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Agricultural Research

Leading Fire Ants Off the Trail

Story on page 6.

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

FORUM

ARS Plays an Integral Role in Combating a Virulent Threat

Agriculture faces constant challenges from nature. Every crop we raise seems to have its own set of insect pests, viruses, diseases, and pathogens just waiting to attack it. Wheat is no exception. One of the most challenging issues facing modern agriculture is a fungal pathogen that threatens the world's supply of wheat. Ug99 is a type of stem rust that was discovered in Africa, has spread to the Middle East, and is considered by many experts to be the most serious threat to the world's wheat in the past 50 years. It also infects barley, with spores that can be carried on the wind or travel on the clothing of someone passing by an infected field. It is one of three rust diseases that infect wheat, and it destroys stem tissue so that carbohydrates are unable to reach the grains, causing them to shrivel. Infected plants become covered with rustlike reddish-orange spores.

Ug99 has yet to reach the United States, but we are not immune. Field testing on wheat, conducted by Agricultural Research Service researchers in Africa as part of an international effort, shows that virtually every type of wheat produced in the United States—from the hard red winter wheat of the Southern Plains to the soft white varieties found in the Pacific Northwest—is susceptible.

An epidemic of another type of stem rust destroyed 40 percent of the nation's spring wheat in the 1950s. Barberry plants harbor that pathogen, and eradicating barberry near the nation's wheat fields helped turn the tide against major stem rust epidemics years ago. But the threat persists.

In this issue, you will read about efforts at the ARS Cereal Disease Laboratory (CDL) in St. Paul, Minnesota, to probe the genetics of some of wheat's wild ancestors in a search for genes that will resist Ug99. The hope is that genes can be bred into commercial wheat varieties that will make them resistant. The work is a preemptive strike to ensure that if the disease ever arrives in the United States, wheat producers will be ready for it.

DAVID HANSEN (D3136-1)

In experimental plots in Njoro, Kenya, Ug99-susceptible wheat is lodging (falling over) because the stems have been weakened by the rust fungus. Meanwhile, the Ug99-resistant wheat lines in the background remain upright.

CDL scientists fill a unique niche in what has become an international priority for agricultural scientists. They were part of a team that released a draft of the Ug99 genome and mapped eight resistance genes in wheat that show potential for breeders. They also maintain a collection of stem rust specimens, gathered over the past 50 years during rust surveys of wheat fields, that has proved useful in comparison studies.

Experiments in St. Paul are conducted in mid-winter when it is too cold for escaping spores to survive. When necessary, the researchers work in specially equipped, "contained" greenhouses to ensure that the pathogen poses no risk of escaping into surrounding fields.

The research at St. Paul described in this issue is only one part of an impressive body of work being done by ARS scientists at a number of other sites around the country to help wheat producers and breeders address the threat posed by Ug99. Critical to the effort is the U.S. Small Grains Collection in Aberdeen, Idaho, which is a treasure trove of wheat seeds and plants. It has become a key resource for breeders and researchers from around the world who are looking for new sources of Ug99 resistance. Aberdeen scientists play a critical role in that search, prescreening wheat from the collection to select lines for field testing in Africa that show the most promise. There have also been contributions from several other ARS facilities, including the Plant Sciences Research Unit in Raleigh, North Carolina; the Wheat, Sorghum, and Forage Research Unit in Lincoln, Nebraska; and ARS wheat genotyping laboratories in Fargo, North Dakota; Pullman, Washington; and Manhattan, Kansas.

Wheat breeders generate new varieties each year, and one question they need to address is whether their new varieties will be resistant to Ug99. Field tests of those varieties conducted in Africa by a Raleigh-based ARS researcher have been instrumental in providing guidance to breeders. The publicly available data, on thousands of carefully selected wheat lines, has become a widely used tool.

Evolving pathogens like Ug99 are a good example of why there is such a critical need for research and for development of new wheat varieties. The challenges presented by nature seem to be never-ending. If we are to feed a growing world, we have to be ready for them.

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Grain Crops
Beltsville, Maryland

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Solving Problems for the Growing World

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TIMOTHY RINEHART (D3126-1)

Blooms of Anna Bella, a recently released ornamental tung tree variety that produces no nuts. Story begins on page 8.

Cover: A fire ant's sting apparatus, where trail pheromone is produced. ARS researchers are studying ways to use the pheromone to help control these stinging pests. Story begins on page 6. Photo by Justin Schmidt. (D3134-1)

Looking to Wheat's Wild Ancestors To Solve a Modern Problem

DAVID HANSEN (D3123-1)

At a nursery in Njoro, Kenya, ARS plant pathologists Matt Rouse and Yue Jin score wheat plants for their responses to infection with Ug99 rust. The scientists spend hours every day walking the experimental plots and rating thousands of wheat lines.

Except for wheat breeders, producers, and scientists, few people have probably ever heard of einkorn wheat *(Triticum monococcum),* an ancient variety still cultivated in some parts of the Mediterranean. Emmer wheat *(T. turgidum),* found at some archeological sites and still growing wild in parts of the Near East, may be equally obscure. But these little-known grains and others like them could hold keys to saving one of the world's most important cereal crops from an unrelenting fungus.

Wheat is under attack by a fungal pathogen that has become an international priority for scientists. Ug99 is a strain of the stem rust fungus *(Puccinia graminis)* that threatens up to 90 percent of the world's wheat and much of its barley. Even worse, the fungus is constantly evolving. Genes that seem to offer immunity one growing season become susceptible to newly developed "races" the next growing season.

Since Ug99 was first reported in Uganda in 1999, at least six related races of the pathogen have been discovered. It has yet to reach the United States, but it is spreading throughout eastern and southern Africa, Iran, and Yemen.

Researchers worldwide are working to identify genes capable of resisting Ug99 for eventual use in wheat and barley. As part of that effort, Agricultural Research Service scientists Matt Rouse and Yue Jin are searching for resistance genes among some of wheat's lesser-known relatives. Both scientists are at the ARS Cereal Disease Research Laboratory in St. Paul, Minnesota. Along with einkorn and emmer wheat, candidates for study include goatgrass *(Aegilops tauschii),* triticale *(x Triticosecale),* and other wild relatives of wheat.

