



# Agricultural Research

Preserving  
the  
Flavor

Story on  
pages 10-11

# FORUM

## Keeping Pace With Agriculture's Challenges

**G**rowers today face a daunting list of challenges. Chief among them are dealing with climate change, limiting agriculture's environmental footprint, and expanding the range of key crops to feed growing populations. To address these challenges, we need to look at them in new ways and with new tools.

Agricultural Research Service scientists are at the forefront of these efforts and have been instrumental in developing new technologies that will help both the growers who produce our agricultural bounty and the scientists who look for ways to help address these challenges. This issue of *Agricultural Research* includes articles that describe some of these efforts.

In Raleigh, North Carolina, an ARS agronomist and his colleagues have developed a technique for creating images of cereal crops that is helping scientists understand their internal structures and how they react to cold and other environmental stressors. It involves taking standard slices of plant tissues, making high-resolution digital photos of them, and using imaging software to create a three-dimensional perspective that gives added depth to their structures, above and below ground. The results are similar to images produced by magnetic resonance imaging and computed tomography, but they can be created from much smaller tissue samples, and they are easier and less expensive to produce.

The team used the imaging technique to study ice formation in oat plants. Understanding the effects of ice formation in cereal crops could help breeders develop

hardier varieties with an expanded range. Climate change has also meant some widely fluctuating winter temperatures in recent years, making it important to understand how winter oats and other cereal crops react to frozen soils. The ARS-published work so far in this area has focused on oats, but similar results have been observed in wheat, barley, and rye, and the technique should prove useful for studying a number of other crop plants. You can read about this research on [page 4](#).

Another ARS research team, in the ARS Fruit and Vegetable Insect Research Unit in Wapato, Washington, has found that spotted wing drosophila flies have a taste for certain chemicals present in wines and vinegars. These insect pests from eastern Asia are damaging tree fruit and berry crops across the United States. After extensive testing, the team determined that a precise blend of chemicals is highly attractive to the flies, and that has led to the development of an effective new lure and trap. These kinds of strategies are essential to ensuring a continued supply of nutritious foods while minimizing the use of insecticides. The story begins on [page 9](#) of this issue.

One of the main ways crops are fertilized is with beef manure. It's effective and costs less than commercial fertilizers, but the odor is unpleasant. Two ARS agricultural engineers in Nebraska decided to find out exactly which compounds are responsible for beef manure odor and to evaluate how diet, soil moisture, and application procedures affect the emission of those compounds. They suggest a simple technique that can maintain the benefits

of manure application but greatly reduce the odor. The research was also part of GRACENet, a wider effort to determine the effects of management practices on soil carbon sequestration, trace gas emissions, and environmental quality. See [page 6](#) for details

When planning a study, such as those mentioned here, researchers consult the scientific literature to see what others have learned about the problem of interest. This can be a time-consuming process, but it can save time and resources in the long run. Many scientific journals are now online, making literature searches much faster. But an ARS ecologist has taken this a step further. He is "geotagging" scientific articles—making them searchable by the geographic location where the research was conducted. You can read about this new search engine on [page 19](#).

Such innovative approaches are a key part of ARS's scientific mission for a simple reason: To address the modern challenges inherent in trying to feed a hungry planet, we have to begin looking at old problems in new ways.

**José M. Costa**

ARS National Program Leader  
Grain Crops

Beltsville, Maryland

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PEGGY GREB (D3313-1)



Sod being installed in July 2014 for the fall opening of the U.S. National Arboretum's "Grass Roots" exhibit, featuring 14 stations on everything from golf course grasses to turf diseases to green roofs. [Story begins on page 14.](#)

- 4** New Imaging Technique Leads to Better Understanding of Freezing in Plants
- 6** Better Information for Improved Beef Manure Management
- 8** Improving Trout Resistance to a Deadly Disease
- 9** Pest's Taste for Fine Wine May Prove Its Undoing
- 10** Mandarin Oranges: Protecting the Flavor of This Popular Fruit
- 12** Experimental Website Helps Teens Eat More Veggies
- 13** Latest Update of USDA's Nutrient Database Released
- 14** The Greatest Show on Turf
- 17** Assessing Cotton Fiber Quality From a Tiny Sample
- 18** A Steady Pace of Cellulosic Ethanol Research in Peoria
- 19** Using Location Information To Improve Literature Searches
- 20** Locations Featured in This Magazine Issue



**Cover:** These clementine oranges are a type of mandarin orange. ARS scientists are studying cold-storage temperatures and more to make sure this popular fruit is as tasty as possible for you. [Story begins on page 10.](#) Photo by Peggy Greb. [\(D3267-1\)](#)

# New Imaging Technique Leads to Better Understanding of Freezing in Plants

Using a new technique to study an old problem, an Agricultural Research Service scientist in North Carolina has uncovered new details about what happens to a cereal plant when it freezes.

Agronomist David P. Livingston, in the Plant Science Research Unit in Raleigh, has developed an imaging technique and has used it to show that when an oat plant freezes, ice forms in its roots and in portions of its crown, which lies just below the soil surface and connects the roots to the stalk.

The results have implications for growers. In winter cereals like oats (*Avena sativa*), the crown is where the plant generates new tissue growth—if it survives the winter cold. Oats won't grow in many northern areas because of cold temperatures. Understanding how ice forms in oats could help breeders develop hardier varieties and expand their range, Livingston says. Climate change has also made it more important to understand how cereal crops react to wide fluctuations of winter temperatures and other environmental stresses.

The process developed by Livingston involves making high-resolution digital photos of standard histological slices of plant tissues and using commercially available software to create a three-dimensional perspective, which gives added depth to their structures, above and below ground. The resulting images are similar to those produced by magnetic resonance imaging (MRI) and computed tomography (CT) scans. The advantages of Livingston's images are that they can be created from much smaller tissue samples than what CT and MRI tests require and are less expensive to produce, because they require less expensive equipment and training.

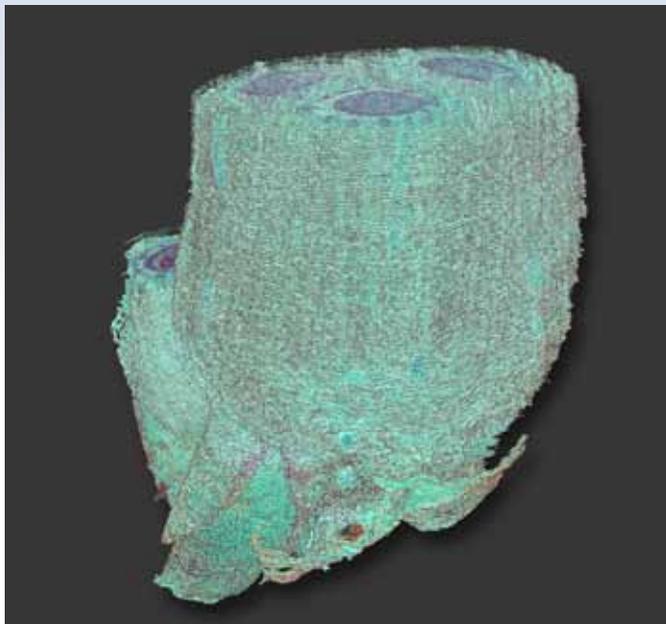
Livingston's studies have so far focused on oats because their production in the United States is limited by their sensitivity to subfreezing temperatures. He has also used the technique to examine wheat, barley, rye, and corn, and he says it could be used to study other crop plants. The technique even works on mammalian systems and has been used to produce three-dimensional reconstructions of tumors in liver biopsies.

Livingston first described the technique in a paper in the *Journal of Microscopy* in 2010, which included images of oat tissue and lung tissue from a mouse. In the more recent study, he used it to examine how oat plants react to freezing temperatures in the soil. He stained frozen tissue samples and took 186 sequential images of them with a digital camera.

