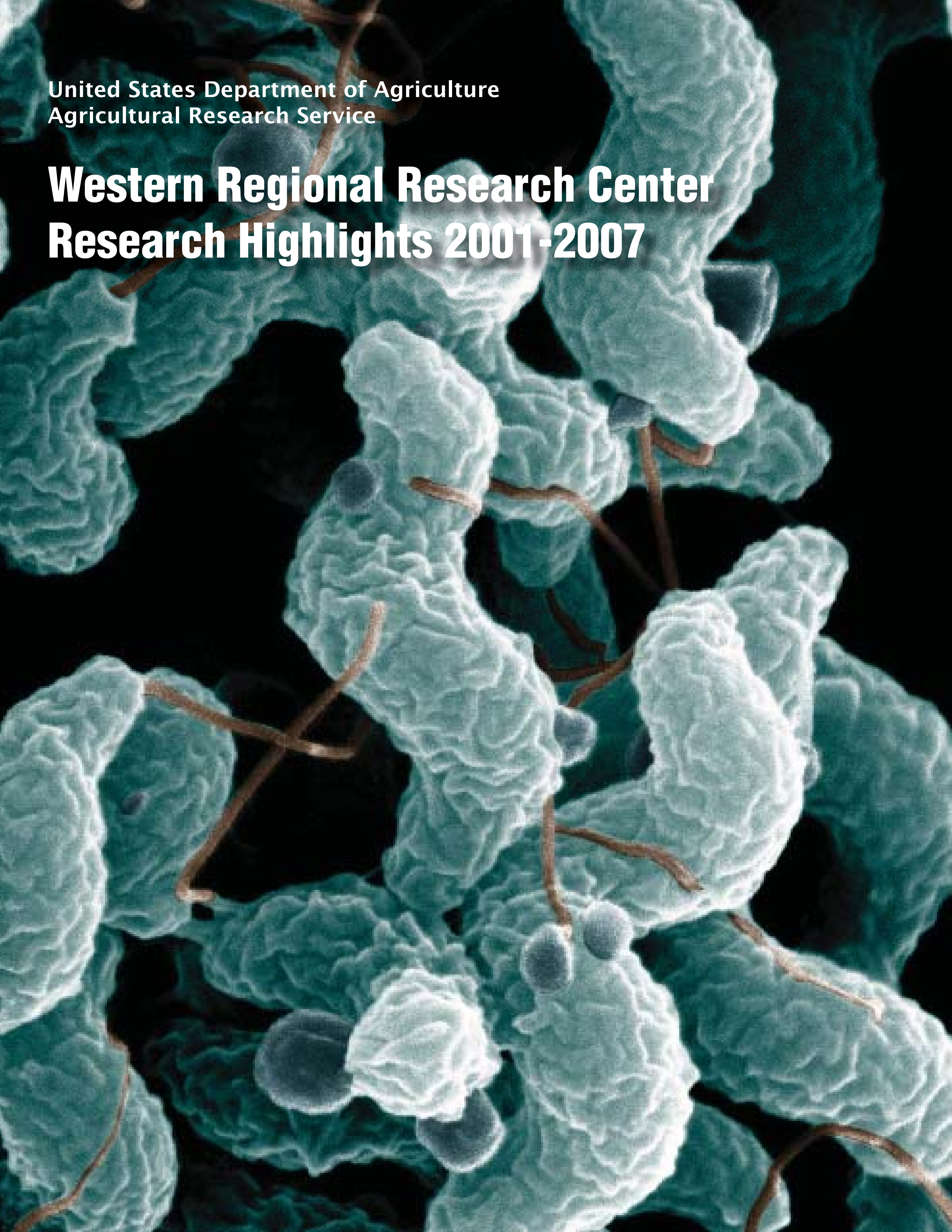
United States Department of Agriculture
Agricultural Research Service

Western Regional Research Center Research Highlights 2001-2007



Foreword

In the pages to follow, you'll find articles about the work of the Western Regional Research Center in Albany, California—the Agricultural Research Service's largest research laboratory in the western United States. The articles were printed in *Agricultural Research,* the magazine of the Agricultural Research Service.

For more than 60 years, the Western Regional Research Center has conducted research that boosts the quality of foods and fibers and broadens use of agricultural products and coproducts.

The Center's mission is three-fold:

- To enhance the healthfulness of foods by understanding food components that promote human health and well-being and by studying ways to combat harmful microorganisms or other contaminants.
- To conduct research that leads to new foods—and now, industrial products and biofuels—from agriculture.
- To protect and improve the quality of the environment by developing biological controls and other Earth-friendly ways to control pests.

In accomplishing these objectives, we develop and apply new technologies in agricultural genomics, proteomics, and biotechnology. We collaborate with scientists in academia, other agencies, and the private sector, and transfer our technologies to an array of commercial partners.

This compilation is a great way to learn more about the Western Regional Research Center, the Agricultural Research Service, and the more than 225 scientific staffers who conduct the Center's research. I hope this publication will give you a taste of Center accomplishments and give you some ideas about how you can interact with us. We're always interested in hearing from potential research partners, as well as prospective employees and others interested in our leading-edge research programs. We value your comments and suggestions.

I encourage you to visit us here at our headquarters in the beautiful San Francisco Bay Area. Please contact me to arrange a visit.

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Cover: ***Campylobacter*** **bacteria are the number-one cause of food-related gastrointestinal illness in the United States. To learn more about this pathogen, ARS scientists at the Western Regional Research Center are sequencing multiple** ***Campylobacter*** **genomes. This scanning electron microscope image shows the characteristic spiral, or corkscrew, shape of** ***C. jejuni*** **cells and related structures. (Magnified about 100,000x.) Photo by De Wood; digital colorization by Chris Pooley. (K11505-1)**

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Issued November 2008

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CITRUS COMPOUND

Ready To Help Your Body!

SCOTT BAUER (K7226-29)

These days, juicy, delicious oranges are practically synonymous with vitamin C. But did you know that America's favorite fruit also provides healthful natural compounds called limonoids? In laboratory tests with animals and with human cells, citrus limonoids have been shown to help fight cancers of the mouth, skin, lung, breast, stomach, and colon.

Now, ARS scientists in northern California—led by chemist Gary D. Manners of the Western Regional Research Center in Albany—have uncovered new details about these compounds. Their research has demonstrated that our bodies can readily access a limonoid called limonin, and all its health-imparting properties, each time we bite into a citrus slice or drink a glass of orange juice, for instance. This is the first time this bioavailability has been shown in humans.

"Limonin is what remains after our bodies cleave a glucose, or sugar molecule, from limonin's parent compound, limonin glucoside," says Manners. "Limonin glucoside is present in citrus and citrus juices in about the same amount as vitamin C."

JACK DYKINGA (K5919-1)

Chemist Shin Hasegawa (retired) prepares to analyze limonoids in orange juice.

Though the similar-sounding names of these limonoids may be confusing, their positive impact on our health is becoming clearer with every experiment.

In some individuals, limonin remains in the bloodstream for up to 24 hours—an impressive length of time—Manners and colleagues found. This longevity, or persistence, may help explain some limonoids' ability to fight cancer cells, which, if not continuously suppressed, may proliferate.

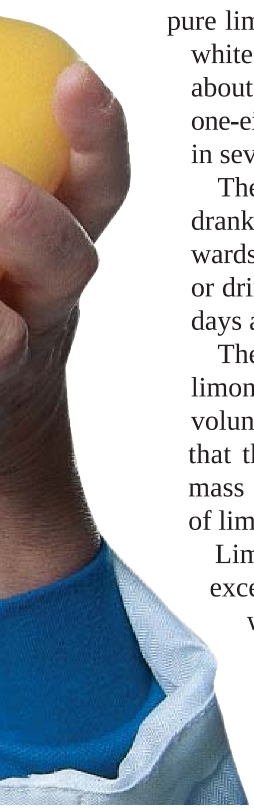
The findings are good news for people who like oranges and the other appetizing citrus fruits rich in limonoids. The world's citrus-juice processors could also benefit; they could extract an estimated 300,000 pounds of these chemicals from peels, seeds, and other processing leftovers each year.

Today, those leftovers, called citrus molasses, are sold as a low-cost ingredient in cattle feed. But thanks to a patented, ARS-developed process, these chemicals can be extracted from citrus molasses and used to fortify foods or beverages.

Citrus limonoids have been shown to help fight cancers of the mouth, skin, lung, breast, stomach, and colon.

Test Targets Limonin Glucoside

For the bioavailability experiment, 16 healthy men and women volunteers downed a dose of pure limonin glucoside. The compound, a white, tasteless, odorless powder, was dissolved in about 4 ounces of water. Doses ranged from about one-eighth to one teaspoon. That's equal to the amount in seven glasses of orange juice.



The participants gave blood samples before they drank the beverage and 3, 6, 12, and 24 hours afterwards. To avoid skewing test results, they didn't eat or drink any citrus products for 3 days before and 3 days after taking part in the study.

The scientists looked for traces of limonin—the limonin glucoside byproduct or metabolite—in the volunteers' blood plasma. The laboratory procedure that the researchers used, liquid chromatography-mass spectrometry, can detect very small amounts of limonin.

Limonin showed up in the plasma of all volunteers except one. For most volunteers, the concentration was highest within 6 hours after they drank the special beverage. Five volunteers still had traces of limonin after 24 hours. That's an indication of the compound's staying power, an important consideration, especially if limonin glucoside were to be used as a pharmaceutical, for example.

In contrast, some natural compounds are bioavailable for shorter periods and have to be taken more than just once a day. An example: the good-for-you phenols in green tea last only 4 to 6 hours.

In this study, Manners collaborated with Andrew P. Breksa III of the Western Regional Research Center; Thomas S. Schooch, formerly of the center; retired ARS chemist Shin Hasegawa, a pioneering investigator of these citrus biochemicals; and Robert A. Jacob, formerly a chemist with the ARS Western Human Nutrition Research Center in Davis, California, and now retired. The group published their findings in a 2003 issue of the *Journal of Agricultural and Food Chemistry*. The Florida Department of Citrus helped fund the work.

Can Limonin Lower Cholesterol?

There's more to come from the California team. Manners and Breksa, along with chemist Darshan S. Kelley and molecular biologist Susan J. Zunino—both of the Davis nutrition center—are gearing up for a first-of-its-kind study of the cholesterol-lowering effects of limonin.

In 2000, Manners, Hasegawa, and their Canadian coauthors reported that limonin may be among the citrus-juice compounds that lower cholesterol. In lab tests, they found that human liver cells produced less apo B—a compound associated with higher cholesterol levels—when exposed to limonin.

Preliminary results of the new cholesterol study are expected later this year. This investigation may provide more details about the health advantages of citrus and greater incentive for us to enjoy these sunny, delectable fruits and juices even more often.—**By Marcia Wood,** ARS.

This research is part of Quality and Utilization of Agricultural Products (#306) and Human Nutrition (#107), two ARS National Programs described on the World Wide Web at www.nps.ars.usda.gov.

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Finding New Uses for Fish Byproducts

Alaska's fish-processing industry produces more than 2.2 billion pounds of fish byproducts every year—the weight equivalent of nearly 10,000 blue whales.

Large processors often convert these byproducts into fishmeal or fish oil, which are generally sold for meager profit. There is little economic incentive for smaller or at-sea processors to do the same, so they generally return byproducts to the ocean, where they are consumed by marine creatures.

But is this the best use of these products? Could fish processors be throwing money into the sea?

Clockwise from upper left: a sheet of gelatin made from Alaskan pollock fish skin, pollock fish skins used for gelatin extraction, shrimp feed pellets made with dried salmon hydrolysate, and salmon hydrolysate powder.

It's possible, say ARS researchers in Fairbanks, Alaska, and Albany, California. Their research demonstrates that fish byproducts have marketable potential.

Take fish livers, for example. They aren't a very popular menu item in the United States. Kids rarely clamor for fish liver snacks, and few people include them in their regular diet. But do they have potential as a dietary supplement?

Peter J. Bechtel, a food technologist in the Subarctic Agricultural Research Unit at Fairbanks, says yes. Working with colleagues, he found that livers from different species of fish all showed positive nutritional properties, but to varying degrees.

"Not all cold-water marine oils are created equal," says Bechtel. Oils from different marine species have different amounts of omega-3 fatty acids, the long-chain fatty acids that have nutritional benefits for humans.

Cold-water marine fish tend to have higher omega-3 content than many warm-water marine fish, which tend to have more omega-3 than freshwater fish. With omega-3 fatty acids of 20 percent or more, pollock and salmon rank at the top, he says.

In one study, the scientists compared livers from seven fish species harvested in Alaska—walleye pollock, pink salmon, bigmouth sculpin, Pacific halibut, arrow-tooth flounder, flat-head sole, and spiney-head rockfish—and examined their composition in terms of proteins and oils. The liver proteins of all the fish had high levels of essential amino acids. Liver lipid content—a strong indicator of omega-3—ranged from 3.3 percent for pink salmon to 50.3 percent for walleye pollock. Bechtel and his colleagues concluded that differences between species could enable development of unique ingredients for food or animal feed, allowing producers to target products to specific markets.

"Fish oil and protein supplements for humans can be made from high-fat livers. And low-fat livers, such as salmon, can be used as supplements for pets and livestock as well as humans," Bechtel says.

Studies conducted by Bechtel and scientists from the University of Alaska confirmed that protein powders made from cold-water marine byproducts could be used as feed ingredients for aquaculture or livestock. And the fish-based proteins compare favorably to products already used in food, dietary supplements, medical treatments, and animal feed.

Global demand for fish protein will exceed supply by 2016, according to the Juneau Economic Development Council. That group is working with the Alaska Department of Environmental Conservation, the Southeast Conference, and the Southeast Sustainable Salmon Fund to find alternatives for the "grind-and-dump" approach to seafood-processing leftovers.

Gelatin: From Fish to Film

Another study, conducted in collaboration with research leader Tara H. McHugh and her colleagues at the Western Regional Research Center in Albany, showed that gelatin recovered from fish skins can be processed into thin, pliable sheets, called films. The scientists made sample films from warm- and cold-water fish, evaluated their physical characteristics, and compared them to mammal-based gelatins.

Most gelatin is made from cattle and swine byproducts. Gelatin made from fish skins has a lower gelling point—about 46˚F to 50˚F, compared to 80˚F to 95˚F for mammals—and is liquid at room temperature, making it impractical for certain products. The scientists concluded that fish gelatins could not substitute universally for mammalian gelatins, but their lower gelling temperature might make them suitable—or superior—for use in products such as frozen desserts.

Experiments led by agricultural engineer Roberto de Jesus Avena-Bustillos, formerly with McHugh's group and now a collaborator based at the University of California-Davis, showed that the fish-derived gelatin films serve as a protective barrier against the damaging effects of moisture and oxygen.

"This suggests that the fish gelatins could be used to reduce water loss in refrigerated and frozen foods," says Avena-Bustillos. The fish-gelatin films "proved to be a better barrier to water vapor than the mammalian gelatins," he says, and offered better protection against oxidation. "Covering a gel-capsule-type medication with a thin coating of fish gelatin would likely slow down natural oxidation."

Working with Avena-Bustillos and his Albany colleagues, Fairbanks-based food technologist Cindy K. Bower added the antibacterial enzyme lysozyme to gels and films made from fish skin gelatins. The lysozyme actively inhibited bacteria—but it also reduced the strength of the gels by as much as 20 percent. Still, researchers concluded that the enhanced gels and films could provide an effective barrier to many bacteria and increase the shelf life of some food products.

"The idea of improving fish-skin gelatin with lysozyme is good but requires more study," Bower says. "Besides antimicrobial agents, we could also add flavorings and aroma compounds."

Expanding the seafood byproducts market with the impressive array of products that ARS scientists are inventing and investigating will enhance the value and scope of seafood-processing plants' product lines. In all, the research provides more options for fish processors, the potential for increased business, and more ways to make environmentally sound use of natural resources from the sea.

"Nobody's opposed to improving efficiency or making more money," Bechtel says. "Our work shows that there are a number of different ways to do that."—By **Laura McGinnis** and **Marcia Wood,** ARS.

This research is part of Aquaculture (#106) and Quality and Utilization of Agricultural Products (#306), two ARS National Programs described on the World Wide Web at www.nps.ars.usda.gov.

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Healthy Foods

COOKING UP TEMPTING, FAT-FIGHTING FOODS AND INGREDIENTS

Moist, chewy, all-natural apple bars can be made not only with apples, but also with delicious combinations of apples and other fruits.

Scientists at ARS are cooking up a storm of new, obesity-fighting foods. These foods are intended to help us prevent unwanted weight gain, yet still enjoy what we eat.

Take apple bars, for instance. These moist, chewy, all-natural snacks are packed with what is likely the richest, most intense apple flavors your taste buds have ever experienced. That's due, in part, to a patented process by which the bars are made. Apples are pureed, mixed with apple concentrate, and shaped, in a food-processing machine, into a bar that's about the size of a typical granola bar, but slightly slimmer.

The process compresses the flavors and nutrients of two freshly harvested apples into one handy bar for kids to take to school in their lunches or grown-ups to enjoy as an afternoon snack.

The good-for-you bars don't crumble, and they stay fresh without artificial preservatives. They're the newest additions to the line of all-fruit snacks from the laboratories of food technologist Tara H. McHugh. She's the leader of the ARS Processed Foods Research Unit at the Western Regional Research Center in Albany, California.

Gorge Delights of Hood River, Oregon, uses crisp, delicious apples from the region's picturesque orchards to make the bars and markets the nutritious treats under the "Just Fruit" label. GFA Brands, Inc., the Cresskill, New Jersey-based marketers for Earth Balance and Smart Balance brands, markets the bars under the Earth Balance name.

Just launched last year, the apple bars—and equally pleasing apple combos with a second fruit—are already showing up in natural-foods stores nationwide, according to GFA president Robert M. Harris.

Unlike other energy-bar type snacks, the apple bars contain no added sugar or sodium, Harris points out.

PEGGY GREB (D433-1)

Research leader Tara McHugh (left) and food technologist John Roberts prepare apple bars at the ARS Western Regional Research Center, in Albany, California.

That's also true of Earth Balance pear bars, another Gorge Delights product line that Harris's company markets.—By **Marcia Wood,** ARS.

This research is part of Quality and Utilization of Agricultural Products, an ARS National Program (#306) described on the World Wide Web at www.nps.ars.usda.gov.

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Science Update

Black Lentils Rich in New Nutrient

A lentil named for the Beluga caviar that it visually resembles has been found to contain a previously unknown anthocyanin. Many such color-imparting compounds naturally found in fruits and vegetables are thought to be health-promoting. Beluga black lentils are less well known in the United States than in Europe and Asia, where they are highly valued for their mild flavor and stores of protein, magnesium, iron, zinc, B vitamins, and dietary fiber.

The new anthocyanin's official name is delphinidin-3-O-(2-O-beta-D-glucopyranosyl-alpha-L-arabinopyranoside). Efforts are under way to evaluate Beluga black lentils and other legumes as potential ingredients in novel, nutritious snack foods. *Gary R. Takeoka, USDA-ARS Processed Foods Research Unit, Albany, California; phone (510) 559-5668, e-mail gary.takeoka@ars.usda.gov.* ✷

Nonfishy Sushi!