Goatgrass is a wild relative of domesticated wheat, and breeders have tapped genes from it to boost the immunity of commercial wheat to a number of pests and pathogens. Triticale, found in some breakfast cereals and other foods, is a hybrid of rye and wheat. It holds promise because rye is nearly immune to Ug99. Also, triticale is considered a potential bridge for transferring genes from rye into wheat.

YUE JIN (K11192-1)

Close-up of stem rust on wheat. A strain called "Ug99" is now in parts of Africa and the Middle East.

Scientists often study a crop's wild ancestors for genes that will confer resistance to emerging pests and pathogens. But what may be noteworthy is the diversity of grasses being evaluated by ARS scientists and others as part of the international effort to combat this fungal menace. Rouse and Jin say the extensive search is necessary because Ug99 evolves so quickly that breeders will probably need several resistance genes for "stacking" into commercial cultivars. That way, when the pathogen attacks, it will have more than one genetic hurdle to overcome. Several genes recently discovered by ARS researchers and their colleagues are being deployed by international breeding teams and are offering some Ug99 protection.

A First in Ug99 Research: A Cloned Resistance Gene

In one study, Rouse and his colleagues focused on locating a gene in einkorn wheat that confers near immunity to Ug99. The gene, known as *Sr35,* was previously discovered in einkorn, but its exact location in the plant's vast genome remained a mystery. The wheat genome is huge, containing nearly two times more genetic information than the human genome. To find *Sr35*'s position, the researchers sequenced areas of the plant's genome where they suspected it was located. In one set of

CEREAL DISEASE LABORATORY (D3124-1)

At the Cereal Disease Laboratory in St. Paul, Minnesota, plant pathologist Matt Rouse (right) displays varying levels of stem rust infection to U.S. Senator Amy Klobuchar.

mutant plants, they knocked out the cloned sequences and found that doing so made those plants susceptible to Ug99. In another set, they inserted the same sequences into previously susceptible plants and found that it made them resistant. The report, published in *Science* in June 2013, was the result of several years of painstaking effort, and it marked the first time that any scientific group had managed to isolate and clone a Ug99 resistance gene.

The achievement should make it easier to insert the gene into a commercial wheat variety, the researchers say. Rouse says *Sr35* will work best if it is stacked with other genes, such as one found in goatgrass, known as *Sr33,* which was identified and mapped by another research team. *Sr33* offers less immunity to Ug99, but broader resistance to other races of stem rust, so the two genes should complement each other, Rouse says.

Continuing the Search

In other studies, Jin and his partners at the University of Minnesota probed the genetic potential of emmer wheat, triticale, and other wheat relatives using similar screening methods. They grew seedlings in an authorized, contained greenhouse in St. Paul, Minnesota, exposed them to Ug99, monitored infection levels, and used molecular techniques to identify genes unique to plants with resistance. Resistant plants were then sent to Africa for field screening. Such time-consuming steps are necessary to ensure that resistance is passed from one generation to the next.

"Until you put the gene in the plant and put the plant in the field, you don't really know how long the trait will last. It depends on the pathogen," Jin says.

Findings from the studies are shedding light on the mechanisms Ug99 uses to infect wheat and on different genetic tools available in some of the grasses to resist it. The researchers raised 567 triticale accessions (lines and cultivars) from 21 countries that were kept at the ARS Small Grains Collection in Aberdeen, Idaho. They exposed them to Ug99 and six other stem rust isolates. They crossed resistant and susceptible plants and analyzed genes of resistant progeny.

Results, published in *Plant Disease* in April 2013, showed that resistance in triticale was usually conferred by single genes. They found similar results, published in *Crop Science* in September 2012, in screening 359 emmer wheat accessions. In goatgrass, they determined that resistance was race-specific and that only 12 of 456 accessions screened were resistant to both Ug99 and other races of stem rust. The goatgrass results were published in *Crop Science* in September 2011.

The researchers say their goal is to leave no stone unturned in their search for Ug99 resistance genes. "We want to access as diverse a pool of genes as possible, so we do everything we can to help breeders deal with this," Jin says.—By **Dennis O'Brien,** ARS.

This research is part of Plant Diseases (#303), an ARS national program described at www.nps.ars.usda.gov.

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Targeting Pheromones in Fire Ants

The painful sting of the red imported fire ant is not easily forgotten. Delivered in large numbers, the stings can kill small animals. Humans that develop hypersensitivity to the ants' venom are at risk as well.

Unfortunately, the fire ant is well established in the southern and southwestern United States and is one of the world's 100 worst invasive species. The pest affects U.S. urban, agricultural, wildlife, recreational, and industrial areas, infesting millions of acres and costing $7 billion in damage repair, medical care, and control each year. As the ants spread northward, Agricultural Research Service scientists at the Center for Medical, Agricultural, and Veterinary Entomology in Gainesville, Florida, stay close on their trail, exploring how they function and developing innovative techniques that could lead to new biologically based and environmentally friendly control methods.

One area under investigation are pheromones, chemicals secreted by ants to induce behaviors in other ants. For example, trail pheromones guide workers from nest to food sources, queen pheromones attract workers, and sex pheromones bring males and females together for mating. Entomologist Man-Yeon Choi and chemist Robert Vander Meer, in the center's Imported Fire Ant and Household Insect Research Unit, have demonstrated for the first time that a neuropeptide hormone, originally discovered in moths, activates the production of trail pheromones. Worker ants lay a chemical trail from food to nest and recruit other workers to follow the trail to retrieve the food.

Following the Trail

The hormone of interest is called "pheromone biosynthesis activating neuropeptide" (PBAN). It was first discovered and identified in moths by ARS scientists in Beltsville, Maryland, in the 1980s. They found that PBAN regulates sex pheromone production in female moths.

