Korea, and is considered a value-added component of the market-class blend known as "western white wheat." For example, in Japan, this high-quality blend of soft white and 10-20 percent club wheat commands a premium price and is the flour of choice for making Kasutera, a traditional Japanese sponge cake.

In the field, however, Cara is one tough cookie. It contains genes (*Yr17, Pch1*, and others) that enable it to withstand attack by the stripe rust fungus, *Puccina striiformis; Oculimacula yallundae*, which causes straw breaker foot rot; and *Blumeria graminis*, the culprit behind powdery mildew.

Though fungicides have proven effective against these pathogens, "We've found that you still need some level of resistance in the crop, because you can't always get the fungicide on the crop in time—especially with fall infections—and fungicides are an added cost to production," says Garland-Campbell.

Based on 60 yield trials conducted in plots where stripe rust was present in Oregon and Washington, Cara outperformed most other commercial cultivars, with 2-year yield averages ranging from 3 to 18 percent better than other cultivars in the trials. Cara's best performance was in five environments in the 16- to 20-inch rainfall zone of the 2011 Washington cereal variety-testing trials. Yields averaged 157 bushels per acre on rain-fed wheat produc-

tion, surpassing those of top-performing commercial club wheat checks Bruehl, Chukar, and Coda, as well as soft white wheat checks Brundage96, ORCF102, and Xerpha—especially in areas with severe rust.

Cara—which was derived from crossing three diverse germplasm sources—is primarily adapted to the Palouse and other rust-prone areas with 15-24 inches of annual precipitation. It grows as a semi-dwarf plant and has sturdy stems that are less prone to lodging, or toppling over such that its grain can't be harvested.

Cara's milling, baking, and end-use properties were evaluated using international protocols set forth by AACC International (formerly American Association of Cereal Chemists). In those evaluations, Cara proved "as good as the best club wheat check cultivars with high break flour and cake volumes," Garland-Campbell and coauthors report in an upcoming issue of *Crop Science*. The milling quality is especially good, says Garland-Campbell. "We like to call it a monster miller."

Like other club wheats, flour milled from Cara has characteristics ideal for making air-leavened cakes, cookies, pastries, and other fluffy-textured baked goods.

PEGGY GREB (K8919-1)

In addition to colleagues in the ARS laboratory and at Washington State University in Pullman, Garland-Campbell collaborated on Cara's development, testing, and release with scientists at the University of Minnesota, Purdue University, Oregon State University, and Connell Grain Growers, Inc., of Connell, Washington.

The researchers are now focusing on improving other desirable traits, including winterhardiness and grain volume. They have two new club wheat cultivars, ARS-Crescent and ARS-Chrystal, on seed increase, making the Pacific Northwest's club wheat crop even more of an asset to growers eyeing lucrative markets abroad.—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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Cara, a winter wheat developed by ARS at Pullman, Washington. This club wheat cultivar boasts high levels of disease resistance and outstanding flour quality.

KIM GARLAND-CAMPBELL (D2772-1)

ANDY LEWIS (D2743-1)

Temperament Plays Key Role in Cattle Health

ARS animal scientist Jeff Carroll (left) and animal physiologist Ron Randel from Texas AgriLife Research and Extension Center measure cattle temperament by scoring their reactions to human observers. This Brahman calf is quite calm, showing virtually no fear of or aggression towards humans and would receive a score of 1, whereas a more aggressive and volatile calf would be scored 4 or 5.

Do you know someone who's temperamental? What about an animal? Amazingly, cattle can be temperamental too, which influences how they should be handled, how they perform, and even how they react to viruses that cause diseases.

For cattle, temperament is defined as the reactivity or fear response to humans or handling. Terms used to describe temperamental animals include "flighty," "excitable," and "high strung." These animals can potentially injure themselves or their handlers.

Beef cattle experience stressful events during routine management practices—weaning, transportation, social mixing, and vaccination. These practices have been shown to induce secretion of the stress-related hormones cortisol and epinephrine. Stress can negatively affect growth, reproduction, welfare, and immune function—predisposing cattle to infectious intestinal and respiratory diseases that cost U.S. cattle producers an estimated $500 million per year. Reducing adverse consequences of stressful incidents and identifying animals that may react differently to stressors may benefit cattle's growth and health.

A team of scientists in the Agricultural Research Service's Livestock Issues Research Unit (LIRU) in Lubbock, Texas, Mississippi State University (MSU), and Texas AgriLife Research—a member of the Texas A&M University System—are studying interrelationships of stress and cattle temperament with transportation, immune challenges, and production traits. They have found that, depending on temperament, cattle respond differently.

Testing Temperament

Most studies were done shortly after weaning to emulate what happens in the industry, says Ron Randel, animal physiologist at the Texas AgriLife Research and Extension Center in Overton, Texas. One of the most stressful times for an animal is after it is weaned.

The team was among the first in the United States to adopt and use the exit velocity system developed in Australia, Randel says. The system measures the rate at which an animal exits a squeeze chute or scale box where it's been restrained or held after transport. A fast exit indicates the animal is showing fear and is stressed by handling and human activity.