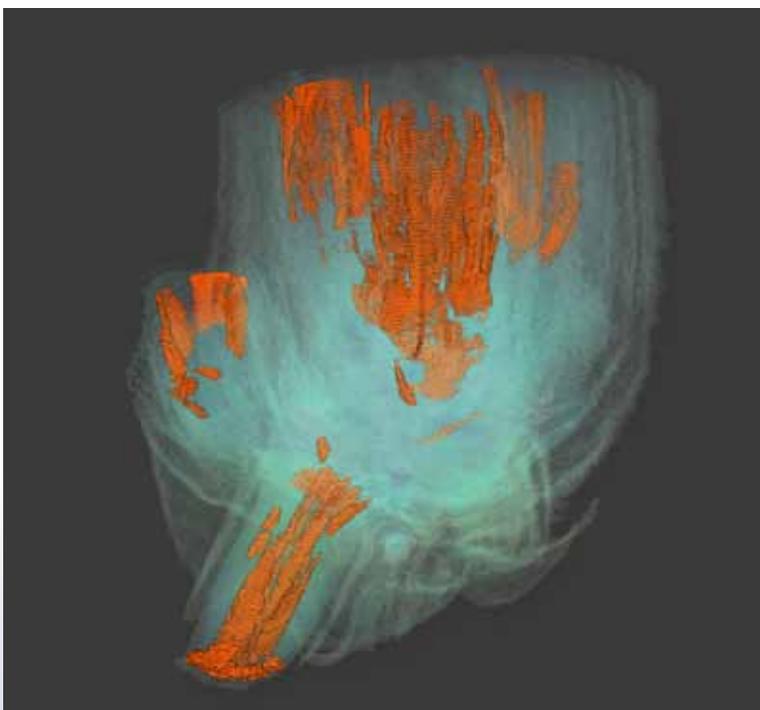
**Right:** Outside view of an oat crown reconstructed in 3D from 186 images taken through a light microscope.

**Below:** During freezing, ice crystals formed in an oat crown (orange coloring added to accentuate the crystals) as seen in this interior view.

DAVID LIVINGSTON (D3317-1)

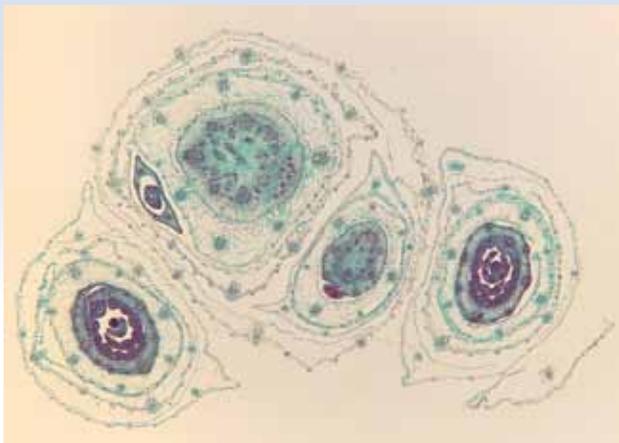


DAVID LIVINGSTON (D3318-1)

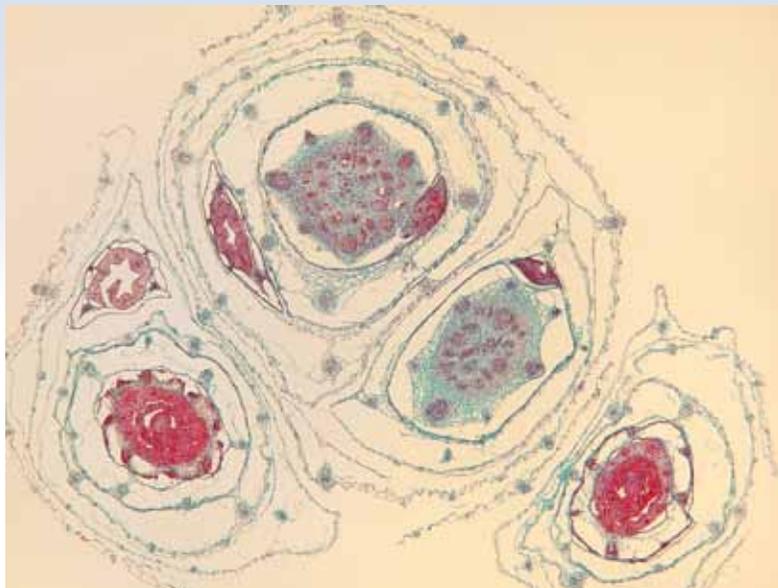


He then aligned the images and used imaging software to clear away the background colors so he could focus on cavities formed by ice crystals in the crown tissues of the oats. He compared the images from frozen plants with images from plants kept at normal temperatures.

DAVID LIVINGSTON (D3319-1)



DAVID LIVINGSTON (D3320-1)



Cross-section views of the plant crown before freezing (left) and after freezing and thawing (right). In the thawed crown, the empty spaces that were not present before freezing are voids within the tissue where ice had formed while the plant tissue was frozen.

DAVID LIVINGSTON (D3321-1)



Right: An oat plant. The white area at the base of the plant is the crown. Left: A longitudinal section of the crown area showing the complexity of tissue within it. The growing point must survive winter conditions for the plant to resume growth in the spring.

DAVID LIVINGSTON (D3322-1)



Along with showing how ice forms in the root, the images revealed that ice formation in the crown is limited to its lowest and uppermost parts, apparently

leaving the middle free of ice—at least free from crystals big enough to visualize. The ice also didn't form in the shape of circular crystals, as portrayed in two-dimensional images. Instead, the crystals were shaped more like elongated curtains.

The results were published in 2014 in *Environmental and Experimental Botany*.—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](http://www.nps.ars.usda.gov).

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# Better Information for Improved Beef Manure Management

PEGGY GREB (D3307-1)



Agricultural engineer Bryan Woodbury (front left) collects a soil sample to characterize soil conditions following the field application of beef manure while agricultural engineer John Gilley (front right) and biological sciences aides adjust small wind tunnel equipment to be used for air quality measurements.

**M**anure disposal is a big concern for cattle feedlot operators,” says Agricultural Research Service agricultural engineer John Gilley. “Fortunately, producers can reduce their use of commercial fertilizer—and their production costs—by using manure to fertilize their fields.”

“Manure makes a great fertilizer because it has so many nutrients,” agrees ARS agricultural engineer Bryan Woodbury. “But more people are moving to rural areas and don’t like the odor, so we’re trying to find a way to help producers control odors more effectively.”

Wherever manure ends up, its decomposition releases volatile fatty acids, aromatics, sulfides, amides, and alcohols that are responsible for the odors. There has been some research on reducing odors when manure is used for fertilizer, but much of the work has been focused on applications of swine manure and poultry litter, which

can be very different than beef manure applications.

Another factor that affects beef manure odor emissions is diet. Some producers supplement livestock feed with wet distillers grains with solubles (WDGS), a coproduct of corn ethanol production. Adding WDGS to cattle feed can increase the dietary levels of phosphorus, nitrogen, and sulfur, which in turn can raise emissions of ammonia and other odor-causing compounds.

However, WDGS feed supplements are a cost-effective way for livestock producers to lower their expenses, so producers are interested in finding approaches to managing manure odors that do not limit their use. One alternative is identifying how different beef manure application methods affect the emission of odor-causing compounds.

Woodbury and Gilley decided to conduct a comprehensive study to identify com-

pounds responsible for beef manure odor and to evaluate how diet, soil moisture, and application procedures affect odor emissions. Woodbury works in the ARS Nutrition and Environmental Management Research Unit in Clay Center, Nebraska, while Gilley works in the ARS Agroecosystem Management Research Unit in Lincoln, Nebraska. ARS agricultural engineer Roger Eigenberg and microbiologist Daniel Miller also collaborated on the study, as did West Texas A&M University professor David Parker and University of Nebraska-Lincoln professor David Marx.