According to Choi, every insect investigated so far has a variation of this neuropeptide—a compound made of two or more amino acids. "More than 200 PBAN-like peptides have been identified in other insects, such as cockroaches, but their pheromone biosynthesis activation function outside of moth species was unknown until now," he says.

SANFORD PORTER (D3131-1)

Left: Entomologist Man-Yeon Choi prepares to inject a worker fire ant with an RNAi solution designed to interfere with a gene so that the ant will produce less trail pheromone.

Below: Close-up of the ant being injected with RNAi through a capillary needle.

SANFORD PORTER (D3131-2)

In one experiment, scientists injected fire ant workers with their specific PBAN to see if this process influenced the biosynthesis of the trail pheromone. Results demonstrated that there was a significant increase in pheromone production after injection.

In addition to the fire ant-specific PBAN gene, they also identified the DNA sequence of the fire ant PBAN receptor gene. With this information, they determined that the receptor gene was being expressed in the Dufour's gland, which produces the trail-making pheromone.

Silencing Genes To Determine Their Function

Having determined the sequence of the genes, Vander Meer and Choi used the relatively new technique of RNA interference (RNAi) to provide another test of the role PBAN plays in fire ant trail pheromone production.

"RNAi technology involves taking normally single-stranded RNA from a gene and creating double-stranded RNA (dsRNA) that can be used to suppress expression of the gene," Vander Meer explains.

Scientists dissolved the dsRNA of PBAN or its receptor in a water solution, injected it into ants, and made observations at 24, 48, and 72 hours after injection.

"When we used RNAi to suppress expression of either the PBAN gene or the PBAN receptor gene, the ants produced less trail pheromone" than ants that did not receive the treatment, Vander Meer says. These results confirmed that PBAN is involved in trail pheromone biosynthesis in the fire ant; therefore this function is not restricted to moths, he says.

Using RNAi for Insect Control

While the above research was not directed at controlling fire ants, prevention of trail pheromone production could limit the ant's survival in terms of collecting resources, migration, and other activities, Vander Meer says. But the team also discovered that the PBAN gene is expressed in every stage of the fire ant's life cycle, which suggests that the gene has functions beyond pheromone production.

Choi and Vander Meer demonstrated this by suppressing expression of the PBAN gene in fire ant larvae, pupae, and adults, and then looking for any observable impact on treated ants. They found significantly greater mortality in PBAN RNAi-injected adults and larvae than in the untreated controls. Injected pupae showed delayed development as well as a high death rate.

"This gene has many different roles. When we disrupt the expression of it with RNAi, we observe multiple effects in immature stages as well as in adults," Vander Meer says.

Most importantly for future fire ant control efforts, brood-tending workers that fed on PBAN RNAi dissolved in sugar water regurgitated the RNAi to their hungry brood, which in turn died at a high rate, he says.

Collaborating with an industry partner, the scientists extended the fire ant PBAN RNAi work to the corn earworm—a moth species and a serious crop pest.

"Our moth study was very extensive," Vander Meer says. "We fed the PBAN RNAi to corn earworms in their standard laboratory diet, and it had dramatic effects." Those effects included a decrease in growth rate and the inability to develop from larvae into pupae. Female moths that survived to adulthood had decreased amounts of sex pheromone.

A patent has been awarded to ARS for the use of RNAi to control ant species. Three additional patent applications have been filed for RNAi suppression of the PBAN gene or its receptor gene in fire ants and moths.

Scientists plan to investigate whether other pheromones are activated by PBAN. Work on the use of RNAi to control insects continues under a cooperative research and development agreement with a private-industry partner.—By **Sandra Avant,** ARS.