If you're one of those folks who don't relish a trip to the sushi bar to feast on raw seafood wrapped in rice and seaweed, help is on the way! Appealing alternatives in brightly colored, healthy wrappers made from familiar vegetables and fruits might soon be served at neighborhood sushi restaurants. These "American-style sushi" delicacies—sized, shaped, and sliced like typical sushi but made with innovative ingredients—could also be great for portion control for the weight-conscious.

The innovative new appetizers would include such combinations as curry-seasoned potatoes in a carrot wrap, diced roast pork and rice in a pineapple-apricot-ginger wrap, carrots and asparagus in a broccoli wrap, spicy tuna and rice in a tomato-basil wrap—even a mini-dessert cheesecake in blueberry or strawberry wrap. The wraps themselves are at least 75 percent vegetable or fruit, with other natural ingredients. Food industry experts and sushi chefs are helping experiment with wrap and filling combinations.

ARS has a patent pending on the technology used to manufacture these wraps and is collaborating with Origami Foods to introduce this product into the marketplace shortly. *Tara H. McHugh, USDA-ARS Processed Foods Research Unit, Albany, California; phone (510) 559-5864, e-mail tara.mchugh@ars.usda.gov.* ✷

STEPHEN AUSMUS (K11611-1)

Healthy food wraps made with colorful nutritious carrots or other vegetables can enliven sushi and other foods at markets and restaurants.

Campylobacter

Unmasking the Secret Genes of a Food-Poisoning Culprit

Microarrays, or gene chips, enable scientists to get a quick look at thousands of genes in a single experiment. Here, technician Sharon Horn monitors robotic equipment as it imprints *Campylobacter* microarrays on glass slides. Photo by Peggy Greb. (K11465-1)

The "juice" that always seems to leak out of those packages of fresh chicken you bring home from the supermarket can make a big mess on your kitchen counter. But more importantly, the juice can pose a hazard to your health. Nasty microbes called *Campylobacter jejuni* can live in that liquid and on the skin of fresh, uncooked poultry.

Thoroughly cooking chicken—by grilling, frying, roasting, or baking—kills this food-poisoning microbe. But if you accidentally splash some of the raw juice on food that you'd planned to eat uncooked, such as leafy greens for a fresh salad, you'd be wise to throw them out. Here's why: If the microbe takes hold on those greens, as it is very adept at doing, you might be in for a case of campylobacteriosis food poisoning that you won't soon forget.

Campylobacter is thought to be the leading cause of bacterial food poisoning in humans and is likely the perpetrator of more than 400 million cases of diarrhea every year. Though being careful when you handle raw poultry should help keep you safe, ARS researchers want to do more to zap this microbial menace before it reaches your home.

At Albany, California, scientists in the ARS Produce Safety and Microbiology Research Unit are making key advances in the international effort to clobber *Campylobacter*. The California team, based at the Western Regional Research Center, is focusing on *Campylobacter's* genes.

Why the interest in the microbe's genetic makeup? Because investigating genes may lead to discovery of faster, more reliable ways to detect the microbe in samples from humans and other animals, food, and water.

In addition, gene-based research opens the door to simpler, less-expensive tactics for distinguishing look-alike species and strains of *Campylobacter* and its close relatives, such as the *Arcobacters.* This will enable experts to quickly finger culprit microbes in food poisoning outbreaks.

Finally, the studies may lead to innovative, environmentally friendly techniques to circumvent the genes that make *C. jejuni* strains so successful in causing human gastrointestinal upset and in some cases paralysis or even death.

Working with the Institute for Genomic Research, Rockville, Maryland, the Albany scientists have decoded the makeup, or sequence, of all the genes and other genetic material in a specially selected strain of *C. jejuni*.

PEGGY GREB (K11476-1)

Technician Guilin Wang sets the conditions for operation of an automated robotic system for purifying DNA. High-quality DNA is required for spotting onto glass slides for microarray experiments.

This research represents the first time that a *C. jejuni* strain from a farm animal—in this case, a market chicken—has been sequenced. That's important, notes research leader Robert E. Mandrell, because chicken is the leading source of the bacterium in food. Earlier *C. jejuni* genome sequencing, performed elsewhere, was based on a specimen from a gastroenteritis patient and was lacking key features, such as the ability to colonize chickens, Mandrell says.

The next step: Zero in on specific genes. "We're particularly interested in the genes that make *Campylobacter* so viable and virulent," says ARS molecular biologist William G. Miller. They're targeting, for instance, genes that carry the code for making oligosaccharides. These compounds likely enable the microbe to stick like glue to chicken skin in the poultry processing plant even though the birds are bathed and rinsed with chlorinated water. The oligosaccharides might be important in invading and colonizing the human body, as well, Miller notes.

With this genome sequence information in hand, the scientists are developing microarrays, or gene chips, that make possible a quick look at thousands of genes in a single experiment. For these analyses, robotic equipment precisely places pieces of the pathogen's DNA in an array of infinitesimally small droplets on glass microscope slides.

"We build and use these microarrays to compare and contrast DNA of various *Campylobacter* samples," explains microbiologist Craig T. Parker. "We're also using microarrays to get a snapshot of genes in action so that we can see when genes are turned on or off." For example, Parker is pinpointing the genes that are active in helping *Campylobacter* overcome our bodies' protective actions. By tracking the action of the microbes' genes, Parker and co-investigators may be able to determine a way to derail them.

Though *C. jejuni* has grabbed center stage because of its known virulence, its relatives are also of interest. The Albany studies of *C. coli, C. lari,* and *C. upsaliensis,* for example, are attracting the attention of member nations in a three-continent collaboration called "Campycheck," formed to evaluate the importance of these lesser-known or newly emerging species. The Albany scientists and colleagues from the ARS Richard B.

Russell Agricultural Research Center, Athens, Georgia, are advisors to Campycheck.

In clinical laboratories, these less-studied pathogens may inadvertently be killed by the antibiotics used to identify the better-known ones. The likely result? An inaccurate picture of their prevalence and virulence. Campycheck may yield a detailed, accurate picture.

The *Campylobacter* studies in the United States and abroad might never completely eliminate the need for careful handling of raw poultry in our homes or the kitchens of school cafeterias, fine restaurants, and other eateries. But the research can reduce our chances of ever encountering this unruly microbe.—By **Marcia Wood,** ARS.

This research is part of Food Safety, an ARS National Program (#108) described on the World Wide Web at www.nps.ars.usda.gov.

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PEGGY GREB (K11462-1)

Research leader Robert Mandrell (left) and microbiologist Craig Parker, both of the Produce Safety and Microbiology Research Unit, examine an image of the results of a microarray experiment comparing over 1,700 genes of *Campylobacter jejuni* strains from farm animals and humans.

What Makes a *Campylobacter* Strain Virulent?

Here's the puzzle: You have two samples of what seem to be the food-poisoning microbe *Campylobacter jejuni***.** A quick look at the specimens with a microarray assay (see main story) shows no immediately apparent differences in their genes. But when you expose piglets—animals susceptible to this microbe—to the bacteria, one strain makes the animals ill, while the other affects them only mildly.

Why the difference?

ARS food safety researchers Craig T. Parker, at Albany, California, and colleague Michael E. Konkel at Washington State University in Pullman, are designing a series of experiments that should enable them to find out. What's more, their work may help other scientists who are investigating the virulence of other major foodborne pathogens.

Even though their preliminary microarray scan failed to reveal significant differences in the *C. jejuni* specimens' DNA, this technology offers another option—one that allows them to delve more deeply.

Instead of beginning with the microbe's DNA, these followup assays begin with RNA—genetic material that's formed when the DNA, or genes, becomes active.

In these tests, the scientists will place the two strains in petri dishes with colonies of a type of human intestinal cell. Called epithelial cells, they're the target of real-life *Campylobacter* attacks. The researchers will take samples of the two strains at successive intervals, looking for changes in RNA that occur over time. RNA extracted from the strains provides tell-tale evidence of genes that went into action. The work is much like that of police detectives who analyze evidence to reconstruct what really happened at a crime scene. The scientists use an enzyme called reverse transcriptase to match up the RNA to a version of the DNA from which it originated. Then, they use the microarray assay to discern the differences between that DNA and the microbe's DNA as it existed at the outset of the experiment. The comparison should reveal genes that were activated in the attack and genes that remained silent.

In earlier work at Pullman, collaborator Konkel uncovered one such *C. jejuni* gene. Named *ciaB,* short for *Campylobacter* invasion antigen B, it cues the microbe to secrete a similarly named protein, CiaB, which apparently plays a crucial role in enabling the bacterium to penetrate epithelial cells. Though undoubtedly key to *C. jejuni's* invasions, it is unlikely to act alone. The West Coast scientists expect to uncover other genes that will lead them into the dark heart of *Campylobacter's* virulence.—By **Marcia Wood,** ARS. ✷

Nuts'

New Aflatoxin Fighter: Caffeic Acid?

PEGGY GREB (K11802-1)

Using high-throughput bioassays, molecular biologist Jong Kim (left) and research leader Bruce Campbell determine the effects gallic acid has on genes that control aflatoxin production.

Crunchy, good-for-you nuts like almonds, pistachios, and walnuts make their way to your local supermarket only after they've passed a rigorous safety test. The assay ensures that the nuts are free of unsafe levels of aflatoxin, a natural, cancer-causing compound.

A fungus, or mold, known as *Aspergillus flavus* is a leading maker of aflatoxin. Now, ARS scientists Bruce C. Campbell and Jong H. Kim and their colleagues have discovered what might be done to stop that from happening.

Campbell leads the Plant Mycotoxin Research Unit at ARS's Western Regional Research Center in Albany, California. Kim is a postdoctoral molecular biologist with the team.

When the mold feeds on certain kinds of tree nuts, the nuts respond by forming compounds called "oxidants," explains Campbell. The oxidants, in turn, cause what's known as "oxidative stress" in the mold. The mold's reaction? Produce aflatoxin.

But compounds called "antioxidants" can, as their name implies, counter the oxidative stress. And, in doing so, they can quell nearly all aflatoxin production, the scientists found.

The studies could lead to safe, Earth-friendly ways to put antioxidants to work in tree-nut orchards. For example, antioxidants could be applied to trees, or perhaps the trees' own supply of antioxidants could be bolstered through plant breeding.

Three Antioxidants Scrutinized

In laboratory experiments, Campbell and Kim grew colonies of the mold on extracts of walnut and pistachio. Next, they exposed the colonies to three compounds thought to be antioxidants: gallic acid, which ARS chemist Russell J. Molyneux and co-researchers discovered has aflatoxin-combating prowess in walnuts, and chlorogenic acid and caffeic acid, two compounds commonly found in coffee beans.

Caffeic acid outperformed the other antioxidants, reducing aflatoxin production by more than 95 percent. The studies are the first to show that oxidative stress that would otherwise trigger or enhance *A. flavus* aflatoxin production can be stymied by caffeic acid.

Caffeic acid is a natural ingredient not only in coffee beans but also in apples, bell peppers, pears, and other crops.

When it suppresses aflatoxin production, caffeic acid is thwarting the mold's aflatoxin genes. Pinpointing these guilty genes could widen the array of new tactics to undermine them. So Campbell and Kim have teamed with ARS plant geneticist Jiujiang Yu of the ARS Southern Regional Research Center, New Orleans, Louisiana, who is currently working at The Institute for Genomic Research in Rockville, Maryland.

Using microarray technology, Kim took successive snapshots that captured the actions of one form of the mold's genetic material, messenger RNA. He did that before the caffeic acid was applied and at 4 and 6 days after.

Yu, Campbell, and Kim found that 48 genes responded to the antioxidants. About half a dozen of these genes were not known to be involved with aflatoxin but may in fact be key players.—By **Marcia Wood,** ARS.

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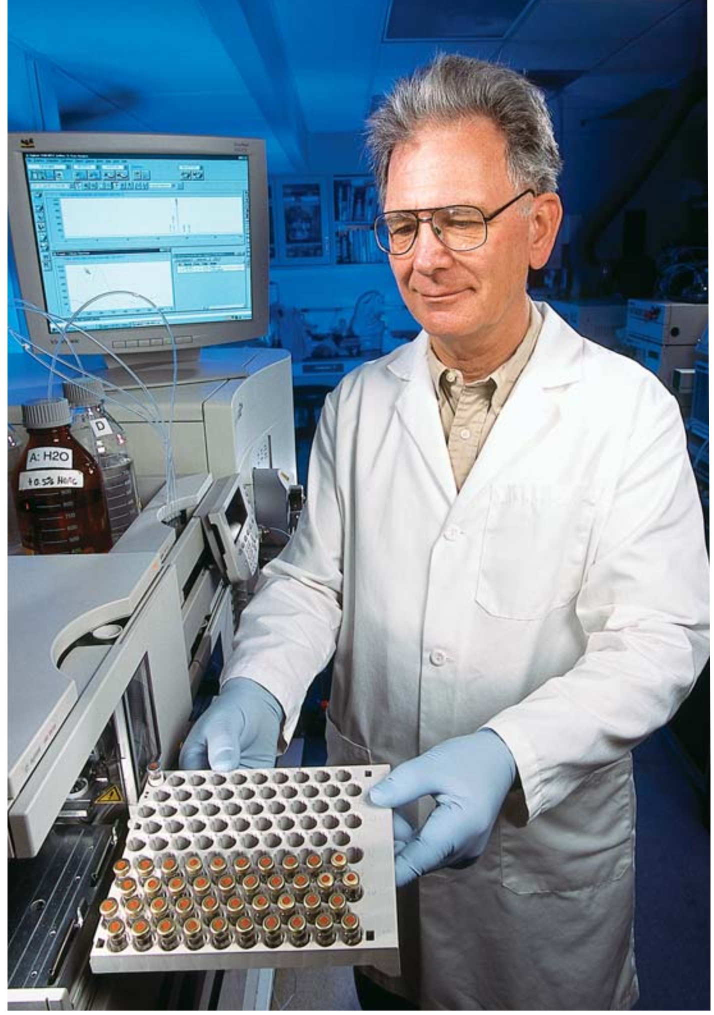
Food Safety

Walnuts' Anti-Aflatoxin Ally
Gallic Acid



PEGGY GREB (K11796-1)

Chemist Russell Molyneux prepares walnut pellicle samples (see photo below) for analysis of gallic acid content.

Rich, crunchy walnuts are always delicious, whether sprinkled on top of a waffle at breakfast, added to brownies you've whipped up for an after-lunch treat, or tossed with a crisp green salad for dinner. Walnuts are a good source of omega-3 fatty acids, thought to reduce risk of cancer. They also provide protein, several essential vitamins and minerals, and antioxidants, yet are free of trans fats and cholesterol. Today, they're ranked as America's third most popular tree nut.

U.S. walnut growers and processors meticulously inspect walnuts so that only the best make their way from orchards to your kitchen. Part of their quality control involves making sure the nuts don't exceed federal food-safety standards for aflatoxin, a naturally occurring compound that can be converted into chemicals harmful to us.

Two kinds of fungi, *Aspergillus flavus* and *A. parasiticus,* are perhaps the best known of the microbes capable of forming aflatoxin. They have the ability to infect not only walnuts, but also almonds, pistachios, peanuts, corn, and cotton.

But ARS scientists in the Western Regional Research Center's Plant Mycotoxin Research Unit at Albany, California, have found that a commercial walnut variety, Tulare, is remarkably resistant to being contaminated by the fungus. The quest to discover Tulare's secret has led the researchers to gallic acid, a natural compound that's locked up in walnut tannin.

PEGGY GREB (K11792-1)

The thin outer coating—or pellicle—of Tulare walnuts is removed before analysis for gallic acid.

Enzyme Disrupts Aflatoxin-Production Process

Ironically, *Aspergillus* itself helps free up the gallic acid in walnut tannin. An *Aspergillus* protein or enzyme known as tannase breaks down the tannin and unlocks the gallic acid.

In Tulare walnuts, the gallic-acid-containing tannin occurs in levels sufficient to render the fungus incapable of making aflatoxin. The nuts' ability to inhibit aflatoxin production increases as the walnut matures, notes ARS chemist Russell J. Molyneux, who led the research.

Comparing Gallic Acid Levels

Molyneux and ARS chemist Noreen E. Mahoney at Albany tracked down gallic acid as the key player in Tulare's aflatoxin-defense strategy. Their petri-dish tests revealed that this variety's anti-aflatoxin activity was better than that of the other commercial walnut varieties—and other tree nuts—they screened.

The assays encompassed nearly a dozen leading varieties of English walnuts, the kind most widely marketed in the United States and Europe today, and two species of black walnuts, not as widely grown because their thicker shells are harder to open and their nutmeats don't loosen as easily from the shells.

The Albany scientists collaborated in the screening with Charles A. Leslie, Gale H. McGranahan, and James McKenna of the University of California, Davis. They found that Tulare walnuts contained one and a half to two times more gallic acid than, for instance, Chico—the most aflatoxin-susceptible variety they examined.

These studies are likely the first to find that an agricultural crop—susceptible to infection by an aflatoxin-producing *Aspergillus* species—can actually prevent the *Aspergillus* from making aflatoxin.

Researchers elsewhere had already shown, in laboratory experiments, that gallic acid had antimicrobial effects. But the investigations Molyneux directed are likely the first to find that an agricultural crop—susceptible to infection by an aflatoxin-producing *Aspergillus* species—can actually prevent the *Aspergillus* from making aflatoxin.

The work paves the way for moving Tulare's gallic-acid-producing prowess into vulnerable walnut varieties. Conventional walnut breeding is one way to accomplish this.

Discovering More About Gallic Acid

Molyneux and Mahoney pinpointed Tulare walnuts' pellicle—the nutmeat's thin outer coating—as the only source of the gallic acid. Exactly how gallic acid disrupts the fungus's ability to produce aflatoxin isn't clear yet, says Bruce C. Campbell, who heads the Albany research unit.

Preliminary research by Campbell and molecular biologist Jong H. Kim suggests that *Aspergillus* may make aflatoxin in response to environmental stress, such as drought.

More details about the gallic acid research appeared in the *Journal of Food Science.*

The aflatoxin investigations were funded in part by the California-based Walnut Marketing Board. California produces nearly all of the nation's walnut crop. The 2003 harvest of 326,000 tons of in-the-shell walnuts was worth about $355 million to growers.

This research may be good news for growers, as it might lead to new, environmentally friendly strategies to undermine aflatoxin production not only in walnuts and other popular tree nuts but also in other crops.—By **Marcia Wood,** ARS.

This research is part of Food Safety, an ARS National Program (#108) described on the World Wide Web at www.nps.ars.usda.gov.