“Compounds that cause odor are really hard to identify, so we knew we had our work cut out for us,” Gilley says.

## From the Feedlot to the Field

In their study, the team used manure collected from feedlot pens where cattle consumed diets containing 0, 10, or 30 percent WDGS. The scientists also evaluated

two application methods—no-till surface manure application and disk tillage that incorporated manure into the soil—and collected air samples before and after water was added to the soil to assess the effect of moisture levels on emissions.

Beef cattle manure was applied at levels that provided 135 pounds of nitrogen per acre, which met the 1-year nitrogen requirement for corn. This meant that some plots received more manure than other plots, because varying levels of WDGS in the cattle feed resulted in different levels of nitrogen in the manure. This approach was selected so that study findings related to odor mitigation practices would have more real-world relevance for producers.

After collecting and analyzing the air samples, the researchers determined that two volatile fatty acids—isovaleric acid and butyric acid—and the aromatic compound 4-methylphenol were responsible for more than two-thirds of detectable beef manure odors. Most of these odors were released within 24 hours after manure was applied to the soil.

Incorporating the manure into the soil and irrigating afterwards reduced most of the odor compounds that were measured. But the manure needed to be incorporated almost immediately after it was applied to obtain the most effective odor mitigation.

The importance of tilling manure into soil was highlighted by emission measurements the researchers obtained for 4-methylphenol. The greatest emissions of this compound occurred from dry soils on no-till plots and were sometimes as much as 10 times more than similar emissions from tilled soils.

The researchers also noted some significant differences between emissions produced by volatile fatty acids and aromatics and emissions produced by sulfide compounds. These differences were especially notable in soils amended with manure produced by cattle that consumed feed with 30 percent WDGS. This type of manure emitted two sulfide compounds (dimethyl disulfide and dimethyl trisulfide) at significantly higher levels than other

manures in the study—sometimes as much as 8 times more.

The researchers also saw something they didn't expect to see: Overall emissions of the sulfide compounds increased when soil moisture levels increased, unlike the other odor compounds, where emissions decreased as soil moisture levels increased. They are currently conducting additional laboratory studies to determine why this occurred. "It was a big surprise to us," Woodbury says.

"Our results basically confirm that producers who want to use beef manure to improve soil quality can incorporate it into the soil to reduce odors and maintain nutrients," Woodbury says. "Now we're working on ways to manage manure in the feedlot that will improve its characteristics as a soil amendment."

"Knowing the principal components that influence odors in feedlots—pen location, moisture, and temperature—will help us with these investigations," Gilley adds.

This research, which was published in the *Journal of Environmental Quality* in 2013, was conducted as part of the GRACEnet (Greenhouse-Gas Reduction through Agricultural Carbon Enhancement Network) program. GRACEnet is an effort to coordinate research projects at multiple ARS locations and determine the effects of management practices on soil carbon sequestration, trace gas emissions, and environmental quality.—By [Ann Perry, ARS](#).

*This research is part of Agricultural and Industrial Byproducts (#214) and Climate Change, Soils, and Emissions (#212), two ARS national programs described at [www.nps.ars.usda.gov](http://www.nps.ars.usda.gov).*

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Biological sciences aide Charles Hinds (left) and microbiologist Daniel Miller apply beef cattle manure to experimental plots in studies to identify compounds responsible for manure odor.

PEGGY GREB (D3308-1)



**Above:** Postdoctoral fellow/veterinarian Dave Marancik (left) and technicians Travis Moreland (center) and Joel Caren test rainbow trout for the pathogen that causes bacterial cold-water disease. **Right:** Molecular biologist Greg Wiens inspects fingerlings bred to have increased resistance to the disease.

Each year, the rainbow trout industry suffers significant economic losses due to bacterial cold-water disease, caused by the bacterium *Flavobacterium psychrophilum*. The disease also affects salmon and other cold-water fish species. It first occurs when fish are small, often leading to rapid death. Larger fish can become chronically infected and consequently have lesions and impaired growth and yield.

At the Agricultural Research Service's National Center for Cool and Cold Water Aquaculture (NCCCWA) in Leetown, West Virginia, scientists have developed a new line of trout that is resistant to bacterial cold-water disease. They've also developed a susceptible line and a control line to use in studies of how breeding changes disease-resistance properties in trout. They have identified regions on several chromosomes that are responsible for disease resistance and have developed a test that detects *F. psychrophilum* after infection.

Molecular biologist Greg Wiens and geneticist Timothy Leeds recently completed a field performance evaluation in collaboration with industry and government stakeholders. In a study that appeared in *Aquaculture* in 2013, Wiens measured performance of the control, susceptible, and disease-resistant lines of fish under farm conditions before and after natural exposure to the pathogen.

"After exposure, the disease-resistant line had a higher rate of survival than the

control or susceptible lines," Wiens says. "In addition, during the outbreak, fewer disease-resistant fish harbored the pathogen in their internal tissues, compared to the control and susceptible fish."

Wiens and postdoctoral fellow David Marancik developed a highly sensitive real-time polymerase chain reaction test that accurately measures small amounts of *F. psychrophilum* in fish tissue. The test recognizes a unique gene sequence that is only found in that pathogen. In the study, published in *FEMS Microbiology Letters* in 2013, more than 200 different isolates of *F. psychrophilum* were detected. These isolates were all collected at farms where fish suffered from the disease.

"No other species of environmental bacteria or fish pathogens were recognized by the test, which demonstrates its high specificity," Wiens says. At the conclusion of the farm trial, the test confirmed that the resistant-line fish did not harbor detectable levels of pathogen.

Scientists at NCCCWA are also investigating mechanisms that cause fish to be disease resistant. After finding a correlation between disease resistance and larger spleen size in rainbow trout, Wiens and geneticist Yniv Palti searched for common genetic regions that influence both spleen size and disease resistance.

## Improving Trout Resistance to a Deadly Disease



STEPHEN AUSMUS (D3207-7)

In their study, published in *PLOS ONE* in 2013, they mapped regions in the trout genome that determine spleen size and found links to chromosomes 19, 16, and 5. They also mapped disease resistance and found a closely linked region on chromosome 19 that had a major effect on bacterial cold-water disease resistance.

"This is the first study to identify a genetic link between a physical trait—spleen size—and specific disease resistance in fish," Wiens says. "We are now working to identify genes and mechanisms of resistance."

Based on the results of several field trials and laboratory-evaluation data, disease-resistant rainbow trout germplasm was released in 2012 and 2013 to stakeholders, who are propagating the line and continuing to evaluate its performance in large-scale trials in conjunction with NCCCWA scientists.—By [Sandra Avant, ARS](#).

*This research is part of Aquaculture, an ARS national program (#106) described at [www.nps.ars.usda.gov](http://www.nps.ars.usda.gov).*

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# Pest's Taste for Fine Wine May Prove Its Undoing

A blend of odors that attracts spotted wing drosophila (SWD) flies has been developed into a new lure product for improved monitoring and control of these tree-fruit and berry pests.

The blend is a combination of four different chemicals found in the aromas of both wine and vinegar. Entomologist Peter Landolt and colleagues from USDA's Agricultural Research Service and the Oregon Department of Agriculture isolated the chemicals and evaluated them extensively in laboratory and field trials.

Based on those findings, Trécé, Inc., of Adair, Oklahoma, has commercially formulated the compounds into a novel blend and controlled-release lure, which is marketed under the trademark "PHEROCON SWD," along with a related trap.

"We developed the attractant because farmers and pest managers need improved methods of attracting, monitoring, and managing the fly to prevent severe potential losses of cherries, berries, grapes, and other fruit crops," says Landolt, who leads the

ARS Fruit and Vegetable Insect Research Unit in Wapato, Washington. "The lure's availability should provide better information to growers who use trap-catch data to make pest-management decisions." Those decisions include where, when, or whether to spray.