Scientists also used pen scoring, a subjective measurement in which small groups of cattle are scored based on their reactions to a human observer. Scores range from 1—calm, docile, and approachable, to 5—aggressive and volatile.

The exit velocity and pen score for each animal were then averaged together to come up with a temperament score, says Jeffery Carroll, LIRU research leader.

Calves were chosen for studies based on their temperament scores, which categorized them as most calm, intermediate, and most temperamental.

Challenging Behaviors

"Depending on whether an animal is classified as being really calm or really high strung, we're seeing differences in the way it deals with illness," Carroll says.

In the study, Brahman calves were classified by temperament and transported 478 miles from Overton to Lubbock. After the trip, blood samples and body temperatures were taken before, during, and after administration of an endotoxin to simulate illness. Sickness behavior was scored on a 1-to-5 scale that measured the severity of calves' behavioral responses to the challenge. A score of 1 indicated normal maintenance behaviors, and 5 indicated the greatest amount of sickness behaviors, such as head distension, increased respiration, and labored breathing.

"You could immediately tell that the calm animals had been given an immune challenge, because they showed visual signs and became ill," Carroll says.

The more temperamental animals continued to act high-strung and flighty after the endotoxin challenge. If a temperamental animal doesn't show signs of illness, managers might not realize that the animal is sick and needs treatment.

"We're not talking about one breed compared to another breed," Carroll says. "We're talking about animals within the same breed type, and the only difference is their temperament."

Results showed that the endotoxin increased body temperature and induced secretion of epinephrine and cortisol, hormones associated with coping with stress, says Tom Welsh, Texas AgriLife Research endocrinologist in College Station, Texas.

When animals are transported, they become stressed, contributing to the incidence of disease, Randel says. Therefore, identifying cattle that are more susceptible to stressors and subsequently have altered immune responses may help to reduce the impact of sickness after transport.

Previous studies indicate that human-animal interactions are probably the most stressful events that the majority of cattle encounter.

"The duration of transportation is not the problem," Randel says. "It's the action of being handled and loaded into the trailer that is producing the stress." If cattle are handled in an appropriate manner and given water and feed at no more than 12-hour intervals, then getting on and off the trailer is the major stressor, he says.

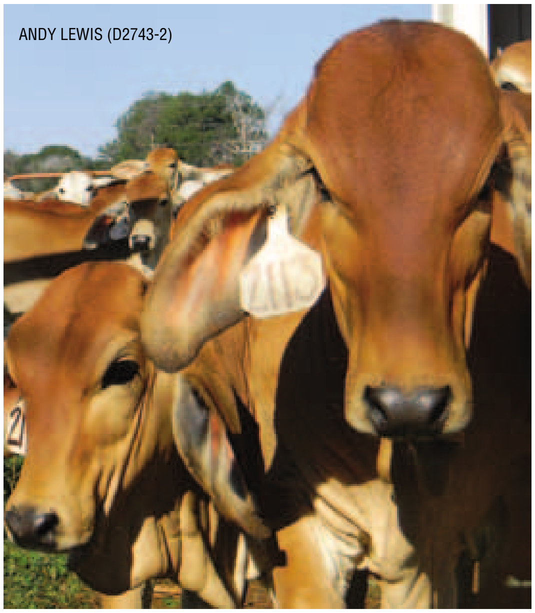
Making the Grade

While the handling process is more stressful for animals, transportation duration and conditions can have negative effects on intramuscular fat, says Rhonda Vann, MSU associate research professor.

"When animals are transported, they will use or mobilize intramuscular fat very quickly for fast sources of energy," Vann says. "The degree of intramuscular fat, or marbling, determines the quality grade of beef. High levels of marbling improve quality grade, whereas lower levels reduce it."

Vann and her colleagues looked at the combined effects of transportation and animal temperament on body composition traits. They took ultrasound images of muscle ribeye area, rib fat, intramuscular fat, and rump fat to evaluate and measure fat mobilization.

Temperamental cattle appeared to use more fat stores when stressed, Vann says.



Brahman calves just before transport to Lubbock, Texas. Each calf's stress response will be monitored before, during, and after transport to determine whether temperament is linked to the amount of stress experienced in transport.

Also, as the hauling distance increased, the percentage of intramuscular fat decreased.

"From a production standpoint, temperament of animals does make a difference in the ultimate quality grade—for example, choice versus select," Vann says. "As stressors and transportation times increase, temperamental animals could potentially have lower quality grades, and that could mean lower profits."

Tactics for Taming

Results show that temperamental cattle require special management practices to reduce stress before, during, and after transportation. Also, because temperament and resistance to bovine respiratory disease are both heritable traits, future research will include developing gene-based methods to select calm, stress-tolerant, and disease-resistant cattle.

"Information derived from our studies will enhance gene-based approaches to improving animal health and performance," Welsh says.

In the meantime, cattle producers can use temperament scoring to select calmer bulls for breeding less temperamental cattle and use pen scoring for replacement females to eliminate the more temperamental cows, Randel says.

"I'm not suggesting selecting for the calmest cattle," he says. "I'm suggesting that producers eliminate animals that are most temperamental to improve herd health and productivity, ensure animal welfare, and to protect animal and worker safety."