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

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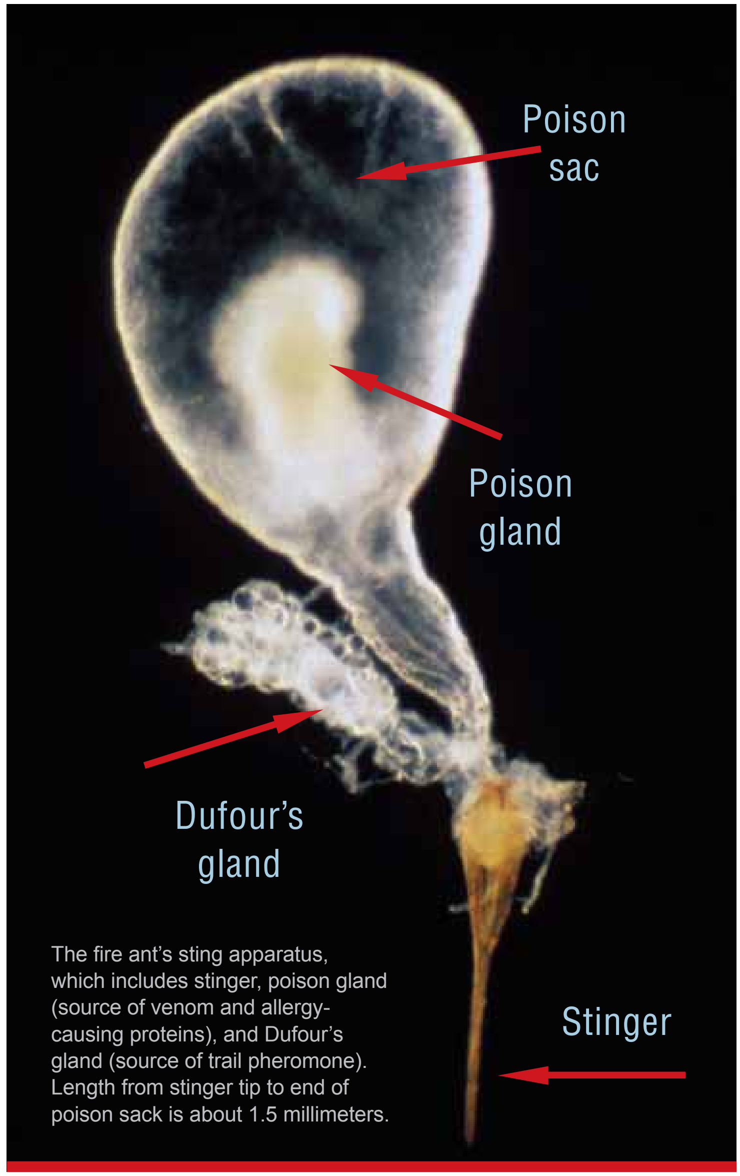
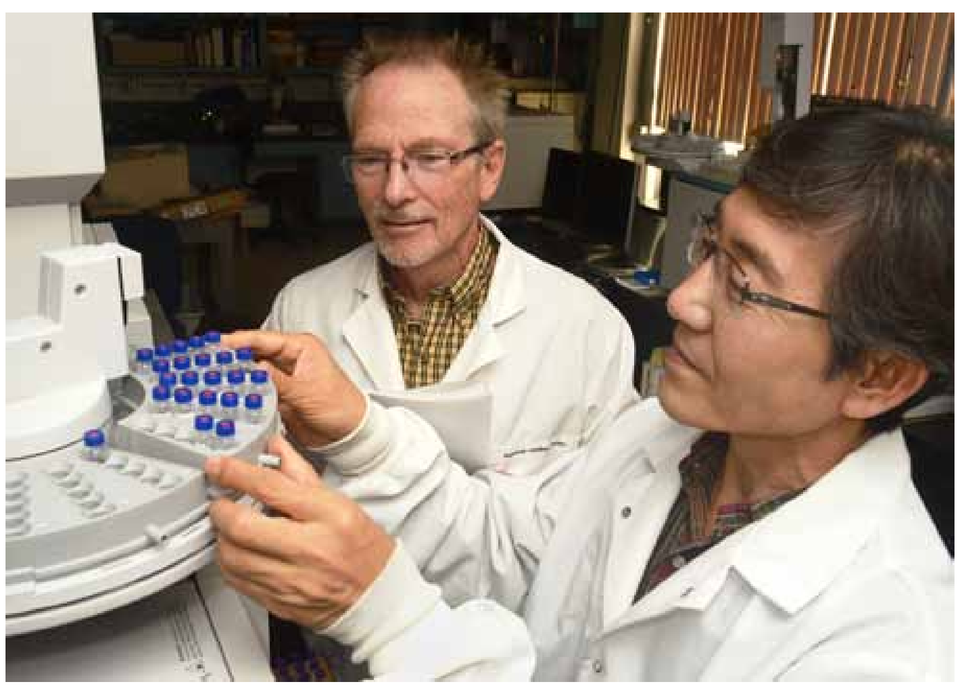
JUSTIN SCHMIDT (D3134-1)



The fire ant's sting apparatus, which includes stinger, poison gland (source of venom and allergy-causing proteins), and Dufour's gland (source of trail pheromone). Length from stinger tip to end of poison sack is about 1.5 millimeters.



SANFORD PORTER (D3132-1)

Chemist Robert Vander Meer (left) and entomologist Man-Yeon Choi use gas chromatography-mass spectrometry to measure changes in ant trail pheromone production after RNA interference.

TIMOTHY RINEHART (D3125-1)

New Ornamental Tung Tree a Story of Loss and Restoration

Tung tree germplasm orchard at the Thad Cochran Southern Horticultural Research Laboratory in Poplarville, Mississippi.

The newest tung tree on the scene, Anna Bella, may herald a new generation of ornamental tung tree varieties suitable for landscape uses in the U.S. Gulf Coast.

From the late 1920s to early 1970s, nut-producing tung trees had been grown commercially on plantations across the region as the source of a prized fast-drying and waterproofing oil for paints, varnishes, lacquers, wood finishes, and other industrial products. A convergence of factors ultimately scuttled the tung oil industry there, but nostalgia for *Vernicia fordii,* as the native Chinese tree is known scientifically, has lingered to this day.

"At one time there was 'tung tourism,' where folks came to the Gulf Coast for the beaches and to drive through the tung orchards," says Timothy Rinehart, a molecular geneticist at the Agricultural Research Service's Thad Cochran Southern Horticultural Research Laboratory in Poplarville, Mississippi. "People still plant tung trees in their yards around here—sometimes as an homage to their childhood, when tung was grown everywhere, and sometimes just because of its beauty."

What makes Anna Bella unique as an ornamental variety is that it is sterile and produces virtually no nuts, which are toxic if ingested (as are the leaves) and pose a mowing hazard if left on the ground.

"The downside to planting tung in the backyard has been the nuts, so we thought a nutless tree would be a great solution," Rinehart explains. "This release was also

TIMOTHY RINEHART (D3126-1)

Blooms of Anna Bella, a late-flowering ornamental variety of the tung tree *(Vernicia fordii)* that is sterile and produces virtually no nuts.

PEGGY GREB (D848-1)

Tung tree fruit (about 3 inches in diameter), seeds, and oil.

JENNIFER CARROLL (D3127-1)

ARS molecular geneticist Timothy Rinehart removes leaf tissue samples of Anna Bella, an ornamental tung tree variety, for DNA testing using molecular markers.

driven by the desire to preserve some of the history of tung before it was lost, to publicly acknowledge ARS's involvement in tung production in the region, and to give specialty nurseries an additional flowering tree for use in the South."

Indeed, the Poplarville laboratory had originally been known as the "USDA Tung Oil Research Station"—a reflection of its commitment to providing commercial tung tree growers in Mississippi, Florida, Louisiana, and other Gulf Coast states with new varieties that offered high yield, frost tolerance, and other desirable traits.

U.S. Tung Oil Industry: Bright but Brief

In its heyday, circa 1945, the U.S. tung oil industry had nearly 10 million trees in production—with mills strategically located across the Gulf Coast that specialized in extracting oil from the seed and processing it further for use, including to coat ammunition during World War II. By 1971, however, the laboratory had redirected its research to small fruits production. This shift was a consequence of an industry overwhelmed by the forces of economics (competition from abroad as well as other oilseed crops) and nature—in the form of Hurricane Camille, which in 1969 destroyed about half of the Gulf region's commercial groves.