Known scientifically as *Drosophila suzukii*, SWD is a nonnative species from eastern Asia that was first detected in the United States in 2008. Since then, it has become an established pest of numerous tree-fruit and berry crops in both the eastern and western United States, says Landolt.

If unchecked, female SWD flies deposit their eggs beneath the surface of host fruit. Subsequent larval feeding inside the fruit causes it to soften, bruise, and wrinkle, ruining its marketability.

Capturing drosophila flies with lures containing wine and vinegar isn't a new approach. But Landolt's team was first to conduct a top-down examination of which chemical constituents in the aromas of these liquids specifically attract the flies.

Initially, acetic acid in vinegar and ethanol in wine were thought to be the primary attractants. Though important, the two compounds weren't the only sources of attraction for SWD flies, the researchers found. In extensive testing, they showed that ethanol alone was less attractive than wine, and acetic acid alone was less attractive than vinegar. Similarly, combinations of ethanol and acetic acid were also less attractive to the flies than wine-plus-vinegar blends, which suggested that other constituents were at work. Indeed, in field tests, wine-plus-vinegar captured 10 times more flies than acetic acid-ethanol blends.

Interestingly, combining acetic acid and ethanol with the wine-plus-vinegar blend worked best of all.

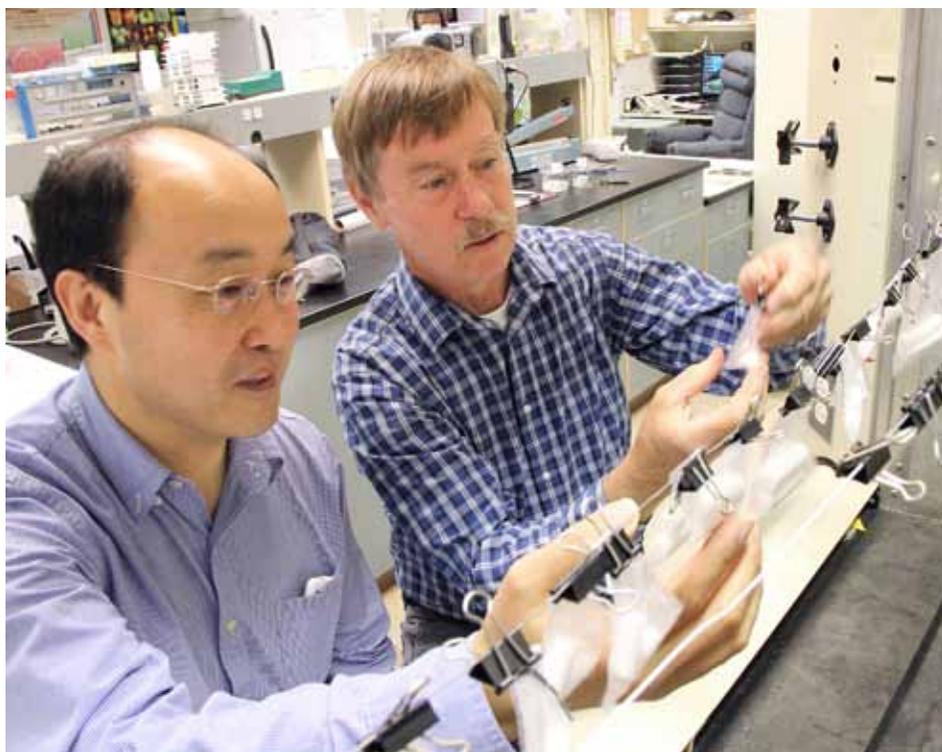
In more recent studies, the team showed that SWD prefers certain types of wine and vinegar over others, with Merlot wine and rice vinegar attracting more male and female flies than Chardonnay wines and apple cider vinegar, for example.

Of 20 total Chardonnay and rice-vinegar chemicals the team evaluated, acetoin and methionol triggered the strongest responses in SWD when combined with acetic acid and ethanol.

A third chemical, ethyl lactate, also attracted the flies but was determined unnecessary for optimum attraction. It was ultimately dropped from the final lure formulation, which contains acetoin, methionol, acetic acid, and ethanol. "If one of those is missing, you get significantly lower attraction," notes Dong Cha, an ARS postdoctoral researcher who, along with Landolt and coauthors, reported the findings in the February 2014 issue of *Pest Management Science*.—By [Jan Suszkiw, ARS](#).

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

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DONG CHA (D3304-1)

Postdoctoral researcher Dong Cha (left) and entomologist Peter Landolt isolated chemicals from wine and vinegar that attract *Drosophila* flies.

# Mandarin Oranges

## Protecting the Flavor of This Popular Fruit

**F**resh and juicy mandarin oranges are good for you and have a delightfully sweet flavor that makes them a favorite of kids and adults alike.

Whether you know them by brand names such as “Cuties” or “Halos,” or perhaps simply as clementines or tangerines, all are mandarins. And all are high in vitamin C and are a good source of vitamin A.

Agricultural Research Service scientist Dave Obenland at Parlier, California, and horticulturist Mary Lu Arpaia and colleagues at the University of California-Riverside are conducting studies to discover more about mandarin flavor and—equally as important—how to protect it as these oranges make their way from the orchard to the fruit bowl on your kitchen counter.

Their research focuses on uncovering more details about the natural chemicals in mandarins that contribute to the fruit’s pleasing flavor and on determining what causes changes in those flavors after the fruit is put into storage. That new knowledge may help citrus-fruit breeders, and the people who grow, store, ship, or sell the fruit, ensure that the oranges keep their delicious taste.

### Chilling Out

Most mandarin oranges that are destined for the produce section of your local supermarket probably spend at least some time in cold storage. Before that happens, however, freshly picked mandarins are typically washed, then sprayed with a thin coating of a wax that’s approved for this food use. The wax helps hold in the fruit’s natural moisture and adds an attractive gloss to its peel.

Next, the oranges are placed in cardboard cartons that are then stacked, in packinghouse storerooms, to await shipment.

The flavor research that Obenland and Arpaia began in 2009 may be



Clementine oranges.

PEGGY GREB (D3267-1)

among the most extensive of its kind for this specialty citrus fruit. The scientists have now tested more than 19,000 mandarin oranges from at least a half-dozen different research and commercial orchards in California, the state that produces the bulk of the nation’s mandarin harvest.

NITTAYA UMMARAT (D3264-1)



Plant physiologist David Obenland adds mandarin juice to a vial for analysis of aroma volatiles. The identity and amount of these natural compounds are important in determining their contribution to the flavor of whole, fresh mandarins.

Individual studies have spanned multiple years and multiple harvests, to take within-year and year-to-year variations into account.

All of their experiments simulate real-world conditions of busy commercial packinghouses or retail settings and encompass a range of time-and-temperature regimens. In some of these tests, for instance, the researchers kept the fruit at cold-storage temperatures of 32, 39, 41, 46, or 50 °F for several weeks and, in some cases, followed that by a week or more of storage at 68 °F.

Storage experiments lasted up to 6 weeks and have yielded more than 5,000 data points. The work has included not just the classic W. Murcott Afourer variety, but lesser known ones, as well.

Some storage-related mandarin flavor research, done by scientists elsewhere, “treated cold storage followed by warmer storage as a single regimen,” Obenland says. “We evaluated the effects of the cold-storage period separately from the effects of the warm storage that followed. This work is, to the best of our knowledge, unique among published scientific studies of mandarin flavor. We found that cold-storage temperatures influence flavor in some varieties, but not others.”

An example: The 10 to 17 taste testers that evaluated mandarins for the research reported that the flavor of W. Murcott Afourer fruit stored at 46 °F for 3 to 6 weeks, followed by a week at 68 °F, was “slightly superior” to that of fruit of that same variety stored at 32 or 39 °F, followed by a week at 68 °F. However, the taste testers did not discern a difference in the flavor of Owari variety mandarins that were stored at these regimens.