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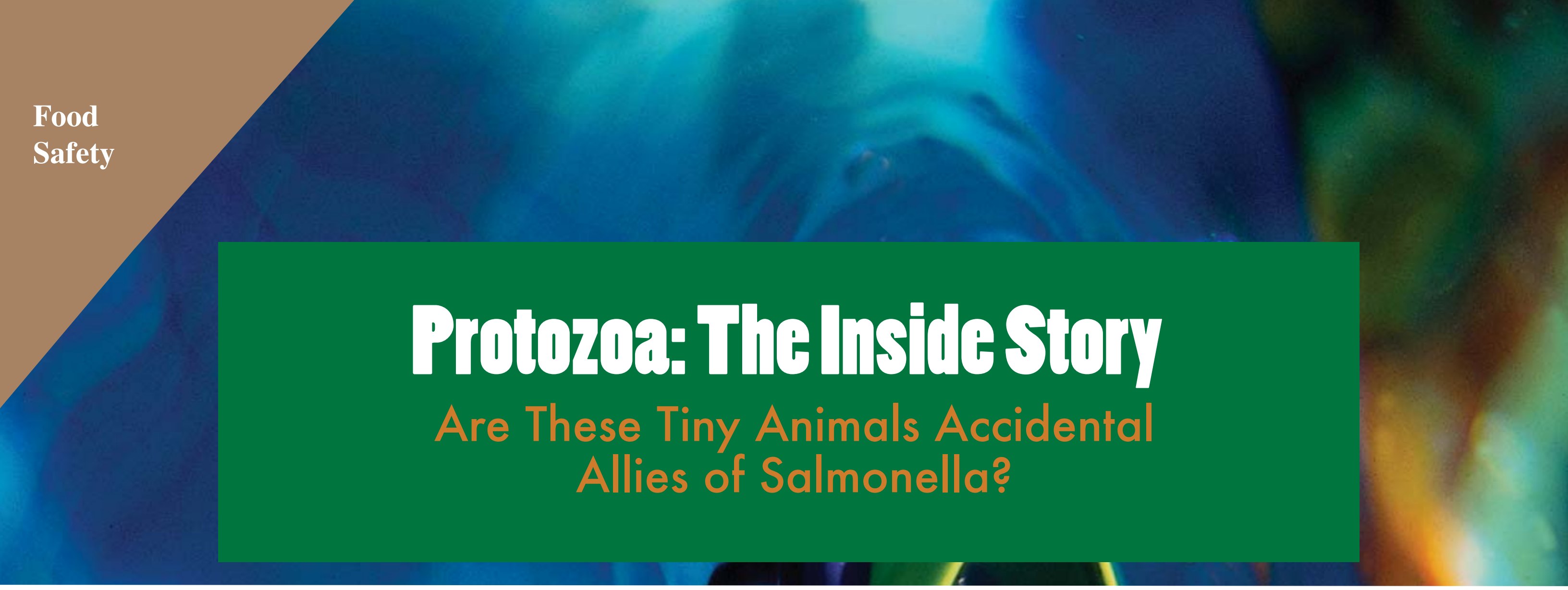
PEGGY GREB (K11798-1)

Chemist Noreen Mahoney performs analysis of aflatoxin from a petri dish (center) containing Tulare walnuts inoculated with *Aspergillus flavus.* The dish in the foreground (green) contains no walnut tissue and displays extensive growth of *A. flavus.*



Protozoa: The Inside Story

Are These Tiny Animals Accidental Allies of Salmonella?

PEGGY GREB (D402-2)

Microbiologist Maria Brandl views microscopic *Tetrahymena* protozoa growing in a liquid medium.

Salmonella enterica, one of the planet's most problematic food-poisoning bacteria, may have an inadvertent ally in the world of one-celled organisms known as "protozoa." Agricultural Research Service microbiologist Maria T. Brandl, based at the agency's Western Regional Research Center in Albany, California, leads a team that's delving into the mostly mysterious interaction between the bacterium and the protozoan. Brandl is part of the center's Produce Safety and Microbiology Research Unit.

Her team's discoveries may lead to new, more powerful, and more environmentally friendly ways to reduce the incidence of *Salmonella* associated with meat, poultry, and fresh produce at the market.

Swallowed Alive, *Salmonella* Survives

At some point in its life, *Salmonella* may encounter a commonplace protozoan known as a *Tetrahymena.* This transparent, nearly invisible animal lives in water—including the water in soil. It uses its hundreds of miniature, oarlike filaments, called cilia, to propel itself through the water, while grazing on any unwary bacteria it happens on.

For instance, it may gulp down *Salmonella*. But that entree choice is really a menu mistake.

Why?

Brandl's laboratory tests showed that the protozoan apparently can't digest *Salmonella.* Instead, it expels *Salmonella* into the environment—alive, well, and fully encased in a miniature pouch. Called a "food vacuole," the pouch is made of an ultrathin, flexible membrane that, Brandl's findings suggest, may afford important protection to *Salmonella.*

But the digestive drama unfolds quite differently for another major foodborne pathogen, *Listeria monocytogenes,* Brandl's experiments show. Once gobbled up, *L. monocytogenes* is deftly digested, disabled, and destroyed by the protozoan.

What happens to *Salmonella* during its brief, gustatorial encounter with the protozoan's innards may strongly affect *Salmonella's* later survival and success, Brandl says. In fact, *Salmonella* may emerge as more resistant than ever to our efforts to kill it.

Salmonella emerges from the innards of the protozoan encased in a food vacuole, which may make it more resistant than ever to our efforts to kill it.

SHARON BERK (D420-1)

A *Tetrahymena* protozoan. The small hairlike projections (cilia) are used by the protozoan to propel itself while grazing on bacteria in water. Note the *Tetrahymena's* mouth (circled), a broad spot covered by many cilia.

When Pathogen Meets Protozoan

Inside a *Tetrahymena, Salmonella* is apparently able to cluster, unharmed, within the food vacuole. After exposing *Salmonella* cells to a *Tetrahymena* for several hours, Brandl found that some vacuoles held as many as 50 *Salmonella* cells. This dense clustering might safeguard the innermost ones from environmental stresses such as ultraviolet rays or harmful temperatures.

Another Albany experiment showed that twice as many *Salmonella* cells stayed alive in water if they were encased in the expelled vacuoles than if they were not enclosed in them.

Sequestering the cells in the vacuoles also protected them against low doses of calcium hypochlorite. Similar to household bleach, it's often used to sanitize food-processing equipment and foods before they reach the supermarket. Brandl found that the encased cells were three times more likely than unenclosed cells to survive exposure to a 10-minute bath of 2 parts per million of calcium hypochlorite.

This study is the first to show that *Tetrahymena* expel living *S. enterica* bacteria encased in food vacuoles and that the still-encased, expelled bacteria can better resist food sanitation.

PEGGY GREB (D403-1)

Research assistant Aileen Haxo (left) and Maria Brandl examine confocal micrographs of green fluorescent-labeled *Salmonella* cells enclosed within food vacuoles of *Tetrahymena* protozoa.

Brandl did the work in collaboration with Sharon G. Berk of Tennessee Technological University, in Cookeville, and with zoologist Benjamin M. Rosenthal at ARS's Henry A. Wallace Beltsville (Maryland) Agricultural Research Center. They documented their findings in a 2005 issue of *Applied and Environmental Microbiology.*

The research exposes how much more there is to learn about *Salmonella's* survival tactics and what growers, food processors, and others can do to keep this microbial menace at bay.

Genes: What's Their Role?

Now, Brandl is taking a closer look at the genes involved in the hidden interaction between *Salmonella* and *Tetrahymena.* Scientists elsewhere are helping solve this puzzle by posting, on the World Wide Web, new information about the estimated 4,000 genes that make up *S. enterica.* Other researchers are now working to decipher all the genes in *Tetrahymena* to learn what jobs they perform inside the little animal.

Brandl's intent is to use microarray technology to capture a record of which genes are turned on or turned off as *S. enterica* is gobbled up by *Tetrahymena,* crammed into a food vacuole, then ejected from the protozoan. She is particularly interested in what genes *Salmonella* turns on "while within the food vacuole. Those genes could be the same ones *Salmonella* activates when inside a human."

Brandl continues, "We may discover new clues to how *Salmonella* behaves outside of hosts such as *Tetrahymena*—and what makes it such a powerful pathogen. Our explorations may also lead to new approaches for controlling *Salmonella.*"

Exposing the hidden details of the encounters of *Salmonella* and its *Tetrahymena* ally may help knock *Salmonella* off the list of major foodborne pathogens.—By **Marcia Wood,** ARS.

This research is part of Food Safety (Animal and Plant Products), an ARS National Program (#108) described on the World Wide Web at www.nps.ars.usda.gov.

Maria T. Brandl is with the USDA-ARS Western Regional Research Center, Produce Safety and Microbiology Research Unit, 800 Buchanan St., Albany, CA 94710; phone (510) 559-5885, fax (510) 559-6162, e-mail maria.brandl@ars.usda.gov. ✷

"Pistachio Blaster" Finds Perfect Nuts

When a pistachio is ripened perfectly, its light-tan shell splits open, revealing a rich-tasting, lime-green kernel that's ready to roast and enjoy. This handy split-shell feature makes it easy for you to loosen the plump nut from its protective housing.

Nicknamed "laughing pistachios" because they look like they're smiling at you, open-shell nuts typically make up about 78 percent of the U.S.-grown harvest.

But some of the remaining harvest is made up of closed-shell nuts that cost pistachio processors an estimated $3 million to $7 million in losses every year. That's due, in part, to sorting-equipment errors that misdirect premium, open-shell pistachios into bins of lower-value, closed-shell nuts.

To help solve this problem, ARS agricultural engineer Thomas C. Pearson has invented the Pistachio Blaster, a high-tech sorter that quickly segregates closed-shell nuts from their high-value, open-shell counterparts with about 90 percent accuracy. The Blaster doesn't damage the nuts, performs at the respectable speed of about 25 nuts per second, and can pay for itself in less than a year, Pearson says.

This super-sorter might be used to sort other crops, such as hazelnuts, also called filberts, and wheat, notes Pearson. He developed it while at the ARS Western Regional Research Center in Albany, California, and is now with the agency's Grain Marketing and Production Research Center in Manhattan, Kansas.

The Blaster relies on what's known as "impact acoustics" to correctly sort the nuts. In a sequence of steps that occur faster than the blink of an eye, the Blaster analyzes sounds made during and immediately after each nut strikes a polished stainless steel block.

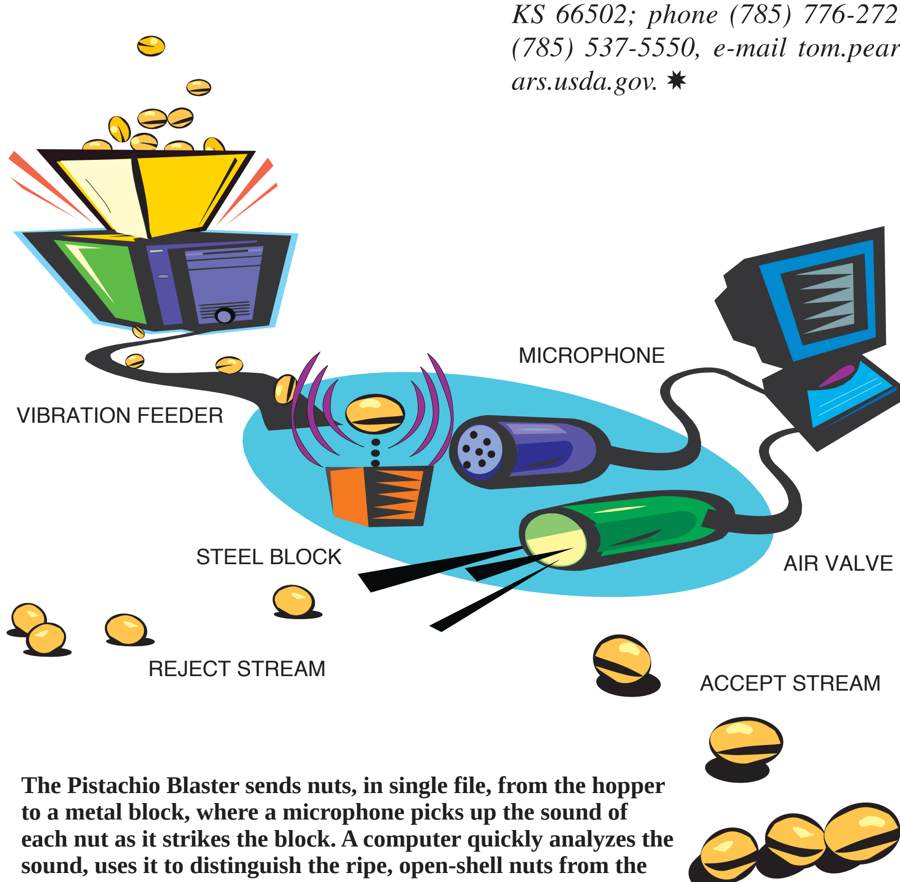
Those sounds, first captured as electrical signals by a precisely positioned directional microphone, are sped to a personal computer, where they are converted into digital data—some 350 pieces of information, or data points, for each nut.

The computer distinguishes the distinctive sound pattern made by the impact of a closed-shell pistachio from that of an open-shell nut, "much like your ear can distinguish a 'plink' from a 'plunk,'" Pearson says. When the analysis reveals the telltale sounds of a closed-shell nut's bounce, the computer sends a signal that causes a blast of compressed air to direct the nut to the reject bin.

One of the nation's largest pistachio processors, Setton Pistachio of Terra Bella, California, holds a license for the Blaster, and is already using several of these novel machines to make sure that more laughing-face pistachios make their way from orchards to you.—By **Marcia Wood,** ARS.

This research is part of Quality and Utilization of Agricultural Products, an ARS National Program (#306) described on the World Wide Web at www.nps.ars.usda.gov.

For further information on U.S. Patent No. 6,541,725, "Acoustical Apparatus and Method for Sorting Objects," contact Thomas C. Pearson, USDA-ARS Grain Marketing and Production Research Center, 1515 College Ave., Manhattan, KS 66502; phone (785) 776-2729, fax (785) 537-5550, e-mail tom.pearson@ars.usda.gov. ✷



The Pistachio Blaster sends nuts, in single file, from the hopper to a metal block, where a microphone picks up the sound of each nut as it strikes the block. A computer quickly analyzes the sound, uses it to distinguish the ripe, open-shell nuts from the lower quality, closed-shell ones, then directs the sorter to send the closed-shell pistachios to the reject bin.

Genome of Bad-Boy *Campylobacter* Sequenced

Inquisitive ARS scientists have laid bare the genetic makeup of a little-known food-poisoning microbe called *Campylobacter lari.* This bad-guy bacterium is a close relative of the better known *C. jejuni,* the culprit in millions of cases of diarrhea in humans every year.

Microbiologist William G. Miller did the genome work in his laboratory at the ARS Western Regional Research Center in Albany, California. *C. lari* has attracted the California team's attention because it is "what we consider an emerging pathogen," Miller says. "It's beginning to show up in other countries, so we need to keep it on our radar here."

Knowing the genetic makeup of a foodborne pathogen such as *C. lari* is a strong first step toward understanding and controlling it. For example, the research opens the door to creating an accurate, affordable, gene-based test to quickly detect the pathogen in samples from people or foods. Such a test would help public health officials track a food-poisoning outbreak to its source.

What's more, the genome is a treasure trove of information for scientists—like Miller—who want to compare and contrast it to other troublesome *Campylobacter* species, such as *C. jejuni* or *C. coli.* Similarities and differences among these genomes will provide important clues to how *Campylobacter* successfully infects us, which may lead to new tactics to outmaneuver the genes that orchestrate infection.

Miller began his genome journey by working forward from a rough draft prepared for ARS by the Institute for Genomic Research, Rockville, Maryland. Digging deeper, Miller filled in gaps and polished rough spots. He now plans to post this important first draft on the World Wide Web early in 2007. Insights that emerge from scientists' scrutiny of this genome will further ensure the safety of the foods we eat.—By **Marcia Wood,** ARS.

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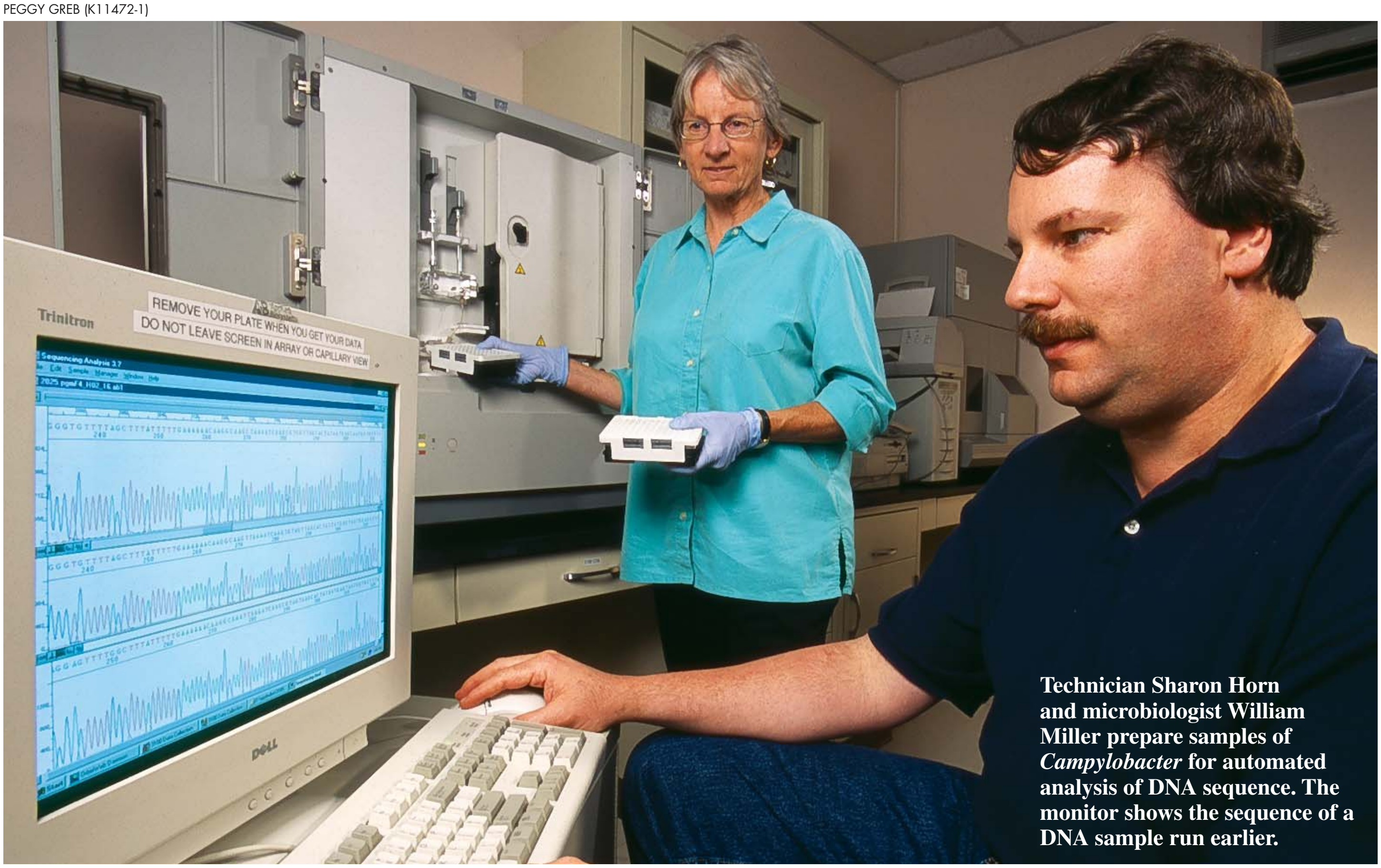
PEGGY GREB (K11472-1)