The station's tung germplasm collection survived, however, and has been maintained ever since. It includes unreleased cultivars with a trait known as "late-flowering," which commercial tung growers desired as a way to avoid costly, bloom-killing spring frosts.

A New Lease on Life?

"A few years ago, we went through the collection and really opened our minds regarding what beneficial uses these trees might have—looking for new life in the collection, so to speak," says Rinehart of initial examinations by colleagues Ned Edwards and James Spiers (retired). "We dug out all the old breeding records, pedigrees, and research publications to make sure we knew what we had."

They came across one tung germplasm line—Anna Bella—that had originally been collected in the 1950s from an unknown source for the late-flowering trait. A lack of records on it led the team to believe it had never been used commercially because of its sterility. However, the researchers were intrigued by the ornamental prospects of this trait, and Rinehart coordinated multistate evaluations with university cooperators to examine Anna Bella's other growth characteristics.

The results were encouraging, so they released the variety "as is" in 2012 and reported its development in the January 2013 issue of *HortScience.*

The tree variety, which is adapted to conditions in the South, can reach nearly 40 feet tall and opens into an umbrella-shaped canopy of lush, heart-shaped leaves. It blooms in late spring, producing clusters of long-lasting white flowers, tinged in the centers with yellow or red. Anna Bella requires little maintenance, bounces back well from pruning, and can withstand common insect pests and diseases.

It is ideal for both single-specimen and row plantings, such as in backyards and along roadsides or property boundaries, the researchers say. And because it produces no seed, the variety is unlikely to become "naturalized," or spread beyond intended planting sites—a characteristic the researchers hope will encourage wider acceptance of the tree species as an ornamental offering.

Rinehart has already received requests from a few specialty nurseries interested in propagating the variety from budwood material and a few hundred "mother plants."

"Since we've started working on Anna Bella, we've been contacted by folks interested in restarting commercial tung oil production in the Gulf Coast region for biofuel and industrial uses," he reports. "After meeting with them to discuss their needs, we identified another tree in our collection that we hope to release as a commercial cultivar."—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 Food Ingredient from Rice Bran Oil May Help Make Foods' Fat Profile More Healthful

ERICA BAKOTA (D3130-1)

Taste testers report that a new, butterlike extract from rice bran oil—used as a partial butter replacement in a standard recipe for white bread—didn't detract from bread taste or texture.

If you enjoy cooking with a variety of different oils, you've probably worked with rice bran oil, perhaps for stir-frying or sautéing. A staple at Asian food markets or other specialty or gourmet grocery stores, this mild-flavored oil is high in vitamin E—an advantage that many other well-known cooking oils can't offer. The oil comes from the outer layers of the rice kernel that are removed when the grain is milled and polished to produce white rice.

At the Agricultural Research Service's National Center for Agricultural Utilization Research in Peoria, Illinois, chemist Erica Bakota and colleagues have shown how to extract natural compounds from the oil to make an interesting new food ingredient. Bakota says the product "looks something like a nut butter" and "shows promise for several uses," including as a replacement for some of the butter, margarine, or shortening currently used in popular baked goods.

The product, or "extract," consists primarily of unrefined rice bran oil and rice bran's natural wax, used in confections. It also contains minor amounts of such compounds as vitamin E; plant sterols, including some that are of interest to medical and nutrition researchers because of their potentially health-imparting properties; and gamma-oryzanol, shown to lower levels of "bad" LDL cholesterol in humans.

Unlike some shortenings and margarines, the extract is free of *trans* fats, which contribute to increased risk of heart disease. Another plus: The extract is shelf-stable: It resists oxidation that could otherwise result in off-flavors and unpleasant odors.

In preliminary experiments, Bakota and co-workers have substituted the extract in place of some of the butter called for in conventional recipes for white bread and granola. Feedback from taste testers (technically speaking, sensory panelists) indicates that the substitutions didn't detract from either the taste or the texture of the bread or granola.

The idea of making a butterlike product from rice bran oil isn't new, nor is the team's choice of solvent (acetone, which is already approved for extracting edible oils). However, Bakota's extraction or fractionation procedure apparently differs from others in that it uses very low temperatures to keep the mixture of acetone and unrefined rice bran oil chilled while the compounds that make up the new extract are separated from the oil.

The result?

"The texture and composition of our product are unique," Bakota says.

Bakota and Peoria teammates Michael J. Bowman, Hong-Sik Hwang, Sean X. Liu, Debra L. Palmquist, and Jill K. Winkler-Moser described the research in a 2013 scientific article published in the *European Journal of Lipid Science and Technology* and also in an article recently accepted for publication in that journal.

ARS is seeking a patent for the extraction procedure, and Bakota, in the meantime, is looking for collaborators interested in exploring applications of the extract. She explains, "We think our product can make the fat profile of many familiar foods much more healthful—and still get high ratings from taste testers."—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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New—and More Economical—Pyrolysis Techniques for Bio-oil Production

Fast pyrolysis, the process of rapidly heating biomass without oxygen, produces energy-dense bio-oil from wood, plants, and other carbon-based materials.

"It's becoming one of the most promising methods for extracting the energy from tough plant materials to produce liquid fuels," says Agricultural Research Service chemist Charles Mullen. Now, innovations by Mullen and his ARS colleagues are bringing researchers one step closer to using pyrolysis in production systems that farmers can use to meet their on-farm energy needs—or to produce renewable fuels for commercial markets.

Using pyrolysis to break down tough feedstocks produces three things: biochar, a gas, and bio-oils that are refined to make "green" gasoline. The oils are high in oxygen, making them acidic and unstable, but the oxygen can be removed by adding catalysts during pyrolysis. Although this adds to production costs and complicates the process, the resulting bio-oil is more suitable for use in existing energy infrastructure systems as a "drop-in" transportation fuel that can used as a substitute for conventional fuels.