## What Makes Flavor?

In some experiments, loss of some flavor was attributed to changes in levels of a few specific chemicals. But the exact biological and chemical mechanisms underlying those changes are, for the most part, not well known and are a target of the team's research.

For instance, the scientists found that statistically significant changes in several flavor-associated chemicals occurred after W. Murcott Afourer mandarins, previously refrigerated at 41 °F, were brought out of the cold and kept at 68 °F.

For that study, Obenland and Arpaia tracked changes in more than two dozen natural chemicals—alcohols, aldehydes, esters, and terpenes, also known as “aroma volatiles”—to learn more about the exact role each may play in the fruit's flavor profile.

In brief, changes in a class of esters—the ethyl esters—occurred soon after the oranges were taken out of cold storage. Statistically significant increases in three of the four monitored ethyl esters (ethyl acetate, ethyl propanoate, and ethyl 2-methylpropanoate) took place within the first 24 hours after the fruits were moved from cold (41 °F) to warmer (68 °F) stor-



Postdoctoral researcher Nittaya Ummarat sets up a device that traps aroma volatiles from mandarin juice for analysis by gas chromatography.

age. What's more, statistically significant increases in the fourth ethyl ester, ethyl 2-methylbutanoate, took place a day later.

Interestingly, all four ethyl esters are thought to contribute to a sweet, fruity aroma, which may have a role in what we perceive as flavor.

However, in the case of these ethyl esters, it may be possible to have too much of a good thing. That's because high levels of these four compounds are suspected of contributing to off-flavor, Obenland says. The team's ongoing studies may help clarify the impact of these ethyl esters in relation to other natural compounds.

Obenland and Arpaia also looked at two other important groups of flavor-related chemicals—sugars and acids. Their data suggests that increased concentrations of esters and alcohols “may have a greater influence on mandarin off-flavor than the ratio of sugars to acids,” Obenland says.

In new research, the scientists are looking at how flavor of W. Murcott Afourer and nine other mandarin varieties changes after the conventional wax-coating process. The coating limits movement of oxygen into the fruit. That can, in turn, lead to buildup of some flavor chemicals. But Obenland says a change in oxygen levels inside the fruit doesn't explain every change in flavor.

In these experiments, mandarin varieties that are the least affected by the coating, or not affected at all, may be of the most interest. Their flavor secrets might help make mandarin oranges—already delectable—even better.

The research team's findings were published in *Postharvest Biology and Technology* in 2011 and 2013.—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](http://www.nps.ars.usda.gov).*

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NITTAYA UMMARAT (D3263-1)

Biological science technician Paul Neipp obtains a mandarin juice sample for analysis of aroma volatiles.

# Experimental Website Helps Teens Eat More Veggies

**S**mart, cool, and sometimes funny, the animated cartoon characters “Darius,” “Kyle,” “Lynn,” and “Maria” were created to help adolescents make better food choices and to be less sedentary. The energetic, likeable cartoon teens are an exclusive feature of “Teen Choice: Food and Fitness,” an experimental, science-based website co-developed and evaluated by nutrition and behavioral science researchers Karen Cullen and Debbe Thompson; Richard Buday and his Houston, Texas, team of animators, scriptwriters—and more—at Archimage, Inc.; along with other research colleagues and hundreds of interested tween and teen volunteers.

To create an engaging, teen-friendly site, the scientists first sought the input of about 100 young volunteers, who shared their ideas about how to make the site easy to navigate, informative, and relevant to real-world obstacles they had encountered in trying to develop better eating habits and to become more physically active.

In a follow-up study, another 400 teen volunteers evaluated the new site. They were asked to visit this Web venue at least once a week for 8 weeks and to peruse the handy information about food and fitness—including easy recipes in the “Teen Kitchen,” and a simplified “Healthy Eating Calculator” for customizing food-group and physical-activity recommendations.

In addition, each volunteer was asked to set a personal goal for becoming more nutrition savvy or more physically active, and to revisit the goal—and their progress towards attaining it—every week.

Importantly, the log-on rate for volunteers averaged an impressive 75 percent, a level of participation that’s regarded as “high” for education-focused Internet sites. One volunteer said the site “wasn’t like those boring online textbooks or anything. But it still got the job done, which I liked. And it didn’t waste time with telling us stuff we already knew.”

The researchers also found that more of the volunteers who had access to the site’s interactive options reported eating three or more servings of veggies in the past week than did volunteers whose access didn’t include the interactive features. Those features included the cartoon videos, interactive goal-setting and problem-solving aids, and a blog.

The teens’ move toward healthier food choices is important: According to Cullen, getting kids to eat more veggies is apparently more difficult than getting them to

The Children’s Nutrition Research Center is a joint venture of USDA’s Agricultural Research Service, Baylor College of Medicine, and the Texas Children’s Hospital. The “Teen Choice” studies are part of the nutrition center’s ongoing research to develop effective strategies for preventing childhood obesity. Currently, more than 30 percent of America’s young are overweight or obese.—By [Marcia Wood, ARS](#).

*This research, funded by ARS and USDA NRI grant #2007-55215-17998, is part of Human Nutrition, an ARS national*

ARCHIMAGE, INC.



Scientists evaluating the “Teen Choice: Food and Fitness” nutrition-education website found that volunteers who had access to science-based videos featuring cartoon personalities “Lynn,” “Kyle,” “Maria,” and “Darius” (shown here, left to right) reported eating more veggies than did other volunteers in the same study.

eat more servings of fruit, for instance.

Cullen and Thompson, both based in Houston at the Children’s Nutrition Research Center, want to make the site publicly available. In the meantime, they’ve documented the development and evaluation of the site in peer-reviewed scientific articles that were published in 2012 in the *Journal of Medical Internet Research* and in 2013 in *Health Education Journal*.

program (#107) described at [www.nps.ars.usda.gov](http://www.nps.ars.usda.gov).

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# Latest Update of USDA's Nutrient Database Released

The 2014 update of the Agricultural Research Service's USDA National Nutrient Database for Standard Reference, Release 27, has been launched. The database is managed by scientists with the ARS Nutrient Data Laboratory in Beltsville, Maryland, and is the major authoritative source of food-nutrient profiles in the United States.

The laboratory is part of the Beltsville Human Nutrition Research Center and provides free electronic access to the National Nutrient Database via the World Wide Web. The entire database is also available in a variety of formats that can be downloaded free of charge for use on personal computers and for upload into professional software programs.

Each year, new food-nutrient profiles are added to the database, and existing nutrient profiles are updated using data generated by USDA-ARS through its National Food and Nutrient Analysis Program and other collaborations, including with the food industry. The analytical cost to produce a food-nutrient profile for a single commercially processed food, representing multiple brands from multiple locations, is estimated to be up to \$50,000, according to experts.

The Internet "dashboard" that users see after launching the online version of the database has been reorganized so that users can more easily select and view food-nutrient profiles from individual food groups. Another new consumer-oriented upgrade allows users to look up the amount of a specific nutrient within any one of the database's thousands of food items. For example, a person whose doctor recommends eating more dietary fiber might sort all foods by fiber content from highest to lowest. A consumer who wants to increase calcium intake might sort by calcium content of foods.

To use the new feature, go to the website listed below and then click on "Nutrient

List" from the menu options. Next click "Select nutrient," and a drop-down list of more than 100 nutrients will appear, such as protein, calcium, carbohydrate, cholesterol, fats, caffeine, and vitamin K. After selecting a single nutrient, choose either "All Foods" or one from the "Food Groups" list. For example, a vegetarian may want to run a report on the protein content of foods ranked from highest to lowest, but only from among the plant food groups. Last, choose "Nutrient Content"

source where consumers can plan, track, and assess their diets based on the Dietary Guidelines for Americans.