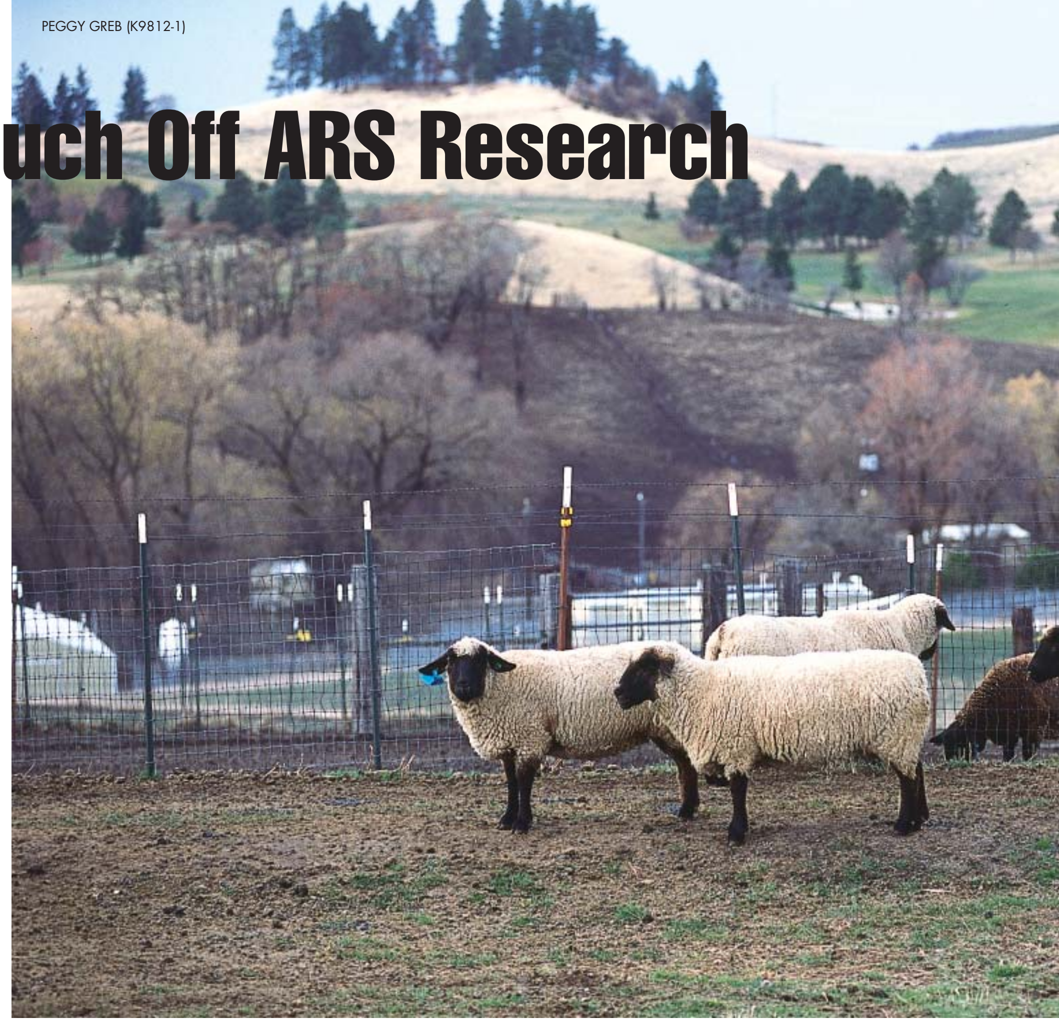


Technician Sharon Horn and microbiologist William Miller prepare samples of *Campylobacter* for automated analysis of DNA sequence. The monitor shows the sequence of a DNA sample run earlier.

Food Safety

TSEs Touch Off ARS Research

PEGGY GREB (K9812-1)



In Pullman, Washington, ARS researchers developed the first practical live-animal test for scrapie, the TSE that afflicts sheep.

A year ago this month, a group of ARS scientists and technicians gave up their Christmas time off and even delayed family vacations to provide characterization of the first case of bovine spongiform encephalopathy (BSE)—commonly called mad cow disease—to be found in the United States.

On December 23, 2003, a Canadian cow shipped to slaughter from a farm in Mabton, Washington, had come up presumptively positive for BSE in testing by USDA's Animal and Plant Health Inspection Service (APHIS), which has diagnostic responsibility and regulatory oversight for BSE issues. APHIS had already used the "gold standard" diagnostic immunohistochemistry test, which was originally developed by ARS. But for the first U.S. case of BSE, APHIS wanted additional scientific information that could be provided by the Western blot test.

PEGGY GREB (K11644-1)

Disease-causing prions accumulate in the brains of host animals. Here, veterinary medical officers Robert Kunkle (left) and Amir Hamir examine prion distribution in brain tissue of TSE-affected animals.

So APHIS put in a high-priority call to veterinary medical officer Juergen Richt and his colleagues at the Virus and Prion Diseases of Livestock Laboratory, which is part of ARS's National Animal Disease Center (NADC) in Ames, Iowa.

"We had experience with the Western blot test and we had all the reagents on hand," explains Richt. "So we put our holiday plans on hold and got everything ready so that APHIS would have verification of the results from the immunohistochemistry test."

On Christmas Eve, Richt and lab technicians Semakaleng Lebepe-Mazur and Deborah Clouser provided APHIS with a report, 22 long hours after the samples arrived in Ames. ARS veterinary medical officers Robert Kunkle and David Alt and technician Dennis Orcutt provided additional DNA sequence information, confirming that the tissue samples actually came from a cow and not a sheep, deer, or other animal.

Then on December 27, APHIS contacted Will Laegreid, animal health research leader at ARS's U.S. Meat Animal Research Center (MARC) in Clay Center, Nebraska, to orchestrate DNA testing and analysis to trace the origin of the BSE-positive cow. His group had previously developed bovine DNA markers for identifying animals that could be used for epidemiological traceback. MARC teams worked around the clock preparing DNA samples. Late on New Year's Eve, after the last critical tissues arrived, the processed samples were driven to the first of two independent, certified laboratories for genotyping. Within days, MARC analysis of DNA evidence confirmed the positive cow was of Canadian origin.

A Mysterious Enemy

Conducting such urgent testing is not a usual part of ARS's work, but very little is usual when it comes to the enigmatic class of animal diseases called transmissible

spongiform encephalopathies (TSEs). These diseases are caused by abnormal prions.

Normal cellular prion proteins occur naturally in many tissues, including brain and other nerve tissue, but their functions are not well understood. These normal prion proteins can change and aggregate to form disease-causing prions.

The prevailing theory is that prions change their shape and fold into an abnormal form that accumulates in the brain and causes lesions. If the abnormal prions are transmitted from an afflicted animal to a new host, they may cause the new host's prions to begin folding abnormally.

Discovery of these prion traits has altered the accepted scientific ground rules for what can cause disease. Prions do not contain DNA or RNA as do fungi, bacteria, viruses, viroids, or any other previously known infectious entities. They are simply proteins, and proteins had not been believed to be infectious on their own.

BSE itself is a fairly new disease; it was first diagnosed in 1986 in Great Britain. The disease has cost the European Union livestock industry at least $107 billion as of this writing. USDA has maintained an aggressive import exclusion and surveillance program since 1986 to minimize the spread of BSE. As of this date, only one imported BSE case has been found in the United States.

Three other animal prion diseases are known today: Scrapie, which affects sheep and goats, was first recognized in Great Britain more than 250 years ago. The disease did not appear in the United States until 1947, when it was found in a Michigan flock. Transmissible mink encephalopathy (TME) is a rare illness that affects mink. It too was first detected in the United States in 1947, on a mink ranch in Wisconsin, and on ranches in Minnesota and Idaho in the 1960s. Epidemiologic data from these outbreaks trace the cases to one common purchased food source. Since then, TME outbreaks have also been reported in Canada, Finland, Germany, and the republics of the former Soviet Union.

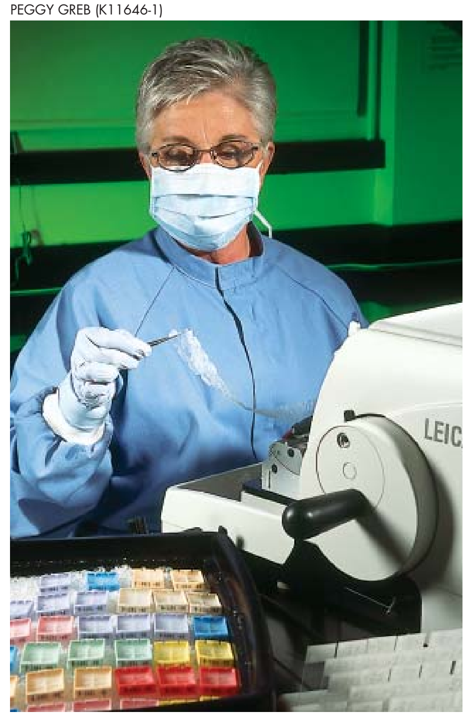
Chronic wasting disease (CWD) is a TSE of deer and elk. CWD has been reported in free-ranging mule deer, white-tailed deer, and Rocky Mountain elk in Colorado, Wyoming, South Dakota, New Mexico, Utah, Wisconsin, Nebraska, and Illinois; and in game-raised elk in South Dakota, Kansas, Montana, Oklahoma, Colorado, Nebraska, Minnesota, and Wisconsin. The disease has also been found in game-raised elk and a few free-ranging deer in Canada.

ARS has one of the world's most comprehensive research programs investigating TSEs. It is the only organization studying all four TSEs in animals. ARS

PEGGY GREB (K11639-1)

A Western blot analysis done by technicians Deborah Clouser (sitting) and Semakaleng Lebepe-Mazur was crucial in tracking the first U.S. BSE case.

PEGGY GREB (K11646-1)



Histotechnologist Jean Donald prepares 5-micrometer-thick sections of tissue collected from TSE-affected animals. The sections are then mounted on glass slides, stained, and examined by pathologists.

is taking a very integrated approach to TSE research, with collaborative projects involving many disciplines and scientists. While each TSE is unique in many respects, there is so much to learn about prion diseases that what researchers learn about one TSE may give insight into another.

Diagnostics

Now-retired ARS veterinarian Janice Miller developed the first immunohistochemistry method for diagnosis of scrapie in sheep in 1993. This test was much more specific and less burdensome than any other at that time. In 1998, ARS microbiologist Katherine I. O'Rourke at the Animal Disease Research Unit in Pullman, Washington, further increased the test's specificity and ease of use by incorporating monoclonal antibodies. Use of these monoclonal antibody reagents was then broadened to be able to diagnose the other TSEs.

Later, O'Rourke had a real breakthrough when she discovered that prions collect in pockets of lymphoid tissue in a sheep's nictitating membrane, or third eyelid. A veterinarian can take a sample of the tissue with only a local anesthetic, which meant that there was finally a practical, live-animal test for scrapie. This live-animal test is now an approved diagnostic test for scrapie in the United States.

A very rapid, ultra-sensitive test that could be used before animals show any symptoms, especially with BSE in cattle and CWD in deer and elk, is still a major research goal.

One approach being taken today by ARS chemist Bruce C. Onisko at the Foodborne Contaminants Research Unit in Albany, California, is use of mass spectrometry to identify extremely low levels of prions. Mass spectrometry reveals structural information from biological compounds by ionizing a molecule of interest, fragmenting it by collisions with an inert gas, and then applying mass analysis to the fragmentation products.

"Antibodies only let us find prions in

PEGGY GREB (K11654-1)

At the ARS National Animal Disease Center in Ames, Iowa, animal caretaker Gary Hansen tends to two jersey steers. The steers are used as controls in a CWD cross-species transmission experiment in which cattle were inoculated intracerebrally with CWD-infected brain tissue.

PEGGY GREB (K11628-1)

Chemist Chris Silva (left) and research leader J. Mark Carter load samples for analysis via nanospray liquid chromatography coupled to mass spectroscopy. This state-of-the-art technology characterizes BSE prions with unprecedented precision.

amounts greater than 1 picomole. For live-animal testing we need to be able to reliably and quantitatively detect concentrations 3 to 4 orders of magnitude less from easily obtainable tissues," explains Onisko. "And we need to be sure we are looking at only the abnormally configured prion protein."

Such a sensitive test would help diagnose animals with abnormal prions before they start showing clinical symptoms. ARS has now applied for a patent for a new diagnostic test based on this technology.

ARS chemist Christopher J. Silva, also at the Foodborne Contaminants Research Unit, is using mass spectrometry to develop a way to test feeds for the presence of animal materials.

"A test for the presence of prions in animal feed is problematic. Epidemiologists in the United Kingdom showed that prions are not evenly distributed in animal feed, so an analytical sample might not be representative of the whole feed lot. Furthermore, could such a test be sensitive enough to detect rendered prions?"

Instead, Silva's work on detecting the presence of prohibited animal materials in animal feeds would serve as an important indirect test for prions. BSE is transmitted to cows through feed containing animal parts from prion-infected cows. Using prohibited animal materials in cattle feed has been outlawed to prevent BSE transmission. "But it would be nice to have a way to double-check that feed is free of prohibited animal materials (and prions), should contamination ever be suspected," Silva says.

Transmission

Another major question that ARS is studying is whether and how TSEs spread between animals, either of the same species or different species. BSE is not communicable from animal to animal except through the recycling of bovine protein, which is now banned. Transmission from cows to humans appears to require contact with specific infected tissues. Routes of transmission have not all been firmly established, but the oral route is most likely.

Meat from BSE-infected cows has not been shown to be infectious or associated with transmission. Exposure in people is most likely through consumption of meat products contaminated with central nervous system tissue. Since 1990, 157 people worldwide are believed to have contracted the abnormal prion-related disease called variant Creutzfeldt-Jakob disease from consumption of BSE-contaminated food.

Scrapie, on the other hand, has never been found to cross from sheep to humans, according to Donald P. Knowles, Jr., research leader at the ARS Animal Disease Research Unit, in Pullman. ARS has found that scrapie from North American sheep, when transmitted to cows by intracranial injection, induced a spongiform encephalopathy with subtle microscopic lesions that didn't mimic BSE and the accumulation of protease-resistant prions. But oral inoculation of cattle with scrapie of North American origin didn't result in any detectable lesions or prion accumulation.

Now, Knowles, Janet Alverson, an ARS veterinary medical officer in Pullman, and Robert D. Harrington, a veterinarian and

Cattle, deer, sheep, raccoons, mice, and **elk,** like the one shown below, are being used in ARS studies on **transmissible spongiform encephalopathies.**

PEGGY GREB (K11659-1)

clinical instructor at the University of Washington Medical School in Seattle, are seeing whether CWD can infect mink. Mink were challenged 1 year ago both orally and intracranially and are under observation for clinical signs.

Most cross-species infection studies begin with intracranial injections of infected material, which is, of course, not a transmission route that could occur naturally. "But we do intracranial inoculations as a positive control for infectivity. If you can't establish a TSE in a species by intracranial injection, you aren't likely to see it spread any other way," says Knowles.

Scientists at NADC, including Kunkle and ARS veterinary medical officer Amirali N. Hamir, are involved with studies that will eventually check for possible cross-species transmission with all three U.S. indigenous TSEs—scrapie, CWD, and TME—in a wide assortment of species. (BSE is not considered indigenous in the United States since the positive cow came from Canada.) NADC has the only biocontainment facilities for working with large animals in which TSEs can take a decade to incubate.

"With these cross-species studies," explains Kunkle, "we learn whether a particular TSE can infect a different species and what it would look like clinically and pathologically if it did, and we build a bank of tissue material to call on for future research."

Intracranial injections have been found to be able to transmit CWD to cattle. "But it doesn't produce the same clinical signs as BSE," Kunkle says. "With the clinical and pathological information we're developing, if CWD can be transmitted to cattle in a natural fashion, we'll have a better ability to recognize it."

With CWD, the situation is especially complicated. Researchers are not even sure yet how the disease spreads from deer to deer or elk to elk. "We know you can put deer in a paddock that infected deer have been kept in and then removed, and the new deer can become infected," says Kunkle. "But we don't yet know whether the prions are shed in urine or feces or something else."

This is obviously different from BSE and TME, but then "prion diseases are a very loosely knit family," he adds.

Another NADC project is to see whether reindeer and fallow deer can become infected with CWD. APHIS is interested in this because these animals may be co-located with farm-raised elk. The study with fallow deer is already under way. Kunkle is also checking to see whether scrapie can be transmitted to pigs by intracranial injection. "It's the same type of basic and precautionary research," he says.

PEGGY GREB (K11632-1)

A sensitive new technique to detect animal products in feed will help formulators and livestock owners identify feed containing only vegetable ingredients. The test's inventor, chemist Chris Silva, weighs potential feed materials before testing.

Faster Models

One of the biggest complications in TSE research is the incubation period—the length of time it takes from exposure until the animal shows symptoms, proving that they have actually developed the disease. Cattle, deer, and elk can take several years or more to show signs of a TSE. To make research more practical, ARS is developing several promising animal models that will have shorter incubation times.

One such model is a collaborative project ARS has begun with Harrington and Knowles. They are developing a line of genetically engineered mice that have an added elk or deer prion gene, making them more susceptible to CWD. The team recently received a National Institutes of Health grant supporting this work for the next 3 years.

"Since such mice may develop CWD in just 1 to 2 years, they would be a powerful research tool," Harrington says. "It will let us validate new diagnostic tests, hopefully give us clues to how CWD is transmitted, and provide an alternative model to study molecular mechanisms of the disease."

Genetic Resistance

With so little known about exactly why and how prions become abnormal, right now the best hope for controlling TSEs appears to be breeding animals that are simply naturally resistant. In Suffolk and other U.S. sheep breeds, O'Rourke has already found sheep with certain genotypes that have very limited susceptibility to scrapie. And she's shown that if an infected ewe is bred to a resistant ram, the lamb will have a resistant genotype and be born free of scrapie.

Selective breeding for genetic resistance to the disease is a valuable tool for the National Scrapie Eradication Program. Genotyping allows infected flocks to be cleaned up while sparing 60 percent of the sheep. The program also encourages producers to select for resistance and to use scrapie-resistant rams in flocks that have risk factors for scrapie. Genetic testing and selection, national sheep and goat identification, regulatory slaughter surveillance of mature sheep, investigation of exposed flocks by use of genetics and the third-eyelid test, cleanup of infected flocks, and the Scrapie Flock Certification Program provide an integrated strategy to eradicate scrapie from U.S. sheep and goat populations.

ARS researchers, including O'Rourke, have also been conducting genetic surveys to determine whether there is variation in the prion genes of other species that might identify susceptible and resistant animals. The makeup of a single amino acid sequence appears to be the difference between an animal that's likely to have its prions altered and one that isn't.

When O'Rourke and Alverson began examining deer and elk for genetic susceptibility or resistance to CWD, they discovered an unusual situation. They found that deer may have four copies of the prion gene rather than the two that would be expected.

"Virtually every mule deer we examined had the gene in duplicate, although only 15 percent of the white-tailed deer have the extra set," O'Rourke explains. "The extra set of prion genes is nonfunctional, but it complicates genetic testing to identify what a susceptible or resistant genotype might be."