In 2013, Mullen and two other researchers—lead scientist Akwasi Boateng and mechanical engineer Neil Goldberg—filed a patent application for a new pyrolysis process that removes much of the oxygen from bio-oils without the need for added catalysts. The three scientists work at the ARS Eastern Regional Research Center in Wyndmoor, Pennsylvania, in the Sustainable Biofuels and Coproducts Research Unit.

The team conducted a pilot-scale study using three types of biofeedstock with different characteristics: oak, switchgrass, and pressed pennycress seeds. They modified standard pyrolysis by gradually replacing nitrogen gas in the processing chamber with recycled gases produced during pyrolysis until they found the best mix to yield the desired products. Compared to the standard pyrolysis process of using nitrogen gas, which did not affect oxygen levels, using recycled gases derived from biomass was very effective in lowering oxygen levels and acidity. With the right mixture, no additional catalysts were needed.

The bio-oils produced from oak and switchgrass by the new process had considerably higher energy content than those produced by conventional fast pyrolysis. The energy content of the oak bio-oil was 33.3 percent higher, increasing from 23.7 to 31.2 megajoules per kilogram, so it contained about two-thirds of the energy contained in gasoline. The energy content for switchgrass was 42 percent higher, increasing from 23.4 to 33.2 megajoules per kilogram—slightly less than three-fourths of the energy content of gasoline. The energy content in bio-oil produced from pressed pennycress seed feedstock did not show a similar increase.

The researchers published the results in 2013 in *Energy Fuels*. A grant of $6.8 million from USDA's National Institute of Food and Agriculture, recently awarded to an ARS-industry-university consortium led by Boateng, will ensure that he, Mullen, and the rest of the team will be able to continue work on improving pyrolysis techniques for producing bio-oil and high-value specialty chemicals. The work funded by the grant will include the generation of a complete bio-oil production life-cycle assessment, from environmentally sustainable biomass collection and handling to the final process of biofuel production.—By **Ann Perry,** ARS.

This research is part of Biorefining (#213) and Agricultural and Industrial Byproducts (#214), two ARS national programs described at www.nps.ars.usda.gov.

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ARS scientists at Wyndmoor, Pennsylvania, are developing this mobile pyrolysis processing system that may one day be used on farms to produce bio-oil.

CHARLES MULLEN (D3129-1)

JANICE WATTS (D3146-1)

Male Chromosome Hinders Female Cattle Reproduction

In the beef industry, if a cow does not get pregnant after breeding, she becomes an economic liability in the herd. Lack of calf production can significantly reduce annual revenue for producers.

At the Agricultural Research Service's Roman L. Hruska U.S. Meat Animal Research Center (USMARC) in Clay Center, Nebraska, scientists are developing genetic markers for economically important traits, such as reproductive ability, which can be used by producers to select efficient animals. They have also found extraordinary answers as to why some cows are not reproducing.

The USMARC research team led by geneticist Tara McDaneld has discovered one reason for reproductive failure is that some females have introduced segments of the male (Y) chromosome in their genome.

McDaneld, molecular biologist John Keele, and geneticist Larry Kuehn collaborated with producers in gathering reproduction data on several female beef cattle populations. They examined records, which indicated whether cows became pregnant in their first spring breeding, on about 6,400 animals from herds in Florida, Nebraska, Colorado, and at USMARC.

The team then used a cost-saving genetic screening method called "DNA pooling" to genotype animals. The technique combines DNA from many individual animals into a single pool for further evaluation.

"We decided to pool the DNA because individually genotyping the 6,400 animals would be very expensive," McDaneld says. "We had two extreme phenotypes—animals that are pregnant and animals that are not pregnant." Multiple DNA pools were constructed for each phenotype—pregnant and nonpregnant. Each pool contained contributions from about 100 animals, and all animals within a pool had the same phenotype.

Segments of the Y chromosome were found only in the pool of DNA from nonpregnant animals. Normally, females inherit an X chromosome from each parent (XX) and males inherit an X and a Y (XY).

"Considering all the animals were females, they should all have been XX," Opposite page: At the U.S. Meat Animal Research Center in Clay Center, Nebraska, geneticists Tara McDaneld and Larry Kuehn (left) look for and record variations in data while molecular biologist John Keele and technician Tammy Sorensen take blood samples for DNA pooling.

McDaneld says. "There shouldn't be any Y chromosome at all in the DNA."

The first thought was that females with the Y chromosome could be "freemartins." This condition, resulting from twinning in cattle, causes infertility in the female calf born at the same time as her male twin. Although the male twin is rarely affected by reduced fertility, the female twin is completely infertile in a high percentage of the cases. The reproductive tracts of freemartins do not develop normally. In addition, because of the blood exchange between male and female fetuses, the Y chromosome can often be detected in the female's blood, Keele says.

To validate their findings, the scientists used a PCR test that amplifies chromosome Y in individual animals. They found that up to 25 percent of the animals with low reproductive efficiency in the Florida population and 20 percent of the low USMARC group were positive for the Y chromosome. None of the animals with prior pregnancies were positive for the Y chromosome.

"We concluded that some females were not getting pregnant because they carried segments of the Y chromosome," Keele says. "And interestingly, there was evidence that some of the Y-containing females were not twins. Hence, they could not be freemartins, yet they inherited Y chromosome material from somewhere, most likely from their fathers."

The USMARC team developed a PCR test that indicates whether a female is carrying Y-chromosome fragments. They also developed single nucleotide polymorphism (SNP) assays that contain genetic markers to identify chromosome Y-associated material in low-reproductive heifers. SNPs are genetic variations that provide information about an animal's genetic value and are often used in breeding programs.

"A lot of money goes into breeding an animal and keeping her long enough for her to get pregnant," McDaneld says. "Beef producers can use these PCR screening tools for chromosome Y before breeding, to test heifers and identify those that are less likely to consistently get pregnant."

"If a female calf is tested at birth and found to carry Y chromosome segments or markers, she can be used for meat production instead of for breeding," Keele says.