This research was partly supported by a cooperative research and development agreement between the U.S. Department of Agriculture and Texas AgriLife Research and was part of a project by Texas A&M graduate student Nicole Burdick, who is now with LIRU.—By **Sandra Avant,** ARS.

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

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State Department's Gift of Dogwoods to Japan in Honor of 100th Anniversary of Cherry Tree Gift

Before these dogwoods can be shipped to Japan, technician Bill Phelan (left) removes potting media while geneticist Richard Olsen and horticulturist Susan Bentz inspect the leaves. Gardener Tom Abell (right) washes the roots to prepare for inspection for pests, pathogens, and overall plant health.

PEGGY GREB (D2774-1)

More than 100 years ago, the Japanese government presented flowering cherry trees as a gift to the U.S. government. Those wonderful living gifts have blossomed along the National Mall's Tidal Basin, as well as at other locations around Washington, D.C., since then.

In April 2012, Secretary of State Hillary Clinton announced a gift of 3,000 flowering dogwood trees to Japan to commemorate the 100th anniversary of Japan's gift of cherry trees to the United States.

The job of finding the right dogwoods belongs to scientists at the U.S. National Arboretum, in Washington, D.C. Agricultural Research Service plant geneticist Richard Olsen has worked to determine the appropriate dogwood cultivars for the varied Japanese climate and to locate the planting material.

"There are many things to consider, including temperature range and insect pests to which particular dogwood candidates may be susceptible," says Olsen.

The planning group—which includes the U.S. Department of State; the U.S. Department of Agriculture's Animal and Plant Health Inspection Service, Forest Service, and ARS; and the U.S./Japan Bridging Foundation, a nonprofit organization—sent the first batch of 150 trees to Japan, and they were planted in Tokyo in November 2012. Altogether, a total of 1,000 will be planted in Tokyo, 1,000 in the Tohoku region in honor of the tsunami victims, and the final 1,000 will be distributed to schools and other organizations throughout the country.

"We are evaluating the performance of dogwood germplasm at the arboretum as well as novel cultivars available in the American nursery industry to find those most suited to the Japanese clime," says Olsen. The arboretum has, for decades, collected dogwood germplasm from across the United States—it is found from Florida to Michigan and as far west as Missouri and Texas.

The arboretum's Woody Landscape Plant Germplasm Repository holds many species of dogwood as well as other trees and shrubs. The repository serves to introduce, maintain, and distribute diverse and wild-origin genetic resources of trees and shrubs for landscape use through collection, exchange, and evaluation. Woody plant germplasm is also evaluated for production potential and further characterized using molecular technologies.

The repository is responsible for maintaining more than 200 woody plant genera. More than 1,400 accessions of seeds are maintained in storage for direct distribution, and another 2,800 living plants are maintained on site at Beltsville and Glenn Dale, Maryland; and in Washington, D.C.—By **Sharon Durham,** 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.

U.S. Agricultural Research Station, Salinas, California
1 research unit ■ 51 employees

Pullman, Washington
6 research units ■ 136 employees

Akron, Colorado
1 research unit ■ 28 employees

Cropping Systems Research Laboratory, Lubbock, Texas
4 research units ■ 113 employees

Roman L. Hruska U.S. Meat Animal Research Center, Clay Center, Nebraska
6 research units ■ 113 employees

Lincoln, Nebraska
2 research units ■ 81 employees

Auburn, Alabama
2 research units ■ 46 employees

Robert W. Holley Center for Agriculture and Health, Ithaca, New York
3 research units ■ 62 employees

U.S. National Arboretum, Washington, D.C.
1 research unit ■ 83 employees

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

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

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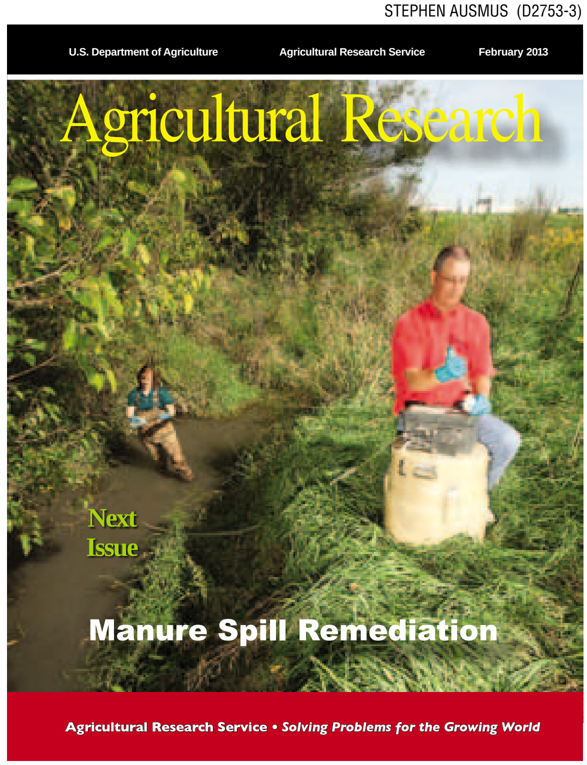

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