So far, it is unclear whether there is any natural resistance in deer or elk populations.

ARS scientist Michael P. Heaton and his co-workers at MARC have recently identified extensive nucleotide variation in the prion genes of U.S. cattle, sheep, and deer.

"This information provides new DNA markers for researchers interested in genetic epidemiological studies of prion diseases. For example, if susceptibility alleles are identified in other populations of cattle, we will immediately know the proportion of U.S. cattle that is most genetically vulnerable to prion disease," Heaton says.—By **J. Kim Kaplan,** ARS.

This research is part of Animal Health, an ARS National Program (#103) described on the World Wide Web at www.nps.ars.usda.gov.

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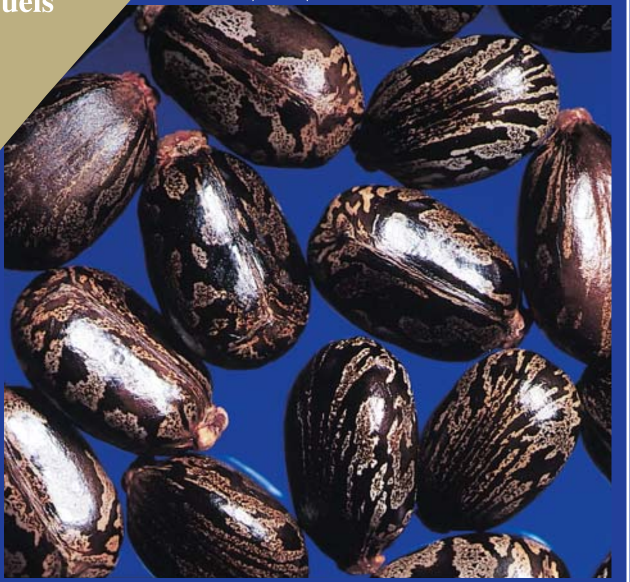
PEGGY GREB (K11635-1)

Biologist Larry Stanker (standing) and chemist David Brandon review results of a rapid immunoassay. They are developing new technology for sensitive detection of BSE, surrogate markers, and risk factors.

BRIAN PRECHTEL (K9200-2)



Castor beans.

HIGH-TECH CASTOR PLANTS MAY OPEN DOOR TO DOMESTIC PRODUCTION

BRIAN PRECHTEL (K9197-1)

Plant physiologist Grace Chen removes castor bean pods to test for genetic transformation.

Inside the beans of the castor plant is a toxin seven times more deadly than cobra venom. Known as ricin, the compound's toxicity is one reason why American farmers no longer grow this crop extensively—even though a lucrative market exists for the castor bean's unique oil.

Components of the oil, known as hydroxy fatty acids, are essential for making high-quality lubricants for heavy equipment or jet engines, for example. Castor oil is also used in paints, coatings, plastics, antifungal compounds, shampoo, and cosmetics.

Allergens Pose Health Hazard

Besides the ricin toxin, there's another compelling reason why this crop has fallen out of favor with U.S. growers. The shiny, beetle-shaped seeds contain powerful allergens. People who work with the off-white meal ground from castor beans may develop allergic reactions, such as hives or asthma. In severe cases, they may go into anaphylactic shock, which can be fatal.

Conventional breeding to rid castor of lethal ricin and troublesome allergens hasn't solved the problem. But biotechnology might, according to Thomas A. McKeon of ARS' Western Regional Research Center in Albany, California. He and colleague Grace Q. Chen, both in the Crop Improvement and Utilization Research Unit, are the first in the world to genetically engineer castor plants.

In preliminary experiments, McKeon and Chen used marker genes to determine whether their tactics for shuttling new genes into plants actually worked. Now the scientists want to give the plants other genes—ones that could, among other things, block production of ricin poison and the powerful allergens.

Biotech Strategies

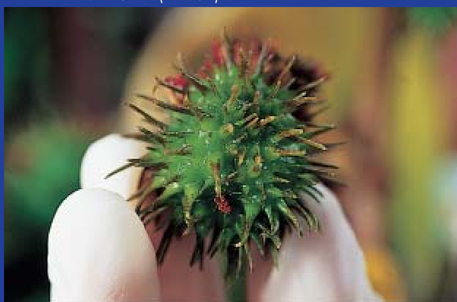
Scientists elsewhere have already isolated and copied a gene critical to ricin production, as well as a gene that produces the key allergen proteins in castor. McKeon and Chen aim to build and insert slightly different versions of those genes into the castor plant, to block the action of the ricin and allergen genes. For example, they want to construct antisense genes, which are genes that make nonsense copies of the authentic ricin or allergen genes.

"Antisense genes," McKeon says, "can interfere with the gene expression needed for producing ricin and allergens. That may leave the plant unable to form these compounds."

Castor plants that are free of ricin and allergens could renew interest in farming this crop. That could happen not only in the southern United States, where it was grown until the early 1970s, but also in the arid Southwest, where it could grow well if irrigated.

"Castor is a semitropical plant that thrives in sunny climates," McKeon says. Although some types of castor grow to be 30- to 40-foot-tall trees in the Tropics, in the United States castor can

BRIAN PRECHTEL (K9198-1)



A castor bean pod.

BRIAN PRECHTEL (K9198-2)

Chemist Thomas McKeon and Grace Chen remove leaf disk samples from genetically transformed castor plants to test for enzyme activity.

be harvested annually when it is only about 4 to 5 feet high. In the past, U.S. production has reached 1,000 pounds of oil per acre. "That's an impressive feat for any oilseed crop," notes McKeon.

Production of a U.S. castor crop could ensure a more reliable supply of the oil for American industries and for defense. This country depends on imports of castor oil, primarily from India. In 1999, America imported nearly 103 million pounds of castor oil, worth about $41 million. The world demand for castor oil is about 1 billion pounds annually, valued at more than $400 million.

Although some other plants, like lesquerella, can produce oil that contains hydroxy fatty acids similar to the ones in castor, these alternative crops are not yet widely grown commercially. Another approach? Synthesize hydroxy fatty acids in chemical factories. Although the technology exists to do that, growing castor plants outdoors in the sunshine is a more economical approach, McKeon says.

Epoxy Oil—A Possibility?

In addition to reviving production of castor, genetic engineering might someday be used to tweak its oil-producing mechanism so that it could yield another valued oil, known as epoxy.

Says McKeon, "There is a potential U.S. market of about $300 million a year for epoxy oil. An epoxy-based paint, for example, offers all the advantages of a premium, oil-based paint, yet does not give off certain volatile chemicals that pollute the atmosphere." That's unlike the solvents in oil-based paints, which can be an environmental hazard.

"We think that production of epoxy oil by castor plants is possible," says McKeon, "because the chemical structure of epoxy oil is very similar to that of castor oil. The modification that's needed to cue the castor plant to make epoxy oil instead of castor oil is minor. That's very different than trying to genetically engineer a corn plant or a soybean plant to make epoxy oil. The oils that those plants make are very unlike epoxy oil."

McKeon and Chen have produced about a dozen genetically engineered castor seedlings in their laboratory and greenhouse. They are applying for a patent for their discoveries (U.S. Patent Application No. 60/167,360, "Transformation of *Ricinus communis,* The Castor Plant").—By **Marcia Wood,** ARS.

This research is part of New Uses, Quality, and Marketability of Plant and Animal Products, an ARS National Program (#306) described on the World Wide Web at www.nps.ars.usda.gov.

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Leftover Straw Gets New Life

Environmentally friendly packaging might soon be made in part from rice or wheat straw left over after harvest. The wedge-shaped corner inserts that hold computer printers snugly in place during shipping, for example, could be molded from a slurry made from these straws.

That's the plan of ARS scientists in Albany, California, and their colleagues at Regale Corporation in Napa. Biobased packaging and other new goods made with straw could become alternatives to today's paper and plastic products.

"These bioproducts may give growers a new, profitable market for their straw," says ARS chemist William J. Orts. He leads the Bioproduct Chemistry and Engineering Research Unit at the ARS Western Regional Research Center. "In addition, the straw could give manufacturers a less expensive raw material for their products."

Limited Options for Leftovers

Today, growers of both wheat and rice face the same problem of what to do with leftover straw. It can be gathered and sold for animal feed or bedding, but those markets are often unprofitable for growers.

Straw can be plowed under, but that costs about $8 to $10 an acre. Alternatively, the straw can be left in place in the field to control erosion and to provide nutrients for beneficial, soil-dwelling microbes. But the leftover straw might also support disease-causing organisms that would attack the next year's crop.

In fact, the added cost of plowing-under old straw and the threat of disease are the main reasons that rice growers in California typically burn their fields after harvest. However, over the past decade, straw burning has been progressively prohibited, except in the case of fields that are already diseased. The ban is meant to boost air quality by reducing smoke.

The amount of straw produced each year is enormous. In California alone, the annual rice crop generates over 300,000 tons of straw. And the state's wheat crop yields an estimated 400,000 tons of straw.

Rice and wheat straw are good sources of cellulose. In turn, cellulose is the basis for strong, biodegradable fibers that can be used for manufacturing, according to Orts. His team is determining the extent to which cellulose fiber from straw can be used in place of wood fiber or plastics derived from petroleum.

"Rice and wheat straw are produced at least once a year," Orts explains. "Trees take longer to mature for harvest, and petroleum is, of course, nonrenewable." So using agricultural fibers such as straw as industrial raw materials may have less impact on the environment than these other options. It should also help growers' profits and the economy of their rural communities.

Pulping Processes Scrutinized

To be used in a product such as packaging for electronics, straw first needs to be put through a pulping process that results in a slurry of straw fibers, water, and additives. At this point, the pulp is molded into the finished shape and dried.

Orts and co-researchers are investigating ways to fine-tune pulping processes so that the straw has the properties that manufacturers, such as Regale, require. Regale executives estimate, for example, that even a 1-second reduction in drying time may mean a savings of many thousands of dollars a year in a manufacturer's energy costs.

A collaborator with ARS in some experiments, the company designs and manufactures innovative custom packaging molded from recycled materials.

At the Albany center, Orts and colleagues are putting rice and wheat straw through both a modified hot-water pulping procedure and the chemical-based kraft one. "By making variations to either pulping process, we might be able to reduce the need for chemicals or to reduce other costs," explains Orts. In addition, changing the minor additives to the pulp might streamline production. Common additives include biopolymers, modified starches, clays, and other natural products.

Other variations may enhance the pulp to make the products stronger and more resistant to the warping effects of humidity, temperature, and time in storage.

PEGGY GREB (K9836-1)

Chemists Greg Gray (left) and William Orts use x-ray diffraction to characterize agricultural fibers for use in biobased products.

Additional improvements could boost bioproducts' resistance to water and grease—a must for acceptance for fast-food packaging.

Equally as important, the watery, straw-pulp slurry must be predictable in how it behaves in the manufacturing process. This uniformity is essential, despite natural variations in the straw from harvest to harvest. The pulp has to be consistent so that the finished product doesn't vary from year to year. Otherwise, the biobased product may introduce too many uncertainties for the manufacturer.

A Manufacturing Mystery

"Right now, alternative agricultural fibers are an underused resource for making products that have tight manufacturing specifications," says Orts. "That's in part because there's relatively little known about how these novel fibers will behave. We need to learn more about alternative agricultural fibers. A key to consistency in manufacturing is characterization—that is, an understanding of how the fiber will perform under various processing conditions.

GUY PEDERSON, REGALE

Agricultural fibers such as leftover straw can be specially processed into useful packaging, like this wine bottle carton.

"We're starting by looking at the characteristics needed for the end product, such as strength, tear resistance, and market appeal," Orts points out. "Then we'll look at the processing that will ensure that the agricultural fibers will have the requisite properties. For this research, we'll be using scanning electron microscopy, x-ray diffraction, differential scanning calorimetry, and thermomechanical analysis.

"The approaches, or recipes, we develop in the laboratory can next be tested on a larger scale under manufacturing conditions in our pilot plant at the Albany center," says Orts. "Then we'll work with our corporate colleagues for industry-scale testing. This scaling up should help us make sure we overcome any potential barriers to commercializing the product."

PEGGY GREB (K9837-1)

Orts, center, and Regale Corp. collaborators Jeff Haugen, left, and Karl Gee remove the bottom part of a wine bottle carton from its mold. The carton was formed from a slurry of straw, water, and additives.

The center's 35,000-square-foot pilot plant is undergoing a $20 million renovation, scheduled for completion in 2006. This updating will make the plant one of the most modern facilities of its kind in the western United States.

Equipment at the pilot plant is suitable for processing several different agricultural fibers—not just rice and wheat straw. "We've started testing rice hulls and flax," says Orts. "We're interested in many different alternative fibers because we want to meet the needs of a wide range of growers and producers—those who have to deal with leftovers."

He adds, "The fibers are neither profitable for producers nor an economical raw material for manufacturers unless they can be used within a relatively short distance from where they were produced. Otherwise, transportation costs take too big a bite out of potential profits."

Because of the need to be near to the agricultural source, these regional plants, sometimes called biorefineries, might be smaller than conventional manufacturing facilities.

The demand for biodegradable products continues to increase. Experts estimate that goods made in part from renewable resources will make up 10 percent of all American manufacturing by 2020 and 50 percent by 2050. "People feel good about buying these 'green' items," says Orts. "Increasing use of agricultural fibers is one way to give consumers more choices of environmentally friendly products."—By **Marcia Wood,** ARS.

This research is part of Quality and Utilization of Agricultural Products, an ARS National Program (#306) described on the World Wide Web at www.nps.ars.usda.gov.

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Biobased Products and Biofuels

EDWARD MCCAIN (K4877-3)

Sunflower Rubber?

Sunflowers—today known for their tasty, crunchy seeds and a healthful salad oil—might tomorrow gain fame as a source of premium rubber. That's the plan of scientists who have joined forces to improve the quality and quantity of latex from sunflower plants.

Latex is made up of rubber particles surrounded by water and other plant compounds. It is a higher value product than solid rubber. As rubber factories of the future, lanky sunflowers would reduce America's dependence on imported natural rubber and on synthetic rubber made from petroleum. The United States imported about 1.2 million tons of natural rubber—worth about $1 billion—in 2000. Although synthetic rubber can be substituted in some instances for natural rubber, high-performance products such as airplane tires require natural rubber.

"More than 2,500 species of plants produce natural latex," says ARS plant physiologist Katrina Cornish at Albany, California. "But few of them have the traits we want. In particular, most are small, grow too slowly, or aren't suitable for being cultivated in uniform stands—or monocultures. And they don't produce enough latex, or the latex they produce is not high quality.

"In contrast, sunflowers grow large rapidly and do well in monocultures. Although the quantity and quality of latex from sunflowers is not yet good enough for commercial use, we expect to improve it further through genetic engineering," Cornish adds. She is part of the ARS Crop Improvement and Utilization Research Unit of the Western Regional Research Center in Albany.

Cornish and colleagues are experimenting with several different types or lines of sunflowers. "We are especially interested in lines that produce the highest amounts of latex in stems and leaves—as opposed to flower heads," Cornish points out. "That's because it's impractical to separate latex in the flower head from oil and other components. We're also focusing on lines that flourish in northern, temperate climates, where most of the U.S. sunflower crop is grown."

At Albany, Cornish is delineating the physical characteristics of sunflower candidates' latex and comparing them to those of latex taken from two other natural sources—the Brazilian rubber tree and a desert shrub, guayule. (See *Agricultural Research*, May 1999 and April 2002.)

Cornish will insert laboratory-built genes for latex production into sunflower tissue. Next, she will test the tissue to determine whether the new genes are working inside the sunflower's cells. Later, greenhouse and field tests will identify the gene-engineered plants that produce the highest amounts of the best quality latex. In experiments after harvest, Cornish and co-researchers will determine how to preserve sunflower latex while it's in storage, awaiting processing.

Cornish is a world authority on how plants produce rubber. She is doing the sunflower project with colleagues from Colorado State University and Oregon State University.—By **Marcia Wood,** ARS.

This research is part of Quality and Utilization of Agricultural Products, an ARS National Program (#306) described on the World Wide Web at www.nps.ars.usda.gov.

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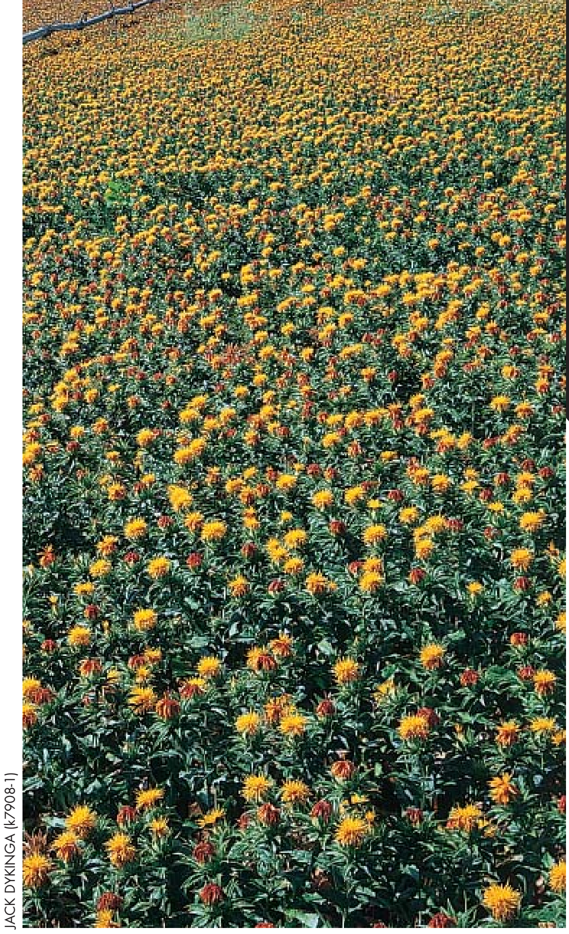
Biodegradable Oils From Alternative Crops



JACK DYKINGA (k7908-1)

A field of safflower, *Carthamus tinctorius.*

Agricultural business is fluctuating and unpredictable. Weather patterns, consumer demand, and geography can lead to market gluts, often in the wheat and corn sectors. One solution to this economic problem is to find new and profitable uses for alternative crops. This strategy could result in farm diversification and less overproduction of crops like corn and soybeans.

ARS chemists Terry A. Isbell and Steven C. Cermak have found a potentially profitable new use for high-oleic oilseeds crops. They've made environmentally friendly, effective lubricants containing estolides, which are fatty acids from oilseeds such as high-oleic sunflower and high-oleic safflower.

Isbell and Cermak, who are at the National Center for Agricultural Utilization Research (NCAUR) in Peoria, Illinois, recently received two U.S. patents on the technology.

The new vegetable-based, biodegradable oils can be used as hydraulic fluid in heavy equipment or as crankcase fluid. Industrial-sized production of the starting material was done at a pilot plant at NCAUR.

These lubricants compare favorably to those produced from soybean and canola. "Tests show that the estolide-based lubricants have excellent pour points in cold temperatures, better oxidative stability than most petroleum lubricants currently on the market, and good lubricity. These properties all exceed those of soybean- and canola-based products," says Isbell. "The exception is the price of the starting material. Soybean oil costs about 13 cents per pound, and oleic acid is 75 cents per pound. But the estolides require far fewer additives than traditional vegetable oil lubricants, which makes their final market cost identical."