The test could also be used to identify heifers that are unable to reproduce. In rare cases, calves are born with female physical characteristics but are in fact genetically male (XY). These calves have defects in the male development pathway, Keele explains, and they are infertile. This condition can now be tested for, and the affected animals can be culled from breeding herds.

A further benefit of this test is to identify potential breeding bulls that have Y chromosome segments in their X chromosome. These bulls will produce normal male calves, but all of their daughters will have a copy of the contaminated X chromosome. Consequently, the reproductive capacity of the bull's daughters will be potentially much poorer.

A genetic test for female reproduction in a bull does not improve his reproductive performance, but rather, it improves the pregnancy rate of his daughters, Keele explains. "Bulls are able to have more offspring than cows; consequently, male selection is more effective than female selection for improving any trait. Testing a sire before using him to produce replacement females will improve reproductive performance in the herd if bulls with the X chromosome genetic defect are identified and culled."

Scientists are examining data from heifer populations for other variations in the genome and are finding other regions that may generate reproductive markers in the future, says Kuehn. For example, they have identified a deletion on chromosome 5 that is associated with females' inability to get pregnant, which they plan to pursue in further research.

Producing calves to sell at the market every year is the main driver for beef cow producers, Kuehn says. Heifers that conceive in their first calving season and then produce a calf every year thereafter are most profitable.

"Although we generally get somewhere from 85 to 95 percent fertility in cow herds, it's the most economically important trait in cow production," Kuehn says, "because if a cow doesn't produce a calf, the producer gets nothing but expenses—feed, labor, and other costs—out of that cow for that year."—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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Geneticist Tara McDaneld (left) and technician Tammy Sorensen prepare a gel for evaluation of genotypes associated with reproduction.

JANICE WATTS (D3147-1)

DUSTIN STRONG (D3128-1)

Fighting a Pernicious Weed with Fire

This research plot in Miles City, Montana, is made up of about 75 percent purple threeawn plants. ARS scientists are looking at ways to use fire to control the weed, which is taking over rangelands in the Northern Plains.

Carefully staged "prescribed fire" can reset a rangeland's biological clock, awaken dormant plants, and breathe new life and diversity into an ecosystem. When fire rolls over a rangeland, it gives perennial sod-forming grasses, which are good sources of forage for livestock, a better chance to take hold.

An Agricultural Research Service team in Miles City, Montana, is looking for ways to use fire to control a weed on the Northern Plains and on western rangelands. The weed is purple threeawn, and it is colonizing disturbed soils and overtaking rangelands used for grazing. When fully grown, the plant has sharp prongs that make it undesirable to cattle, and the cattle do not thrive if it becomes a staple of their diet.

The researchers—range ecologist Lance Vermeire, range technician Dustin Strong, and their colleagues—are in the ARS Range and Livestock Research Unit in Miles City.

Fire is commonly used on rangelands in the Southern Plains (Oklahoma, Texas, and Kansas), but it was not as readily adopted by settlers west and north of those areas. Because of that, less is known about its effectiveness as a management tool in the areas where purple threeawn is becoming more of a problem. The research is largely funded by the U.S. Bureau of Land Management, which manages much of the rangeland in the United States.

Effects of Fire

To find out whether fire is useful in controlling threeawn, Vermeire, Strong, and their partners are probing a number of issues, including which season is best for using fire. The researchers want to determine whether fire not only reduces the abundance of purple threeawn, but also creates more balanced ecosystems where desirable grasses can flourish. Their intent is to give land managers a way to keep rangelands viable so that cattle grazing on them can stay healthy and well fed.

"We don't just want to discourage the bad weeds; we also want to promote the good grasses, such as western wheatgrass, needleandthread, and other native perennial grasses," Vermeire says.

The researchers grew purple threeawn and two desirable grasses—western wheatgrass and blue grama—in a greenhouse to study the effects of fire under controlled conditions. Some purple threeawn was grown in isolation, and some was intermixed with the two grasses. They applied fire to some plants and let others grow unimpeded, and they clipped both the burned and control plants to specific heights to simulate cattle grazing. Combusted materials, clipped material, and final biomass of all plants were measured to assess plant production levels.

Results showed that fire killed 36 percent of the purple threeawn and reduced its biomass by 61 percent, reductions the researchers say were significant. Though this study was done in a greenhouse, the researchers say the results indicate that fire treatments would likely have significant impacts for reducing purple threeawn on open rangelands. The study was published in *Rangeland Ecology and Management* in 2013.

MARK JACOBSEN, BUREAU OF LAND MANAGEMENT (D3128-3)

Thermocouples (the silver wires) and data loggers are used in prescribed burns in research plots planted with purple threeawn. The loggers record plant temperatures every second the fire burns.

MARK JACOBSEN, BUREAU OF LAND MANAGEMENT (D3128-2)

The thermocouples run between the purple threeawn plants and the data loggers (seen here wrapped in wet burlap).

The Right Timing

Another key question is when to use fire. Most prescribed fires are set in rangelands during spring or fall, but a plant's response to fire varies with its stage of development and activity level. Most native grasses in Montana are cool-season plants that have adapted to the natural cycle of frequent summer wildfires. They go dormant in the summer, making them less susceptible to summer fires. Purple threeawn, however, is a warm-season species that grows during the summer, which should make it more susceptible to summer fires.

In a study at two Montana field sites, plots were either burned during the summer or fall or were not burned at all, and each of those treatments had either no nitrogen or one of two levels of nitrogen added. Precipitation levels varied widely: Spring 2011 saw record rainfall, but spring 2012 was one of the driest on record.

The results showed that while fall fires reduced purple threeawn production, summer fires were much more effective, particularly after a wet spring. "Few rangeland treatments have shown such immediate effects with just a one-time application," Vermeire says. In comparison to the control plots, the weed's overall biomass was reduced 90 percent by the summer fire and 73 percent by the fall fire after the wet spring. By comparison, it was reduced 73 percent by the summer fire and 58 percent by the fall fire after the dry spring. Adding nitrogen to the soil had no effect on purple threeawn production at any of the sites nor on growth of the more desirable perennial grasses after the dry spring, but it doubled grass production after the wet spring.