The Nutrient Data Laboratory has continued to make updates to sodium content as a line item in food-nutrient profiles to stay abreast of the food industry's ongoing, self-regulatory efforts to reduce the salt content in foods. Other current changes include additions and updates of foods from both full-service and casual-dining restaurants.

STEPHEN AUSMUS (D2444-6)



The 2014 update of the USDA National Nutrient Database for Standard Reference is now available to the public through multiple interactive online programs and mobile apps, most notably USDA's [ChooseMyPlate.com](http://ChooseMyPlate.com), a free interactive website for creating a customized healthy dietary plan.

to sort by, "Household" as your measure, and hit "Go."

Users include the food industry, government nutrition-monitoring groups, commercial weight-loss enterprises, institutional food-service operations, university and foreign investigators, and more. The data is also downloaded and imported into multiple interactive online programs and mobile apps, most notably USDA's [ChooseMyPlate.com](http://ChooseMyPlate.com), a free re-

The database can be accessed by going to [ndb.nal.usda.gov](http://ndb.nal.usda.gov).—By [Rosalie Marion Bliss, ARS](http://RosalieMarionBliss.ARS).

*This research is part of Human Nutrition, an ARS national program (#107) described at [www.nps.ars.usda.gov](http://www.nps.ars.usda.gov).*

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## The Greatest Show on Turf

Groundbreaking ceremony November 15, 2013, at the U.S. National Arboretum (USNA) for the “Grass Roots” exhibit. **From left to right:** Scott Aker, USNA head of horticulture; Kevin Morris, president of the National Turfgrass Federation; Zoe ClarkWest, Rain Underground (the landscape architecture firm that designed the exhibit); Dr. Colien Hefferan, then director of the USNA; Geoffrey Rinehart, “Grass Roots” exhibit coordinator; and Michael Stachowicz, National Park Service National Mall turf grass manager.

**A**gricultural research is often thought of in terms of fresh produce, like lettuce, corn, apples, and other nutritious foodstuffs. However, turf is another agricultural commodity deserving of research and improvement.

Agricultural Research Service scientists are working diligently to improve turf grass as a commodity. Along with this research is a new program called “Grass Roots,” a 4-year initiative that focuses on

turf grass—its uses, management, benefits, and value.

### Research at Its Roots

At ARS’s U.S. National Arboretum, plant geneticist Scott Warnke and his colleagues are using molecular genetics to determine the genetic workings of turf grasses like creeping bentgrass. Creeping bentgrass is one of the species best adapted to use on golf course fairways and putting greens because of its tolerance to low

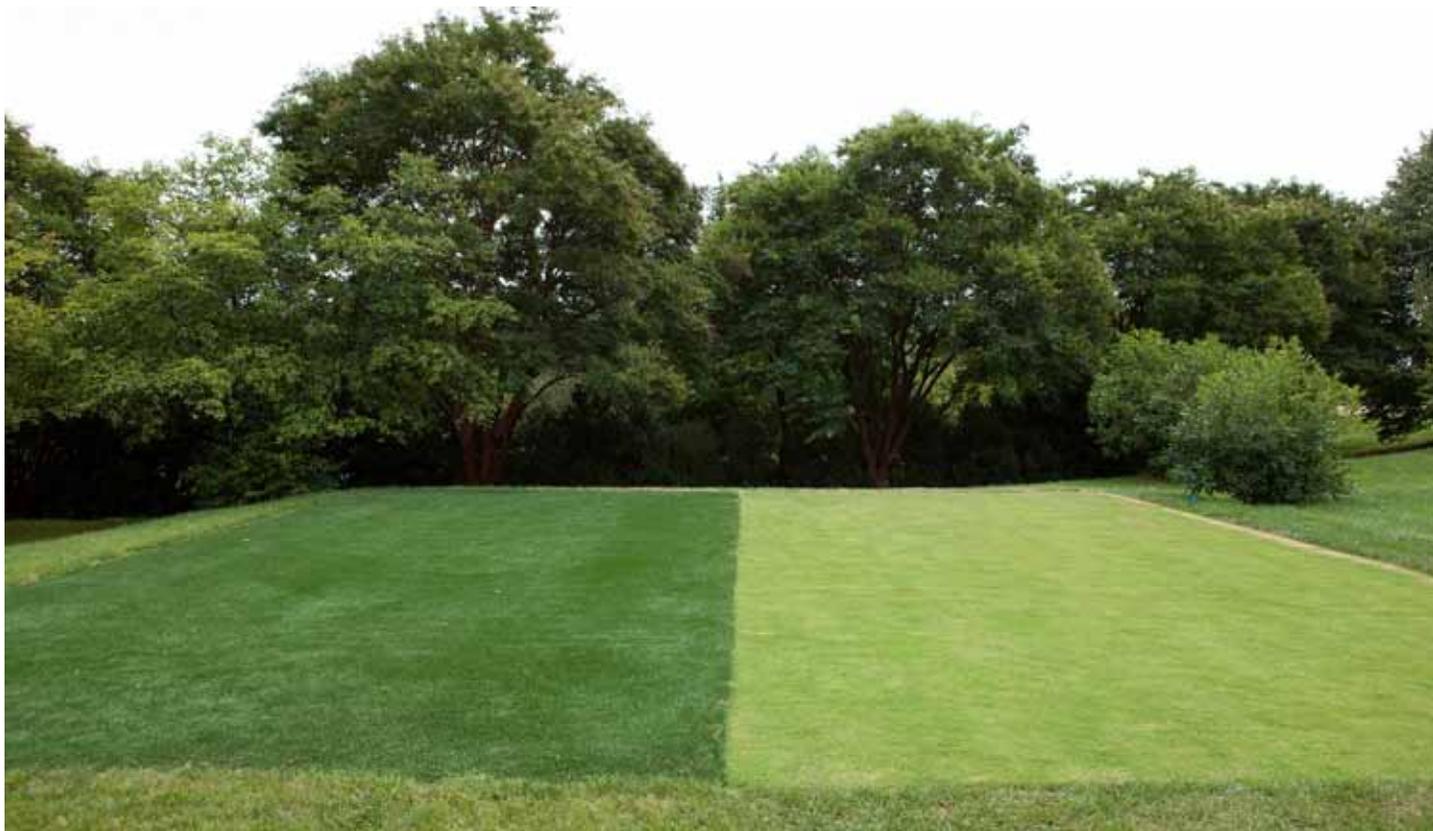
mowing. Low mowing causes most other grasses to burn under the intensity of the summer sun.

Warnke, who is in the Arboretum’s Floral and Nursery Plants Research Unit in Beltsville, Maryland, and his collaborators at Rutgers University and the University of Massachusetts completed the first linkage map for creeping bentgrass.

“The new linkage map will help eventually in marker-assisted breeding of economically important traits, like disease resistance to dollar spot and brown patch, which are common diseases of bentgrass,” says Warnke. “This type of research will help scientists develop improved turf grass germplasm that requires less pesticide, fertilizer, and water. This germplasm will be released to plant breeders at public and private institutions for further development and eventual release of new cultivars.”

The new “Grass Roots” exhibit at the U.S. National Arboretum features examples of many uses of turf grass, including a small demonstration golf hole (in the background) built to professional specifications.





The sports field display features a side-by-side comparison of an artificial field (left) and Latitude 36 bermudagrass, commonly used for new athletic fields. **Below:** “Grass Roots” intern Reuben Weiser lifts a piece of Zenith zoysiagrass during sod installation for the exhibit while intern Megan Wiemer (background) obtains more sod.