Their superior properties make estolides good candidates for many lubricant applications, particularly where enhanced performance and biodegradability are required, says Isbell.

This research was done under a cooperative research and development agreement (CRADA) with Lambent Technologies of Chicago. The company was acquired in 1998 by Petroferm of Fernandina Beach, Florida.

Meanwhile, ARS scientists on the West Coast are exploring ways to boost domestic production of castor plants, which yield versatile, top-quality, high-priced oil. Chemist Thomas A. McKeon and plant physiologist Grace Q. Chen are doing the work at the ARS Western Regional Research Center in Albany, California.

Castor oil is used for making premium lubricants for heavy equipment and for jet engines. It is also used in paints, coatings, plastics, antifungal compounds, shampoo, and cosmetics.

McKeon and Chen are using techniques of modern biotechnology to remove castor's ability to manufacture its potent toxin, ricin, and to keep the plants from synthesizing allergens that can cause hives, asthma, or anaphylactic shock. The team was the first in the world to genetically engineer castor plants. They are seeking a patent for their work.

The two are now experimenting with another approach to shuttling strategic genes into castor. And they're investigating whether castor can yield new chemicals that could replace petroleum-derived compounds. The Dow Chemical Company, headquartered in Midland, Michigan, is funding part of the work under terms of a CRADA with ARS. Castor Oil, Inc., of Plainview, Texas, is also a partner in this research.

Castor thrives in sunny climates, so it could be produced, for instance, in the southern United States.—By **Sharon Durham** and **Marcia Wood,** ARS.

This research is part of Quality and Utilization of Agricultural Products, an ARS National Program (#306) described on the World Wide Web at www.nps.ars.usda.gov.

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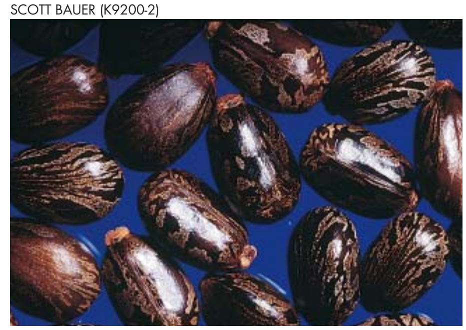
SCOTT BAUER (K9200-2)



Castor beans.

PEGGY GREB (D750-1)

Elite bioenergy switchgrass growing in eastern Nebraska.

Genetic Snapshots Help Brighten Switchgrass's Future

Corn has captured public attention as *the* crop with potential to quench America's thirst for ethanol and other such biofuels. Another fuel-friendly crop is switchgrass. Indeed, ARS-led research in the Midwest indicates an acre of biomass (stems and leaves) from this warm-season perennial grass has the potential to yield 300 to 800 gallons of ethanol.

That's a promising estimate, but more research is needed to improve the conversion technology used and to make the plant biomass easier and less costly to convert into ethanol. Conversion is done by breaking down the plant's cell walls into sugars and then fermenting them.

One approach is to develop new switchgrass varieties with traits geared to producing ethanol rather than traditional uses, like feeding cattle, anchoring soil, or restoring grasslands.

"The ideal switchgrass for bioenergy production would have low input requirements, good stand establishment—especially the first year—high yield, and excellent conversion-to-ethanol properties," comments Gautam Sarath. He's a molecular biologist in ARS's Grain, Forage, and Bioenergy Research Unit (GFBRU), Lincoln, Nebraska.

Building Living "Libraries"

To expedite breeding efforts, Sarath and collaborators generate tens of thousands of genetic "snapshots" of switchgrass in action—from the moment it sprouts from seed to the time it prepares for overwintering.

The snapshots are actually fragments of genetic material called messenger RNA (mRNA). In plant cells, mRNA delivers instructions for making proteins and carrying out other tasks assigned by DNA—the so-called blueprint for life.

Extracting mRNA from switchgrass offers a glimpse of how this molecular workhorse does the bidding of DNA at particular growth stages or physiological moments in development. In a later step, a technique called "microarray analysis" allows scientists to visually identify which genes were active when they plucked the mRNA from the grass's tissues.

The mRNA is difficult to work with outside its natural setting—cells. So, the researchers create a more stable version—complementary DNA (cDNA). Using standard biotech methods, they insert the cDNA into specially engineered plasmids, which can be propagated in *E. coli* bacteria. Plasmids are circular molecules of DNA found outside chromosomes.

Thus engineered, the bacteria are cultured on plates, where they form thousands of colonies. At this stage, they become known as "libraries," because each bacterial colony contains a plasmid with a unique cDNA from switchgrass.

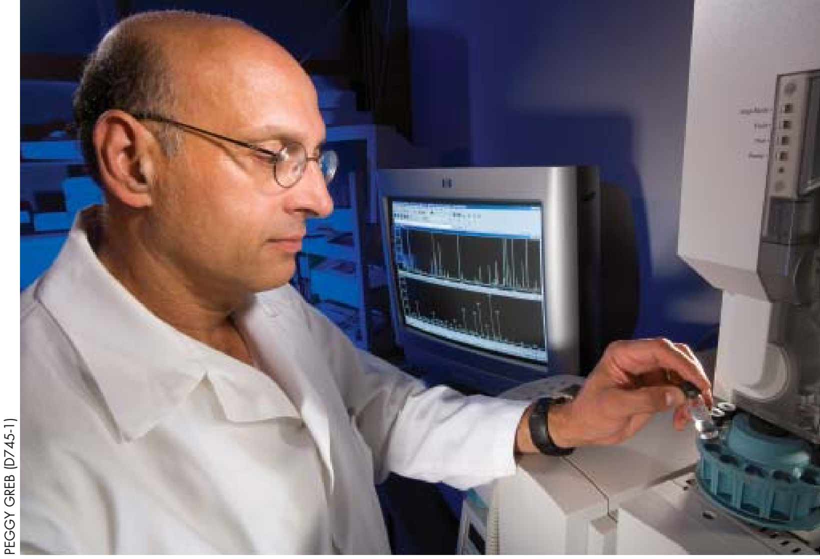
Since 2003, Sarath and collaborator Paul Twigg of the University of Nebraska-Kearney have produced several cDNA libraries from switchgrass. From these, Christian Tobias, a molecular biologist at ARS's Genomics and Gene Discovery Research Unit in Albany, California, has determined the structure or sequence of some 12,000 previously unknown switchgrass gene fragments.

Genetic Diamonds in the Rough

In a preliminary analysis of the sequences, Tobias and co-investigators grouped about 65 percent of the new sequences into clusters based on commonalities in their structures. Each of these groups may prove to be a unique gene. The sequence fragments were then compared with databases containing well-characterized genes to provide insight into the possible function of each new switchgrass sequence.

“A closer examination of fragments within clusters revealed that some seemed to have some slight variations. These variations are of interest because they might lead us to a trait that we want to investigate further,” Tobias points out. “These sequence variations reflect and reveal a portion of the genetic variability within the world’s switchgrass gene pool and can be both associated with desirable traits and used in breeding and switchgrass-improvement programs.”

Tobias and Sarath posted the gene sequences to publicly accessible databases on the Internet in 2005. This treasure trove of new discoveries is the most extensive catalog of switchgrass genes yet available for scientists everywhere to use. Researchers can, with the aid of computers, quickly compare and contrast the structure of switchgrass genes to those of other grasses or other forms of life.



PEGGY GREB (D745-1)

Molecular biologist Gautam Sarath loads vials containing hydrolyzed switchgrass cell-wall samples for analyses of lignin content by gas chromatography-mass spectrometry. The data will be used to identify elite switchgrass plants for improvement through breeding.

Genes from one organism that look like those from another may perform the same job in both. And if that job has already been discovered for the one organism, “you have a head start in correctly identifying its role in switchgrass,” explains Tobias.

Using this comparative approach, Sarath and Twigg have pinpointed a cluster of 12 to 14 genes regulating production and deposition of lignin, a molecular “glue” that binds components of plant cell walls. Sarath notes that bioenergy researchers are keen on weakening lignin’s grip—either through conventional breeding or genetic engineering—to free up more sugars from cell walls for fermenting into ethanol. (See story on page 4.)

The team’s original cDNA libraries came from a single switchgrass variety. Others will be added, including lowland bioenergy-switchgrass types from a breeding and economic-evaluation program run by GFBRU research leader Kenneth P. Vogel and rangeland scientist Robert Mitchell.

PEGGY GREB (D748-1)

In Nebraska, technician Marty Schmer harvests switchgrass to evaluate yield potential. These trials will guide breeding and management efforts to increase switchgrass yields.

Helping Hands

The scientists are sending these libraries and RNA to the U.S. Department of Energy’s Joint Genome Institute in Walnut Creek, California. There, fast, state-of-the-art gene-sequencing instruments will identify up to a half-million switchgrass sequence fragments, called “expressed sequence tags” (ESTs), within the next 3 years. These sequences will be compared with those from other plants—particularly other grasses, such as corn and rice—providing invaluable data.

“These ESTs will give us the tools to really understand, or look for, genes important for breeding purposes,” adds Sarath. For this ambitious venture, he and Tobias have already supplied several of the requisite cDNA libraries for the institute’s ultrafast analyses.

Meanwhile, ARS researchers elsewhere are exploring innovative new ways to improve switchgrass for biofuel and other uses. Some examples of this research include:

• Studying how plant cell walls are made in order to learn how best to break them down—research that should make forage crops like switchgrass more digestible for livestock and more degradable for biofuel production (Hans Jung, dairy scientist, St. Paul, Minnesota).

• Developing switchgrass management tools and conducting greenhouse-gas life-cycle assessments to provide the best combination of biofuel yield and quality, and environmental benefits (Paul R. Adler, agronomist, University Park, Pennsylvania).

• Using enzyme treatments to extract phenolic acids as value-added coproducts (Danny E. Akin, microbiologist, Athens, Georgia).

• Putting both dilute acid and enzymes to work in obtaining fermentable sugars from switchgrass and other species (Bruce S. Dien, chemical engineer, Peoria, Illinois).

From seed to fermentable sugars, such research is helping expand switchgrass’s horizons beyond the prairie and into the bioenergy plants of tomorrow.—By **Jan Suszkiw** and **Marcia Wood,** ARS.

This research is part of Bioenergy and Energy Alternatives, an ARS National Program (#307) described on the World Wide Web at www.nps.ars.usda.gov.

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The Gene Pool Deepens

The original cDNA libraries that led to the team's switchgrass gene discoveries (see previous page) came from a variety called Kanlow. As its name implies, Kanlow is best suited for Kansas prairie lowlands. But researchers need to know about the work of genes in the flowers, leaves, stems, and roots of switchgrass plants from throughout its native range.

To make that happen, scientists like ARS plant pathologist Joseph M. Krupinsky at Mandan, North Dakota, have contributed switchgrass tissue to colleague Paul Twigg, to create RNA and cDNA libraries with a richer and more diverse assembly of switchgrass genes. Samples also come from Kenneth Vogel's program at Lincoln.

In all, the new RNA and cDNA libraries are pools or mixtures of specimens, specially selected to add needed breadth, depth, and diversity that otherwise might be missed were the analyses based solely on a single commercial variety such as Kanlow.

The researchers have included individuals encompassing most of the genetic diversity within the 48 conterminous United States.—By **Marcia Wood,** ARS.

STEPHEN AUSMUS (D854-1)

Switchgrass.

Science Update

KEITH WELLER (K3024-19)

Shiitake mushrooms.

Boosting Ethanol Production—With Shiitakes?

The ability of shiitake mushrooms to dissolve the fallen logs that they call home may one day speed conversion of farm-based feedstocks into ethanol for fuel. That's because scientists have found and copied a shiitake gene, called *Xyn11A,* that lets the mushroom produce an enzyme known as xylanase. This enzyme helps convert decaying wood into sugars that shiitakes use for food.

In the laboratory, researchers successfully transferred the gene into a yeast, which was then able to produce xylanase—something it can't normally do. Next they'll try to modify the *Xyn11A* gene so it enables the yeast—or some other organism—to make more of the xylanase enzyme in less time. Such a boost in efficiency might one day help make production of plant-based fuels an even more practical alternative to petroleum products. *Charles C. Lee, USDA-ARS Bioproduct Chemistry and Engineering Research Unit, Albany, California; phone (510) 559-5858, e-mail charles.c.lee@ars.usda.gov.* ✸

Wheat

A New Option for Carry-Out Containers

Those lightweight, polystyrene containers that some restaurants give you for carrying home leftovers or take-out meals are known in the foodservice industry as "clamshells." Their hinged-lid construction indeed resembles the architecture nature uses for clams, oysters, and other familiar bivalves.

Every year, billions of these clamshells and other foodservice containers made from petroleum-based foams end up in already overstuffed landfills. Slow to decompose, they become yet another environmental burden.

But the containers, along with other disposable foodservice items such as plates, bowls, and cups, can also be manufactured with biodegradable ingredients.

ARS plant physiologist Gregory M. Glenn is working with EarthShell Corp., the California-based innovators of potato-starch-based foam products such as burger boxes, to create environmentally friendly disposables made with starch from wheat, the world's most widely planted grain. His wheat-starch-based prototypes are sturdy, attractive, convenient to use, and just as leakproof as their polystyrene counterparts. Glenn is with the Bioproduct Chemistry and Engineering Research Unit at ARS's Western Regional Research Center in Albany, California.

Why use wheat starch in packaging? Because it offers manufacturers of foodservice products another choice among starches when they're buying raw materials. That purchasing flexibility can help keep their prices competitive with the polystyrene products. Another important cost savings: The machinery already used to make EarthShell's potato-starch-based containers is suitable for the wheat-starch products as well. That sidesteps the need for costly retooling at manufacturing plants.

"The machines are presses or molds that work something like giant waffle irons," explains Glenn. "First, a wheat-starch batter is poured onto the heated mold, which is then closed and locked. Moisture in the batter generates steam that, in turn, causes the batter to foam, expand, and fill the mold. The steam is vented and, when the baking is finished, the mold is opened, the product is removed, and the cycle starts again. This whole process takes less than a minute."

A water-resistant coating, added later, helps the container keep its strength and shape when it's filled with a hot, juicy cheeseburger or creamy pasta alfredo leftovers, for example. But once the container hits the backyard compost pile or municipal landfill, it biodegrades in only a few weeks.

Perhaps having our ready-to-eat meal packed for us in a guilt-free throwaway container, such as a wheat-starch-based clamshell, will make eating those foods even more enjoyable.—By **Marcia Wood,** ARS.

This research is part of Quality and Utilization of Agricultural Products, an ARS National Program (#306) described on the World Wide Web at www.nps.ars.usda.gov.

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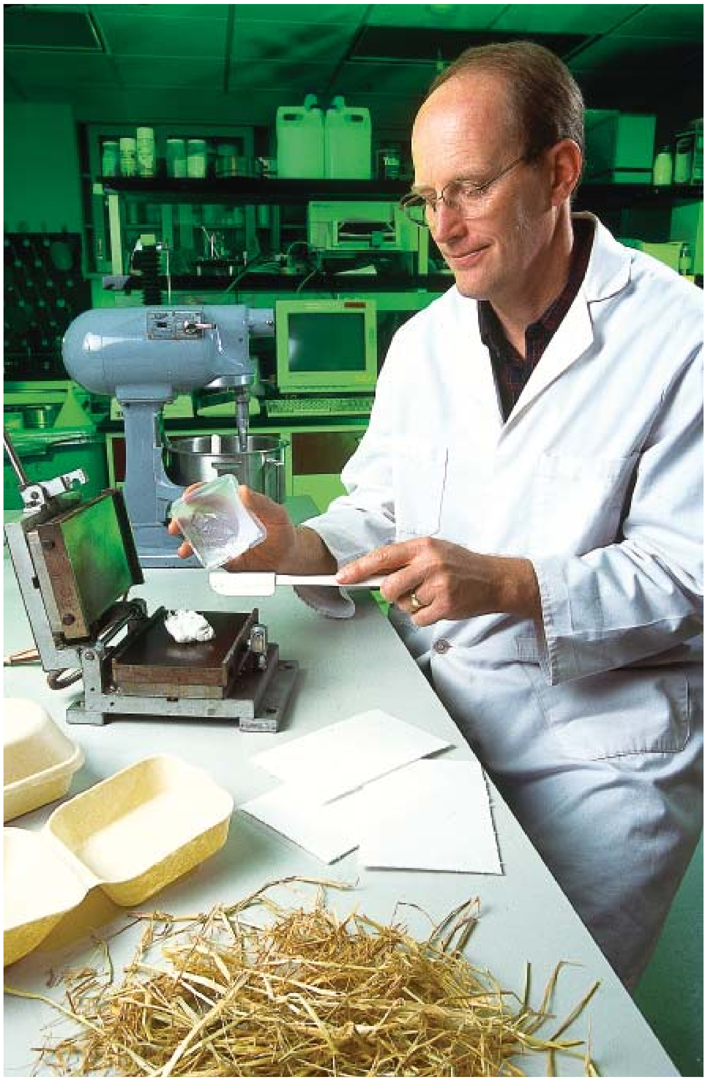
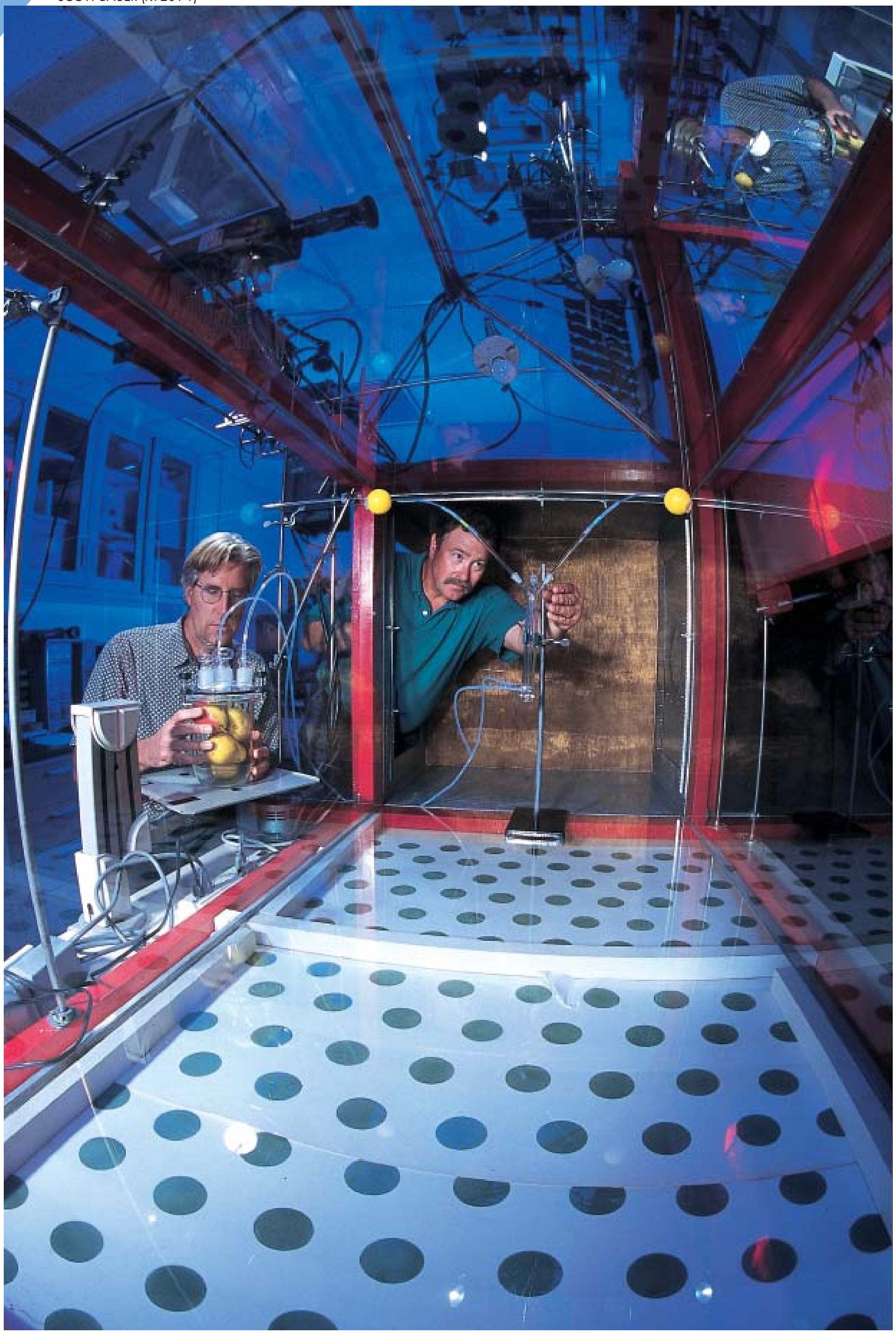
PEGGY GREB (K9838-1)



Plant physiologist Gregory Glenn prepares to make wheat-starch biodegradable containers.