The study was published in *Rangeland Ecology and Management* in 2013.

Heat Dosage and Duration

When conducting prescribed burns, land managers can control certain factors such as the temperature, duration, and the amount of heat applied. The duration of a fire, for instance, can be prolonged by allowing plant litter to accumulate or by burning in light winds or when the plant material is moist. "Dosage" could be defined by how hot and how long a fire burns, the researchers say.

In another study at the Montana sites, the researchers placed data loggers at the base of purple threeawn plants to record the temperatures during prescribed fires. The purpose was to assess the effects of temperature, heat duration, and heat dosage. The loggers recorded temperatures every second the fires burned.

The results, published in 2013 in *Fire Ecology,* showed that heat dosage and duration are more important than maximum temperature. The scientists conclude that summer fires, with their higher dosage and duration levels, provided more benefit than fall fires.

Vermeire said the results could also be attributed to purple threeawn's growth cycle. "Because purple threeawn grows and reproduces during the summer, setting it on fire in summer rather than fall is more likely to shut down that process," he says.—By **Dennis O'Brien,** ARS.

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

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Nitrite's Significant Role in Nitrous Oxide Emissions from Soil

STEPHEN AUSMUS (D1514-13)

University of Minnesota technician Sonya Ewert (left) and ARS soil scientist Rodney Venterea use a gas chromatograph to determine amounts of greenhouse gases in samples collected from fields.

Nitrous oxide can contribute significantly to atmospheric warming because, pound for pound, this greenhouse gas absorbs 300 times more radiation than carbon dioxide. It is also the most important stratospheric-ozone-depleting chemical emitted by human activities. Therefore, scientists are trying to identify strategies to reduce nitrous oxide emissions from agricultural soil.

Many of these efforts have focused on the role of nitrate, a form of nitrogen produced by soil microbes following the application of nitrogen fertilizers, because soil organisms can convert nitrate to nitrous oxide. However, Agricultural Research Service soil scientist Rodney Venterea suspected that nitrous oxide emissions are even more strongly correlated with levels of nitrite—a form of nitrogen that is not commonly measured.

Venterea conducted a study in maize over two growing seasons, examining the effects of different nitrogen fertilizer sources and application methods on nitrous oxide emissions. The fertilizers were conventional urea, polymer-coated urea, urea infused with microbial inhibitors, and a 50/50 mixture of conventional urea and urea-with-inhibitors. Each fertilizer was applied to experimental fields using either uniform broadcasting or concentrated banding.

Venterea's results indicated that nitrous oxide emissions were strongly linked to soil nitrite levels, which accounted for 44 to 73 percent of the variation in nitrous oxide emissions. Neither soil nitrate nor ammonium levels had similar correlations with nitrous oxide.

"The most important finding here is that practices which reduce nitrite have good potential to also reduce nitrous oxide," Venterea states.

The results also showed that the lowest levels of nitrite and nitrous oxide occurred with the fertilizer that contained urea infused with microbial inhibitors. The 50/50 mixture also reduced both nitrite and nitrous oxide. Using this 50/50 combination might be more cost-effective for reducing nitrous oxide because it could cut the cost of just using the more expensive urea infused with microbial inhibitors.

"There are two reasons why people don't study nitrite more," says Venterea. "Nitrite is generally found at lower levels than nitrate, and measuring it requires more effort. Because nitrite is very reactive, we need to process soil samples quickly so the nitrite doesn't disappear from the sample."

However, this higher reactivity is also what makes nitrite a key factor in generating nitrous oxide. "Even though nitrite may be present at lower levels, it can be more important than nitrate because it can produce nitrous oxide quickly under a broader range of conditions. This is especially true when soil is in an oxygenated condition, which it tends to be most of the time, except after large rainfall events."

Venterea, who works in the ARS Soil and Water Management Research Unit in St. Paul, Minnesota, published his results in 2013 in *Soil Biology and Biochemistry*. This work was conducted as part of GRACEnet (Greenhouse-Gas Reduction through Agricultural Carbon Enhancement Network), a national network of ARS research projects investigating the effects of management practices on soil carbon sequestration, trace gas emissions, and environmental quality.

Venterea concludes, "More consideration needs to be given to nitrite. We still do not fully understand the factors that control nitrite dynamics in soil."—By **Ann Perry**, ARS.

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

Rodney Venterea is with the USDA-ARS Soil and Water Management Research Unit, 1991 Upper Buford Circle, St. Paul, MN 55108; (612) 624-7842, rod.venterea@ars.usda.gov. *

A covered gas flux chamber from a study done in a field of maize during two growing seasons. The study examined the effects of different nitrogen fertilizer sources and application methods on nitrous oxide emissions.

RODNEY VENTEREA (D3145-1)

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

Pullman, Washington
6 research units ■ 110 employees

Small Grains and Potato Germplasm Research Unit, Aberdeen, Idaho
1 research unit ■ 42 employees

Livestock and Range Research Laboratory, Miles City, Montana
1 research unit ■ 20 employees

Roman L. Hruska U.S. Meat Animal Research Center, Clay Center, Nebraska
4 research units ■ 117 employees

Red River Valley Agricultural Research Center, Fargo, North Dakota
5 research units ■ 129 employees

Lincoln, Nebraska
2 research units ■ 67 employees

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

St. Paul, Minnesota
3 research units ■ 48 employees

National Center for Agricultural Utilization Research, Peoria, Illinois
7 research units ■ 208 employees

Thad Cochran Southern Horticulture Laboratory, Poplarville, Mississippi
1 research unit ■ 36 employees

Center for Medical, Agricultural, and Veterinary Entomology, Gainesville, Florida
4 research units ■ 145 employees

Raleigh, North Carolina
4 research units ■ 62 employees

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

Eastern Regional Research Center, Wyndmoor, Pennsylvania
6 research units ■ 203 employees