The genus *Agrostis*, commonly known as the bentgrasses, is an extremely diverse and highly outcrossing genus. Only 5 species of the more than 200 in the genus are used as turf grass in the United States: colonial (*Agrostis capillaris*), velvet (*A. canina*), dryland (*A. castellana*), redtop (*A. gigantea*), and creeping (*A. stolonifera*). These species have very different characteristics. One of the keys to improving important traits in *A. stolonifera* would be to transfer these traits from another species. For example, *A. castellana* is far better at withstanding drought and disease, but is less suitable for use as a golf turf than *A. stolonifera*.

This research, and other work similar to it, has led to the “Grass Roots” initiative, a collaborative effort between ARS’s U.S. National Arboretum and the National Turfgrass Federation. The program consists of five components: an outdoor interactive exhibit; extensive information on the Web that complements the outdoor exhibit; workshops, product demonstrations, and symposia; the development of the National Greenscape Corridor, a cooperative effort linking the National Mall, Arlington

National Cemetery, and the National Arboretum; and a tabletop book titled “Personal Grass Roots,” featuring stories from many well-known people about their early childhood experiences with lawn mowing or groundskeeping and how this shaped their future.

#### Grass Roots Outdoors

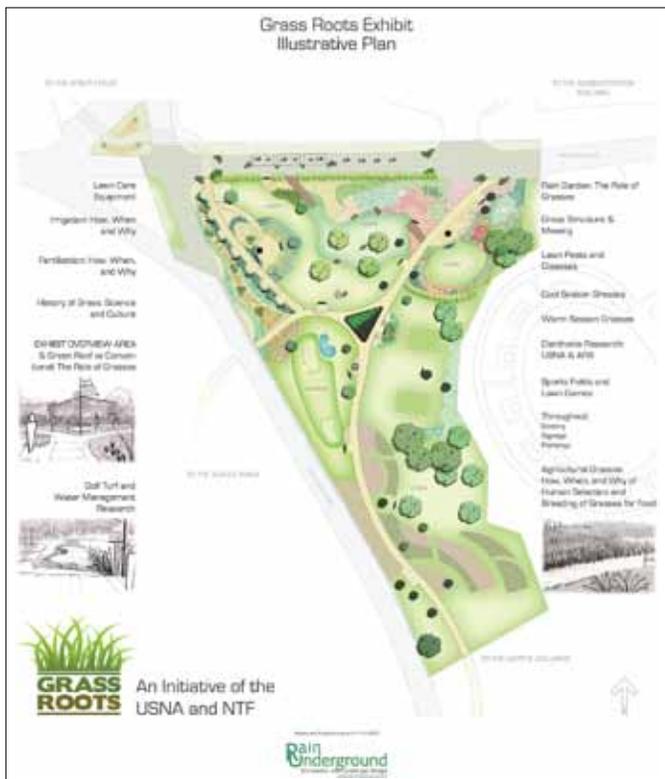
The outdoor exhibit design was developed by a team of people led by head horticulturist Scott Aker, which included Arboretum staff and turf grass researchers. The design and the exhibit language were then reviewed by an industry advisory panel.

“The Arboretum began construction of the exhibit in November 2013, and it will open by the fall of 2014,” says research leader and plant geneticist Margaret Pooler.

The outdoor exhibit is a 1-acre space, adjacent to the Arboretum’s administration building and visitor center, located in Washington, D.C. There are 14 stations, in-

cluding golf turf, irrigation, fertilization, maintenance equipment, lawn history,





**Left:** The “Grass Roots” exhibit features 12 displays demonstrating the many uses of turf grass and includes a “History of the Lawn” walk, a small golf hole, a rain garden, and various grasses grown as agricultural crops. **Right:** Grass Roots intern Megan Wiemer waters the golf green exhibit.

sports fields and lawn games, lawns of the future, turf diseases and pests, grass types, and a “green roof.”

The green roof station is the centerpiece of the exhibit and includes a welcome station that provides an overview of the “Grass Roots” exhibit. The station compares runoff between a conventional roof and a green roof—one that contains turf grass plants—and shows that the green roof absorbs and cleanses water and also cools the temperature underneath the structure.

A station called “Danthonia” will inform visitors about the development of a potentially new turf grass species and how science plays a major role in improving it. *Danthonia spicata* is a native grass common on the East Coast of the United States. Research on *Danthonia* is focused on its unique reproductive biology and its ability to tolerate low-fertility soils. “Future turf grasses will have to be more drought tolerant, pest resistant, and persistent under harsh environmental conditions, and *Danthonia* has all these traits,” says Warnke.

The irrigation station conveys a message any visitor can use: “Save water.” It features information to help consumers learn about smart water usage, including a display of

different water-efficient sprinkler heads. “These tips can help visitors save water and result in healthier turf,” says Pooler.

The sports turf station will introduce visitors to the complexities of sports turf and provide space to play various lawn games like croquet and golf. Many people think of sports turf as just a large field of grass, but sports turf is a complex, highly engineered system that protects people and the environment. “Sports turf can act as a buffer between sports facilities and the underground water supply,” says Warnke. “It acts as a natural filtration system, taking in pollutants and nutrients before they reach the underground water table.”

#### National Greenscape Corridor

The greenscape corridor encompasses three distinct locations that display the different uses of turf: Arlington National Cemetery, the National Mall, and the U.S. National Arboretum.

Each location demonstrates a specific function. Arlington National Cemetery honors service men and women by providing a final resting place and quiet, grassy spaces for reflection and remembrance. The National Mall provides an expanse of turf for residents and visitors to play,

assemble, or just enjoy a wonderful setting in the Nation’s Capital. The U.S. National Arboretum conducts the science of turf grass development and management and develops new technologies in the turf grass field.

Other locations in Washington, D.C., such as parks, schools, colleges, and golf courses, to name a few, could possibly become part of the National Greenscape Corridor, eventually linking popular tourist destinations through these common greenscapes.

“This initiative illustrates how we can connect ARS science to something that is familiar to just about every person in America.” says Pooler.—By **Sharon Durham, ARS.**

*This research is part of Pasture, Forage, and Rangeland Systems (#215) and Plant Genetic Resources, Genomics, and Genetic Improvement (#301), two ARS national programs described at [www.nps.ars.usda.gov](http://www.nps.ars.usda.gov).*

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# Assessing Cotton Fiber Quality From a Tiny Sample

At a time when there is an uptick in U.S. cotton exports, it's not surprising that the Agricultural Research Service's Southern Regional Research Center (SRRC), located in New Orleans, Louisiana, upgraded its cotton textile pilot plant. There, materials engineer Christopher Delhom has successfully "reimagined" model spinning equipment, outfitting it to be able to spin as little as 30-60 grams (1-2 ounces) of cotton fibers grown from selected experimental seeds. The tiny batch of fibers can be quickly tested to gauge the new varieties' fiber performance and viability for use on standard equipment and in textiles.

Says James Rodgers, who heads the Cotton Structure and Quality Research Unit at SRRC, "We can use this miniature processing to accomplish in 2 weeks what would take months to test on a full-scale industrial fleet of textile machinery."

Cotton grows and performs differently based on region and seed genetics. Large-scale processing equipment is customized to accommodate those regional features. But a small change in seed breed can greatly affect the quality of the cotton's fibers during processing and through to finished fabric. Getting timely information about the processing performance of newly bred cotton varieties is key.

"The machinery is capable of taking a very small fiber sample grown from test seeds and processing those fibers all the way through the milling process into yarn and fabrics," says Rodgers. In the past, this kind of testing took place at a pace of less than 200 samples per year, using samples weighing from 25 to 150 pounds each.

Delhom knew that cotton breeders from academia, industry, and even governments were waiting up to a year for standard facility equipment to free up before fibers grown

from large samples of their experimental seeds could be tested. "New-variety fiber samples need to be spun into yarns and tested for their viability for use in fabrics," says Delhom. The fast turnaround of the "miniature spinning" approach allows for prescreening of promising varieties to come up with a subset of candidate varieties to test later on full-scale machinery.