Fruit **Perfume** Lures Female Codling Moths

SCOTT BAUER (K9201-1)



In the laboratory, entomologists Alan Knight (left) and Doug Light set up a flight tunnel experiment to test which pear odors attract female codling moths.

The luscious aroma of ripe pears could spell doom to codling moths. Agricultural Research Service scientists have discovered that a certain chemical in pears provides just the right perfume to attract female codling moths to traps. That's a breakthrough that may revolutionize integrated pest management (IPM) in apple, pear, and walnut orchards worldwide—and will help growers to continue providing safe food more economically.

Codling moths are the most severe and widely distributed pest of apples, pears, and walnuts in the world. The moths were accidentally introduced into the United States from Europe in the 1700s and have been a menace ever since.

Uncontrolled, the larvae—the worm in the apple—can destroy up to 95 percent of an apple crop and up to 60 percent of a pear crop. Feeding by larvae creates holes in walnut hulls and shells that can allow fungi to enter and infect the kernels. Some fungi produce toxins that at high levels are a food safety concern. Both federal and international regulations prohibit growers from selling toxin-contaminated nuts.

The Main Attraction

"Sex attractants called pheromones have been the main tool available to growers for monitoring codling moths in orchards," says ARS entomologist Douglas M. Light.

Pheromones, which are chemicals given off by female moths to attract males for mating, have allowed scientists and growers to trap and monitor male moths in orchards.

A synthetic version of the codling moth pheromone has been available for 30 years and provides the basis for mating-disruption programs. Growers can disrupt the ability of males to find a mate by flooding the orchard canopy with pheromone. Or they can time insecticide sprays according to the number of moths found in traps baited with pheromone.

Now Light has found a new, potentially more useful tool.

"We've found a natural chemical in a fruit that is as effective as pheromones in attracting moths. But more importantly, this compound not only attracts males, it lures females," Light says. He works in the USDA-ARS Plant Protection Research Unit at the Western Regional Research Center in Albany, California.

While pheromone-based programs target male moths, the real goal has always been to reduce the female's ability to reproduce. Females can lay 50 to 100 eggs, and two or three generations can hatch each growing season. Attracting females directly would allow growers to eliminate the females and their unlaid eggs and to monitor mating cycles more precisely.

"IPM programs are based on the relationship between the time when male moths appear in pheromone traps and the time eggs begin to hatch," says ARS entomologist Alan L. Knight. To predict insect development, scientists use a measurement tool called a degree-day model. For codling moths, eggs are believed to hatch after the accumulation of 250 degree days—which ranges from 15 to 30 calendar days—after the males emerge and are detected in sex-pheromone-baited traps.

"There's a lot of potential error in estimating egg hatch based on the number of males trapped," says Knight. "We think that being able to monitor females should be a lot more accurate."

In field tests, Knight verified that the pear-derived attractant is more effective than pheromones in monitoring—and potentially predicting—mating and egg laying.

New Strategies Needed

"In the past, growers would use long-lasting insecticides that would cover the range of possible days when moths could lay eggs and larvae could hatch," Knight says. "But as environmental regulations require use of less toxic and shorter-lived pesticides, it becomes important to know exactly when the females mate and begin their egg laying."

Once the larvae hatch, there is only a short time before they crawl to and bore into the developing fruit, where they are safe from external controls.

Although apples and pears generally suffer more from codling moth attacks, Light was looking for a way to protect walnut orchards. He wondered if using one of their preferred foods—pears—would lure the insects away from the walnuts.

Finding What Rings Their Bell

Light worked with a team of ARS chemists at the Albany laboratory to obtain 37 combinations of the chemicals that make up pear odors and flavors. Improvements in chemical detection methods over the last two decades allowed the scientists to isolate and identify the many components that make up a wide range of fruit and nut odors and flavors.

One of the mixtures showed extraordinary promise as a codling moth attractant. They discovered that the key attractant was a chemical known as the pear ester, or ethyl (2E, 4Z)-2,4-decadienoate.

To pursue the commercial potential of his discovery, Light established a cooperative research and development agreement with Trécé, Inc., of Salinas, California. Clive Henrick, vice president of research and development at Trécé, used synthetic chemistry techniques to confirm that one key chemical in pears attracted the moths.

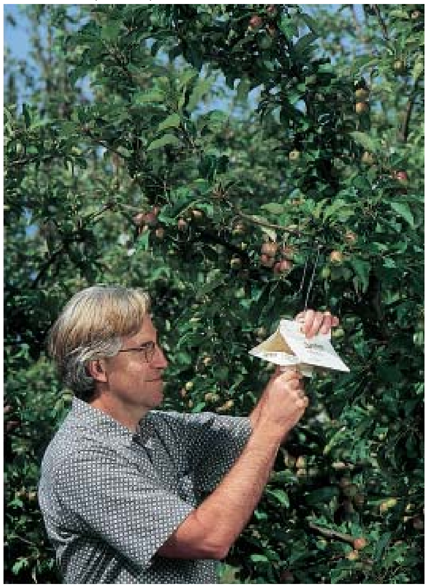
Pear ester, they found, acts as a kairomone. Unlike a pheromone, which involves only one species, kairomones are chemicals emitted by one species—in this case pears—that attract and benefit another, such as codling moths. The moths have apparently evolved to detect this odor and use it to locate a preferred food.

"There are a lot of known kairomones, but most are worthless for com-

SCOTT BAUER (K9206-1)

Codling moths of both sexes are strongly attracted to a chemical in pears.

SCOTT BAUER (K9204-1)



Entomologist Alan Knight places a pear-based kairomone trap in an apple orchard to attract codling moths.

SCOTT BAUER (K9205-1)

The high level of attractiveness of the pear ester could be useful in developing "attract and kill" traps that reduce pesticide use while removing moths from orchards before they reproduce.

SCOTT BAUER (K9202-1)

Technician Kathy Reynolds, entomologist Doug Light (center), and Trécé field development manager Scott Lingren inspect a trap baited with pear ester for use in codling moth monitoring in a walnut orchard.

mercial applications to monitor insects under field conditions," Henrick says. "This one is fantastic."

ARS and Trécé have applied for a patent on use of the pear ester for codling moth monitoring and control.

Doing More With Less

Trécé also plans to include the attractant in a lure containing insecticides. This attracticide will kill moths that contact it. "This approach will use less than 10 percent of the amount of insecticide that would normally be used," Henrick says.

Researchers estimate that 90 to 95 percent of male codling moths in an orchard must be trapped or prevented from finding a mate to reduce the number of fertile eggs laid to an economically manageable level. "But for each female trapped, dozens of eggs are immediately eliminated," says Knight.

"Right now, there is no effective and economical way to determine how many females are in an orchard," says Light. "Female lures may help growers reduce pesticide use either by disrupting mating or by helping growers time their pesticide use more precisely."—By **Kathryn Barry Stelljes,** ARS.

This research is part of Food Safety (#108) and Crop Protection and Quarantine (#304), two ARS National Programs described on the World Wide Web at www.nps.ars.usda.gov.

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South African Insects May Help Against Cape Ivy

A fly and a moth from South Africa may soon be enlisted to help protect natural treasures in California, Oregon, and Hawaii. That's because these insects might serve as biological control agents for Cape ivy, *Delairea odorata.*

Previously known as German ivy, *Senecio mikanioides,* Cape ivy was introduced from South Africa as an ornamental vine in the late 1800s. It soon escaped and now infests prized natural coastal areas in California up into Oregon and native upland forests on the island of Hawaii. Willow-dominated riparian areas in California have been among the most overrun so far.

"In its homeland, Cape ivy is hard to find," says ARS entomologist Joseph K. Balciunas. "That suggests there are natural insect enemies helping to limit it in South Africa." He works in the Exotic and Invasive Weed Research Unit at ARS' Western Regional Research Center in Albany, California.

"Because Cape ivy is uncommon there, the South Africans didn't know much about the plant's distribution," Balciunas says. He teamed up with Beth Grobbelaar and Stefan Neser, of the Plant Protection Research Institute in Pretoria, South Africa, to search for natural enemies. "In 2 years of surveys, we've more than doubled the knowledge about native locations where Cape ivy grows. We've also identified several insects that could serve as biological control agents here," he says.

In January, Balciunas brought two of those candidates into the insect quarantine facility in Albany. (See "Foreign Agents Imported for Weed Control," *Agricultural Research,* March 2000, p. 4.)

The Cape ivy gall fly, *Parafreutreta regalis,* lays eggs in the tips of stems, where vines and leaves would normally develop. When the larvae hatch, they feed inside the shoots, causing the plant to produce galls (swellings) about the size of a large marble. Although the fly may not cause much direct damage to the ivy, it could slow the vine's ability to spread by decreasing leaf and shoot production.

The other new arrival is a tiny moth, *Acrolepia* sp., recently discovered by Neser. Its larvae—less than one-quarter inch long—form tunnels between the layers of plant tissue in the stems and leaves as they are feeding.

"Native willows could benefit immediately if these insects keep the ivy close to the ground by decreasing vine and leaf production," Balciunas says. Cape ivy harms willows by overgrowing saplings and blocking out light the trees need to survive.

Balciunas will test the insects in quarantine to make sure they don't feed on desirable plants—a process expected to take about 3 to 4 years. "Cape ivy is the only plant in the genus *Delairea,*" he says, "which makes it more likely that we'll find an insect that feeds specifically on the weed—a key criterion for a safe biological control agent." He also plans to test several other beetles and moths that the team discovered in South Africa.—By **Kathryn Barry Stelljes,** ARS.

This research is part of Crop Protection and Quarantine, an ARS National Program (#304) described on the World Wide Web at www.nps.ars.usda.gov.

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BETH GROBBELAAR (K9423-1)

This tiny (about a quarter-inch long) stem-boring moth, *Acrolepia* sp., is being evaluated as a biological control for Cape ivy.

Squelching Water Primrose

With its beautiful, bright-yellow flowers, water primrose might seem like the perfect aquatic plant to enhance a serene backyard pond.

But if it's a South American water primrose that you have in mind—*Ludwigia hexapetala* from Uruguay, for instance—don't be tempted. This species and other invasive water primroses are weeds that can crowd out native plants.

In South America, both the shrub-forming and small-statured water primrose plants are well-behaved members of watery natural ecosystems. In the United States, however, these aggressive, nonnative *Ludwigia* plants infest ponds, lakes, streams, rivers, canals, and reservoirs along both East and West coasts, and at some inland sites, as well.

Now, ARS researchers in the United States and Argentina are targeting the alien water primrose species in a new effort to keep people, pets, livestock, and farmland safe from the threats these plants harbor.

Along with providing a haven for mosquitoes that could carry West Nile virus, the plants pose a hazard to growers because the dense mats that the weed forms can easily clog water channels, causing overflows in adjacent fields, orchards, and vineyards.

But insects that attack unwanted water primrose species may help stop the plant's spread. Ray Carruthers and Lars Anderson, with ARS in California; Cristina Hernández and Willie Cabrera Walsh in Argentina; and colleague Robinson Pitelli in Brazil pinpointed some promising candidate insects in a 2005 trek through wet and wild regions of Argentina and Brazil. The intent of their journeys? Find critters that dine on invader water primrose species—but not other plants.

The scientists' work confirmed ARS studies in South America, completed in the 1970s. That earlier work, put on hold for studies on more threatening weeds, had also spotlighted the potential of a beetle with the tongue-twisting name of *Lysathia flavipes* and several similarly hard-to-pronounce weevils—*Ochetina bruchi, Auleutes bosqi,* and some *Onychylis* and *Tyloderma* species. That's according to Juan Briano, who heads ARS's South American Biological Control Laboratory in Hurlingham (near Buenos Aires), where Hernández and Cabrera Walsh are based.

Hernández leads the Hurlingham quest to identify some of the other primrose-eating insects that the scientists brought back from their expedition. And she's heading the exacting "host-specificity" testing that determines whether the insects will attack water primrose exclusively. This research yields the all-important guarantee that these candidate biological control agents, if brought into the United States to tackle water primrose, won't wander from an infested pond to have a taste of something a little different—someone's prize rose bushes, for instance.

Meanwhile, thousands of miles away in Davis, California, ARS ecologist Brenda Grewell is hunting for answers to tough questions about water primrose's little-known life cycle. Her aim: find times of high natural vulnerability. These could become critical windows of opportunity for clobbering the plant. She'll determine, in other studies, the best strategies for reestablishing the native vegetation that water primrose pushes out and the best ways to ensure that—once primrose is gone—it stays away.

From these and other meticulous studies underway in North and South America, science-based strategies for bringing water primrose under control are likely to blossom soon.—By **Marcia Wood,** ARS.

This research is part of Crop Protection and Quarantine, an ARS National Program (#304) described on the World Wide Web at www.nps.ars.usda.gov.

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BECKY MILLER (D485-1)

BRENDA GREWELL (D484-1)

ARS plant ecologist Brenda Grewell stands amid early spring growth of water primrose in California's Laguna de Santa Rosa. The invasive plant can disrupt entire ecosystems.

Right: Close-up of *Ludwigia,* a member of the evening primrose family that sports beautiful yellow blossoms throughout the summer.

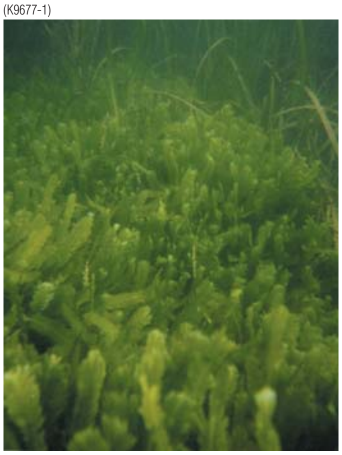
More Troublesome Water Weeds Targeted by Researchers

A pleasant saltwater lagoon on the sunny coast of southern California made botanical history last year when a dreaded marine alga, *Caulerpa taxifolia,* showed up there. The infestation at Agua Hedionda lagoon in Carlsbad, about 30 miles north of San Diego, was the first discovery in the Western Hemisphere of the Mediterranean strain of this notorious alga.

Caulerpa was next detected in Huntington Harbor, a small, isolated marina near Long Beach, California.

ARS plant physiologist Lars W. J. Anderson, together with industry, university, state, and federal colleagues, worked quickly to quash the weed before it could spread farther up the coast.

(K9677-1)



Marine alga, *Caulerpa taxifolia.* Photo by Rachel Woodfield, Merkel & Associates.

In preliminary experiments, Anderson and co-researchers tried several commonly used aquatic herbicides but found that liquid chlorine, injected under 10- to 20-foot-square black plastic tarpaulins spread atop the weed, worked best at Agua Hedionda. At Huntington Harbor, they used solid, puck-shaped disks of chlorine instead of liquid. Both killed the algae. "Nothing else seemed to work," Anderson says. He is with the ARS Exotic and Invasive Weeds Research Unit, stationed at Davis, California.

C. taxifolia is one of about a half-dozen water weeds that Anderson and other scientists in the research unit are investigating. The unit is headquartered at the ARS Western Regional Research Center in Albany, California.

PEGGY GREB (K9658-1)

Close-up of yellow starthistle *(Centaurea solstitialis).*

Conquering *Caulerpa*—A Marine Miscreant

Sometimes referred to as "killer algae," *C. taxifolia* flourishes in warm saltwater harbors, bays, and lagoons. It spreads remarkably fast, crowding out native species of algae and sea grasses. "*Caulerpa* essentially ruins conditions for a whole host of marine animals, such as small mollusks," says Anderson.

"No one knows exactly how it ended up at the lagoon or at Huntington Harbor," he says, "but we suspect that someone dumped the contents of an aquarium." *Caulerpa* was listed by the federal government as a noxious weed in 1999, which means that it is illegal to sell it in the United States. However, there is still a market for it. People like to have this weed in their fish tanks because it grows so well. They may not even be aware of the effects it can have if it escapes to the outdoors.

Today, the black plastic tarps still remain in place. The scientists and their collaborators continue to monitor the outbreaks regularly. "We know that chlorine kills the top of the *Caulerpa* plants," Anderson explains, "but the real question is what's happening below, in the mud. We know that the plant forms structures called rhizoids that could produce new growth. We need to be sure that the chlorine kills them."

In testing the viability of the rhizoids after aboveground parts of the plant have been killed, Anderson is collaborating with Susan Williams, director of the University of California at Davis Bodega Marine Laboratory, just north of San Francisco. "We're also looking at other algicides," notes Anderson.

Arundo donax Raises Cain

Unlike the algal newcomer *Caulerpa,* a bamboolike invader called giant reed or giant cane has been an unwanted intruder in California for at least 100 years. Known to scientists as *Arundo donax,* this member of the grass family can grow 3 to 7 inches a day, reaching 30 feet in height. Plants sport feathery white plumes called panicles.

Arundo is used as an ornamental plant for landscaping and as a source of reeds for musical instruments, such as bassoons and bagpipes. Its cellulose can be used for papermaking.

Found from Maryland to California, *Arundo* thrives along streams and ditches. A fierce invader of freshwater ecosystems, *Arundo* crowds out riparian regulars like alders, cottonwoods, and willows that would otherwise provide a home for wildlife and cooling shade vital for fish and other aquatic creatures. The plant recovers rapidly after wildfire, sending up hardy new sprouts from its roots. What's more, bits of *Arundo* that break off and travel down creeks and streams can readily start new infestations.