The pilot plant's miniature-spinning equipment is being used to process fiber samples in the National Cotton Variety Trials, which is an ARS-led national trial of varieties involving U.S. breeders. "We are helping producers decide which commercial varieties to plant," says Delhom.

Rodgers notes that the fiber test results are very important to breeders, because they need to know which varieties to focus their efforts on. They want to know how the fibers will act inside the machinery used to make textiles and clothing.

Delhom's team is also miniature-spinning a large number of samples for an ARS geneticist and, on the gin side, for an ARS agricultural engineer to evaluate the effect of various gin and harvester settings and gin trials.—By [Rosalie Marion Bliss, ARS](#).

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

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MELISSA DUNN (D3302-1)

In the textile mill at the Southern Regional Research Center, materials engineer Chris Delhom (left) and technician E.J. Deshotel examine yarn packages in front of the miniature spinning equipment.

# A Steady Pace of Cellulosic Ethanol Research in Peoria



Biochemical engineer Bruce Dien and microbiologist Loren Iten use mini-batch reactors to pretreat biomass at high temperatures to prepare it for fermentation to ethanol. Here, they are pretreating corn fiber, but the process is the same for switchgrass.

At the Agricultural Research Service's Bioenergy Research Unit in Peoria, Illinois, field work and bench investigations keep ARS scientists on the scientific front lines of converting biomass into cellulosic ethanol.

For instance, one recent research focus has been on determining how switchgrass plant maturity at harvest affects ethanol yields. Chemical engineer Bruce Dien led a study that evaluated samples of two different switchgrass varieties that were harvested at three different points in plant development and then pretreated with diluted ammonia. This approach is similar to a treatment used sometimes for enhancing forage quality.

Dien's team observed that even though plant glucose and ethanol conversion efficiencies decreased as the plants matured, overall ethanol yields were relatively consistent—between 176 and 202 liters per metric tonne (42 and 48 gallons per ton) of biomass. After evaluating the different yields obtained from the two varieties, the scientists concluded that biomass producers could optimize ethanol production from their crops by planting the variety Kanlow—a lowland switchgrass type—and harvesting either at midseason or after a frost. Results from this

study were published in *Environmental Technology* in 2013.

Chemist Michael Bowman led another study that focused specifically on switchgrass xylans. Xylans are polymer chains composed primarily of the sugar xylose. Bowman studied xylan levels at three different stages in switchgrass development to see whether xylan structures change as the plant matures.

Bowman determined that structural features of xylan remained the same throughout different stages of maturity, even though the amount of xylan differed from one stage to another. This is good news for biorefiners because it suggests that they can use the same enzyme mix to break down xylans for all switchgrass biomass, no matter when the crop is harvested. Results from this study were published in *Metabolites* in 2012.

Molecular biologist Ron Hector, meanwhile, led work on the microorganisms needed to ferment xylose into ethanol. Xylose is more difficult to convert to ethanol, compared to glucose. Scientists already knew that an enzyme called “D-xylose isomerase,” or XI, is one of several enzymes required to convert xylose into ethanol. But, to date, it has been difficult to harness XI's conversion potential because of the difficulty of expressing XI in yeast strains and other technical issues.

However, Hector and his colleagues isolated the XI enzyme from several different rumen and intestinal bacteria and used them to engineer yeast strains that were able to ferment xylose into ethanol. Then they took the most promising yeast strain from this first round of trials—which contained the XI enzyme from the rumen bacterium *Prevotella ruminicola*—and improved its growth and fermenting capacities through further adaptations.

The result was a yeast strain that grew almost four times faster than other strains that contained XI enzymes and could produce ethanol at significantly greater yields than other yeasts engineered to ferment xylose to ethanol.

The scientists published their findings in *Biotechnology for Biofuels* in 2013, and a U.S. patent was recently issued for the XI enzyme described in this article.—By [Ann Perry, ARS](#).

*This research is part of Biorefining, an ARS national program (#213) described at [www.nps.ars.usda.gov](http://www.nps.ars.usda.gov).*

*To reach the scientists mentioned in this story, contact Ann Perry, USDA-ARS [Information Staff](#), 5601 Sunnyside Ave., Beltsville MD 20705-5128; (301) 504-1628, [ann.perry@ars.usda.gov](mailto:ann.perry@ars.usda.gov).\**

# Using Location Information To Improve Literature Searches

Agricultural Research Service ecologist Jason Karl is creating new options for helping researchers to conduct literature searches that go beyond using traditional search terms such as keywords or authors. With the help of a diverse team of collaborators, he has developed a search engine called “JournalMap” that uses research locations and physical site variables to identify scientific papers of interest.

Articles in the JournalMap citation index are “geotagged” based on locations reported in the study and then plotted on a world map. This means that scientists can use JournalMap to search for environmental literature thematically and geographically just by selecting a location on a map.

This new approach to combing through scientific literature can help researchers find published research and data for their investigations in similar ecosystems where such information may be lacking. In some cases, simply identifying the locations of previous research can provide enough

information to obtain pertinent ecological data from online geospatial databases.

The environmental factors tagged in JournalMap include climate variables such as daily temperatures during the growing season, average annual precipitation, and aridity index; landform characteristics, such as elevation and slope; soil characteristics, such as surface texture and depth; and types of land cover.

Karl and his collaborators are also working with Taylor & Francis, a publisher of more than 1,600 journals, to build literature geotagging into the publication process and to enable geographic literature searching of entire journal archives. Initially, this effort focused on geotagging the archives of three journals, including the *Journal of Natural History*, which has been published since 1838. The partnership now includes automatically geotagging articles when they are submitted for publication and standardizing the way locations are reported. Through this partnership, Karl

and his collaborators are also promoting location-reporting standards and article geotagging with other scholarly publishers.

The JournalMap citation index currently contains more than 10,000 published papers from around 600 journals, with more articles being added regularly. Karl and his collaborators are continuing to refine JournalMap by expanding the content of available journals and papers. Authors and researchers are also able to upload their own geotagged articles to the JournalMap citation index and create their own georeferenced article collections on the website, [www.journalmap.org](http://www.journalmap.org).

Karl, who works in the ARS Range Management Research Unit in Las Cruces, New Mexico, partnered with the Idaho Chapter of the Nature Conservancy and Web application developers at The Other Firm to develop JournalMap. The researchers published a report on the development of the citation database in *BioScience* in 2013.—By [Ann Perry, ARS](#).

*This research is part of Pasture, Forage, and Rangeland Systems, an ARS national program (#215) described at [www.nps.ars.usda.gov](http://www.nps.ars.usda.gov).*

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JournalMap is a new search engine developed by ARS scientists and collaborators. Users can click on a geotag and be taken to a list of scientific papers related to that geographical area.



(D3305-1)

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

### [San Joaquin Valley Agricultural Sciences Center, Parlier, California](#)

3 research units ■ 112 employees

### [Yakima Agricultural Research Laboratory, Wapato, Washington](#)

1 research unit ■ 44 employees

### [Las Cruces, New Mexico](#)

2 research units ■ 43 employees

### [Roman L. Hruska U.S. Meat Animal Research Center, Clay Center, Nebraska](#)

4 research units ■ 118 employees

### [Lincoln, Nebraska](#)

2 research unit ■ 69 employees

### [Children's Nutrition Research Center, Houston, Texas](#)

1 research unit ■ 7 employees

### [National Center for Agricultural Utilization Research, Peoria, Illinois](#)

7 research units ■ 254 employees

### [Southern Regional Research Center, New Orleans, Louisiana](#)

6 research units ■ 160 employees

### [National Center for Cool and Cold Water Aquaculture, Leetown, West Virginia](#)

1 research unit ■ 34 employees

### [Raleigh, North Carolina](#)

4 research units ■ 60 employees

### [Henry A. Wallace Beltsville Agricultural Research Center, Beltsville, Maryland](#)

27 research units ■ 806 employees