PEGGY GREB (K9656-1)

Ecologist Ray Carruthers examines saltcedar *(Tamarix parviflora)* along Cache Creek.

Despite the weed's long history in the United States, there's very little published information about its basic biology and ecology, according to David F. Spencer. He is with the ARS team at Davis.

To help fill in the gap, Spencer and colleague Greg G. Ksander, also at Davis, are gathering data needed for equations that can be used to predict *Arundo's* growth at various stages of its life cycle under various environmental conditions. Explains Spencer, "Some of *Arundo's* life stages may be more susceptible than others to certain control tactics, like herbicides or biological control. And environmental conditions such as temperature are likely to influence growth and development, but we don't have specific details."

As a starting point, Spencer and Ksander looked at the effects of temperature and soil nitrogen levels on sprouting. "We want to determine the environmental conditions that cue *Arundo* to produce new sprouts from its rhizomes." Those thick, underground stems are critical to *Arundo's* long-term persistence.

In tests of rhizome sections and stem cuttings kept indoors for 12 weeks at 44.6˚F, 57.2˚F, or 68˚F, the researchers found that new shoots emerged and survived at 57.2˚F and 68˚F and emerged sooner at the higher temperature. At 44.6˚F, no shoots grew from rhizome sections; only a single shoot sprouted from a stem cutting but soon died.

In a second experiment, the scientists varied the level of the nitrate form of nitrogen applied to rhizome sections during weekly watering. Notes Spencer, "Some studies done at other labs have indicated that nitrate in the soil, which may fluctuate seasonally, stimulates some seeds to germinate."

The researchers exposed rhizome sections to 46.4˚F or 60.8˚F for 14 weeks and applied concentrations of nitrate ranging from 0.3 to 6.0 milligrams per liter of water. "New shoots emerged at 60.8˚F but not at 46.4˚F," reports Spencer. "Neither the number of shoots that emerged nor the length of time it took for them to appear was influenced by the amount of nitrate that we applied."

In an experiment with rhizomes planted outdoors at Davis, the scientists found that shoots first appeared in late March, when the average weekly temperature was 52.7˚F. New shoots continued to emerge until November.

"With this new information, we can develop equations that relate sprouting to temperature over time, or what's known as accumulated degree-days," Spencer says. "Because nitrate—at

PEGGY GREB (K9657-1)

Ecologist David Spencer (right) and technician Greg Ksander collect an *Arundo donax* leaf sample for tissue nitrogen analysis.

the levels we applied—didn't stimulate sprouting, the equations won't have to include nitrate fluctuations."

Fish and wildlife specialists, water district managers, and other streamkeepers throughout the United States can use the information from the equations. It will help them decide the best timing for whatever management technique they choose to bring this aggressive weed under control.—By **Marcia Wood,** ARS.

This research is part of Crop Protection and Quarantine, an ARS National Program (#304) described on the World Wide Web at www.nps.ars.usda.gov.

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PEGGY GREB (K9655-1)

Ecologist Ray Carruthers measures plant and flower head density of yellow starthistle while technician Justin Weber uses a sweep net to sample populations of biocontrol agent *Chaetorellia succinea.*

Tackling a Tough Trio

As if giant reed weren't enough of a problem, this rugged invader often grows interspersed with another waterside menace, saltcedar, or *Tamarix parviflora.* These weeds may leave off further inland, but then another unwelcome intruder, called yellow starthistle, *Centaurea solstitialis,* often takes over.

"These exotic species are thought to be the three most serious plant invaders in riparian ecosystems and adjacent uplands in the West," says ARS ecologist Raymond I. Carruthers. He is leader of the ARS Exotic and Invasive Weeds Research Unit, based at the Western Regional Research Center in Albany, California.

Funded in part by a grant from the Initiative for Future Agriculture and Food Systems, Carruthers co-manages a unique research project aimed at helping land managers tackle all three problem weeds at once in what's known as an ecosystem-level approach. At least three dozen federal, state, and private agencies, universities, and organizations are working together to develop the best biological control strategies for combating these weeds and restoring native plants.

"People have joined this team effort because they're convinced that biological control has the ecological and economic potential to fight established invasive species," says Carruthers.

Program participants are sharing the work of evaluating the risks and effectiveness of biological control agents such as weed-eating insects. They are also helping develop innovative techniques to prevent the weeds from successfully reinvading. And they're helping make sure that land managers have the newest and best available information about successful weed-management strategies.

"Results of this project," notes Carruthers, "may provide a model that people elsewhere can use to get the upper hand with other groups of invasive plants."—By **Kathryn Barry Stelljes,** formerly with ARS.

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Beneficial Beetles Take a Bite Out of Saltcedar

Can a tiny beetle spell doom for a rugged, aggressive weed that's already invaded streambanks and river channels throughout the American West?

Yes!

That's what ARS scientists and their colleagues have found in their investigations of a leafbeetle, *Diorhabda elongata,* that they've brought to sites infested with saltcedar, also known as tamarisk.

This salt-tolerant, fire-resistant, drought-hardy water guzzler was imported in the 1800s to help hold stream and river banks in place.

Today, saltcedar's range extends from the Great Plains to the Pacific and from Canada to northern Mexico. It has crowded out native plants such as cottonwoods and willows, disrupting the natural surroundings needed by these plants as well as by birds, fish, and other forms of life.

Saltcedar resprouts vigorously even after the hottest blazes. It blocks river and stream access—by river rafters or thirsty wildlife and livestock—by forming dense, often impenetrable thickets, first as bushy shrubs and, later, as pink-blossomed trees that grow to 30 feet.

In addition, saltcedar plays havoc with watercourses. When rivers and streams overflow their banks, saltcedar bushes can trap natural flood debris, blocking waterflow and causing new, erosive channels to form. These channels sometimes undercut farm roads and fields, causing them to collapse.

But the jaunty beetle, imported from China, Kazakhstan, other parts of Asia, and the Mediterranean region—all lands where the troublesome tree is native—has taken an impressive bite out of saltcedar in several study sites in the West.

To this insect, America's vast stands of saltcedar are just one enormous, seemingly endless banquet. The plant's scale-like leaves offer a nutritious treat for the insects, whether they're in their caterpillar-like larval stage or have matured into quarter-inch-long adult beetles.

PEGGY GREB (K9656-1)

Ecologist Ray Carruthers examines saltcedar *(Tamarix parviflora)* along Cache Creek.

Besides thriving on emerging leaves and shoots, the beetles also nibble on the bark of small twigs, all the while posing no hazard to people, pets, or crops. That's according to ARS entomologist C. Jack DeLoach and ARS ecologist Raymond I. Carruthers. DeLoach is based at the Grassland Protection Research Unit in Temple, Texas. Carruthers works in the San Francisco Bay area at the ARS Exotic and Invasive Weeds Research Unit, part of the Western Regional Research Center at Albany, California.

Scoring Big in Lovelock, Nevada

The beetles' greatest success since first being turned loose in saltcedar stands in 2001 has been along the Humboldt River in Lovelock, Nevada, about 80 miles northeast of Reno. Here, an original colony of some 1,400 laboratory-reared beetles has "expanded exponentially and has now defoliated about 5,000 acres of saltcedar," says Carruthers.

Today, the helpful insects number in the millions. What's more, they've extended their range, spreading at least 100 miles along the Humboldt River—defoliating and stunting saltcedar. Some of those trees are beginning to die.

"We've seen a similar impact in some other study sites in Colorado, Nevada, Utah, and Wyoming, with hundreds of acres of trees hit hard by the beetles," notes Carruthers. He and DeLoach expect that figure to climb to thousands of acres by summer's end.

Experts like Carruthers and DeLoach

into agricultural lands in western states. He enlisted the help of colleagues in Asia to search for insects to control the plant.

Entomologist Robert Pemberton—then at an ARS laboratory in South Korea and now with ARS in Fort Lauderdale, Florida—collected *D. elongata* in China, making him the first to identify this insect's promise as a biological control agent for the United States.

Then followed nearly two decades of pioneering investigations of the beetle and the weed by collaborators Bao Ping Li in China, Roman Jashenko and Ivan Mityaev in Kazakhstan, and DeLoach.

In ARS-led outdoor tests, begun in 1998, beetles were confined with saltcedar and other plants in carport-sized screened cages. "The beetles proved effective in controlling saltcedar and at the same time safe for the environment," Carruthers says. Because the beetles controlled saltcedar but didn't feed on other plants, the scientists received federal and state approvals to turn the beetles loose at test sites in the West.

These studies represent the first time—in the more than 170 years during which tamarisk has run rampant in the United States—that any natural organism had been lined up to challenge this pest.

PEGGY GREB (K11824-1)

Diorhabda elongata **larva (typically 7 to 12 mm long) feeding on saltcedar.**

view biological control agents such as the *D. elongata* leafbeetle as perhaps the best long-term solution to getting tough with tamarisk.

Of course, the insects represent a considerable investment. Initially, they have to be collected abroad. Then they have to be extensively studied to make sure they don't inadvertently damage other plants. Finally, they have to be put to work outdoors and carefully monitored. Ideally, the beetles will provide an impressive return on these investments.

A Little History

The beetle saga began in the 1970s when ARS entomologist Lloyd A. Andres at Albany, now retired, became concerned about saltcedar's unchecked incursion

But much more remains to be done. Though the beetles have established many flourishing colonies, they originally failed to reproduce at southerly test sites, such as those in Texas.

Deceptive Daylengths Confuse Beetles

Entomologists Phil A. Lewis, formerly at DeLoach's lab, and Daniel Bean of the University of California at Davis, working at Carruthers's lab, discovered the reason for this failure to survive: Mistaking the region's shorter days as a signal of winter's approach, the beetles went into hibernation, called diapause, in July—2 months too early. The mistake was fatal. The following year, they never woke up to complete their life cycle. Without enough fat reserves in their bodies to tide them over until saltcedar leafed out in spring, they starved to death.

To help solve this problem, ARS scientists and collaborators collected *D. elongata* from Uzbekistan through Greece to Tunisia, where daylengths better align with those of the American Southwest.

"These southern-adapted beetles," says DeLoach, "are now defoliating saltcedars in California, Texas, and New Mexico," where ARS and university scientists are monitoring their progress.

Spying From the Sky

Now, the researchers are streamlining tactics to track the spread and success of the hungry insects. For example, ARS remote-sensing specialists Jim Everitt of Weslaco, Texas, and Gerald Anderson of Sidney, Montana, are using a trio of technologies to spy on the beetle and the plant.

This technological mix includes color aerial photography and two types of scanner-derived aerial imagery, hyperspectral and multispectral.

All have helped the saltcedar scientists assess the beetle's work. Monitoring the beetle's progress also entails scouting—on foot. That job may be less of a hassle in the near future, thanks to a new, ARS-developed lure that beetles can't seem to resist.

The intensive studies of the diminutive beetle have revealed other secrets about it. For example, what's been regarded as merely one species of beetle—with biotypes adapted to different areas—may actually be several different species. Too, the work has underscored the need for other biocontrol agents to complement the beetle. Those studies are being carried out at the ARS European Biological Control Laboratory, in Montpellier, France.

PEGGY GREB (K11827-1)

Technician Julie Keller and entomologist John Herr assess results of a host-specificity experiment measuring feeding damage by the biological control agent *Diorhabda elongata* on saltcedar (*Tamarix* spp.) and on nontarget native plants in the genus *Frankenia.*

OKLA W. THORNTON, JR. (D061-1)

Browning, defoliating saltcedar trees in Big Spring, Texas, in July 2004, 3 months after *Diorhabda* beetles from Crete, Greece, were released.

Setting New Records

ARS's tamarisk-taming studies link with efforts of others in the national Saltcedar Biological Control Consortium. Specialists from federal, tribal, state, regional, and environmental organizations are involved in tackling this weed. That makes this consortium likely the largest of its kind ever established in this country.

Also unparalleled in the history of U.S. biological control of weeds are the international scope of the research, the variety of ecosystems ARS scientists have scrutinized in their studies of the beetle, and the number of states encompassed in the outdoor tests.

Perhaps in another few years, this carefully orchestrated campaign to stop saltcedar's spread may become one of America's most remarkable biological control successes.—By **Marcia Wood** and **Don Comis,** ARS.

This research is part of Crop Protection and Quarantine, an ARS National Program (#304) described on the World Wide Web at www.nps.ars.usda.gov.

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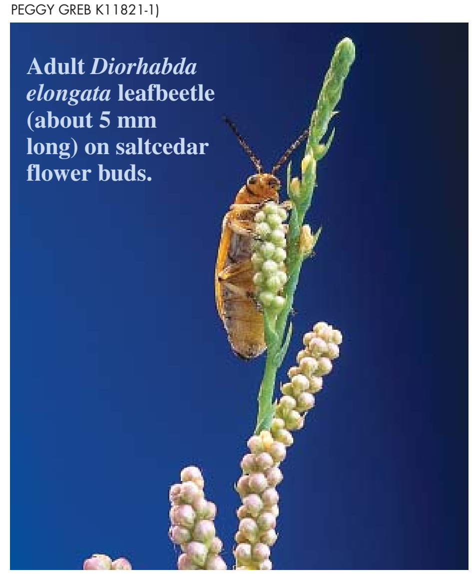
PEGGY GREB K11821-1)



Adult *Diorhabda elongata* leafbeetle (about 5 mm long) on saltcedar flower buds.

APHIS, BOB RICHARD (K8836-1)

A Chinese leaf beetle, *Diorhabda elongata.*

Beetles Sock It to Saltcedar

The first biological control agents to be set loose against invasive saltcedar, *Tamarix* spp., are Chinese leaf beetles, *Diorhabda elongata.* The 10- to 30-foot trees infest over a million acres along western waterways, displacing native plants and wildlife, increasing soil salinity, diverting natural streamflow, and increasing the frequency of wildfires.

Since July 1999, caged beetles have been carefully monitored at 10 locations in 6 western states. Now, their postrelease activity will be closely followed to ensure their establishment and evaluate their impact, population growth, and safety. The researchers want to protect native species in the release areas and to facilitate revegetation with native plants.

The project operates in conjunction with more than 30 federal, state, and local agencies; universities; and private organizations. A $3 million grant in 2000 from USDA's Initiative for Future Agriculture and Food Systems supports work on a complex of invasive weeds, including saltcedar. *C. Jack DeLoach, USDA-ARS Grassland, Soil, and Water Research Laboratory, Temple, Texas; phone (254) 770-6531, e-mail jack.deloach@ars.usda.gov; Raymond I. Carruthers, USDA-ARS Exotic and Invasive Weeds Research Unit, Albany, California; phone (510) 559-6127, e-mail ray.carruthers@ars.usda.gov.*

Minty-Fresh Fumigants

Environmental concerns are causing many key components of currently used fumigants to lose their federal registrations. And some stored-product insect pests are beginning to develop resistance to some widely used fumigants. These two trends could spell trouble for bulk grains that must be held in storage until markets open up for their sale and use.

So researchers have been testing oils extracted from 16 medicinal plants and spices for their effects against one important pest, the rice weevil, *Sitophilus oryzae*. This tiny insect causes major problems in granaries worldwide, reducing the grain's nutritional value and ability to germinate and exposing it to odor, mold, and heat damage.

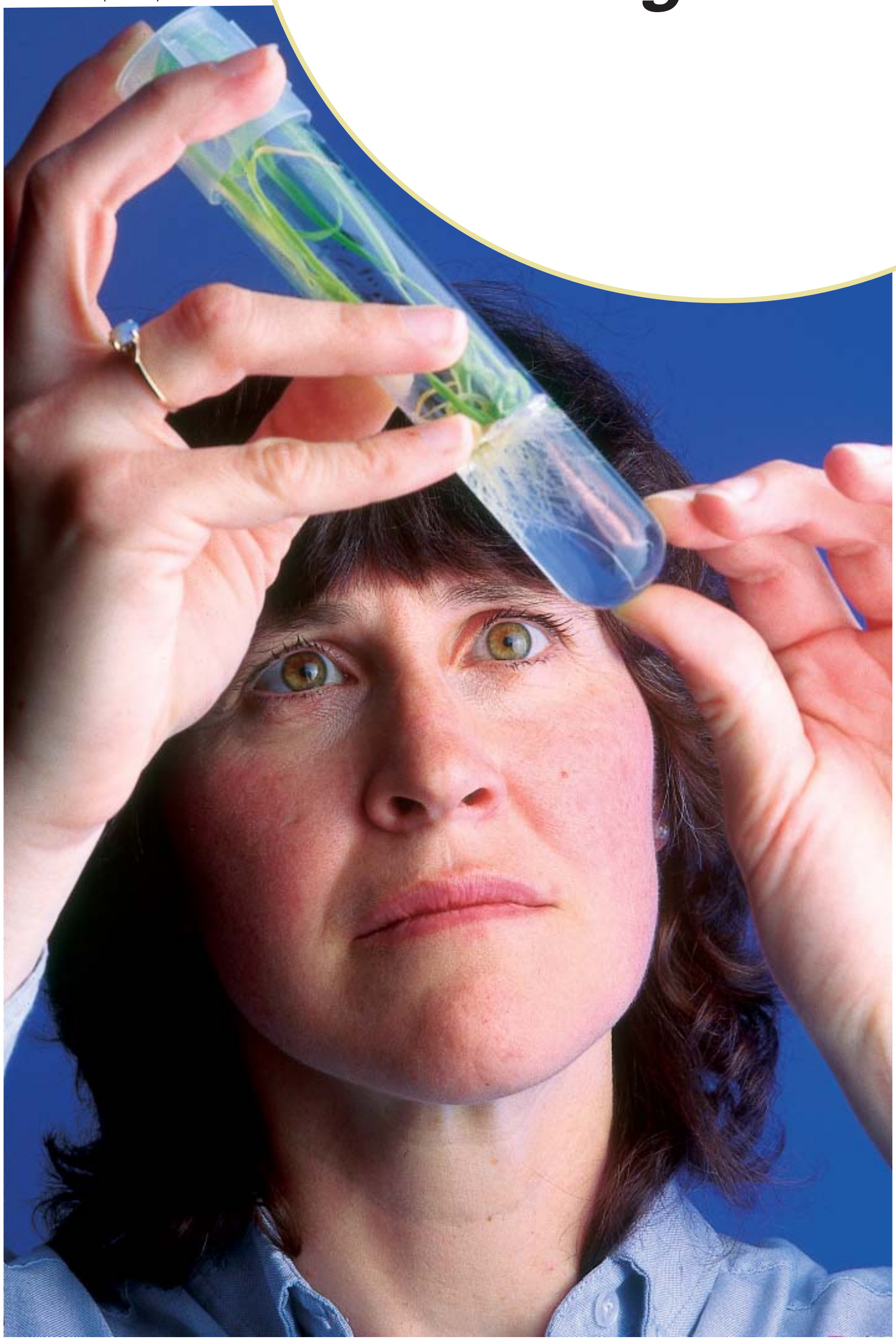
The most potent antiweevil oils proved to be found in Japanese mint, *Mentha arvensis*. Its active ingredient, menthone, could provide the basis for a new, environmentally friendly fumigant. But additional testing is needed to determine its impact on grain's smell and taste. *Bruce C. Campbell, USDA-ARS Plant Mycotoxin Research Unit, Albany, California; phone (510) 559-5846, e-mail bruce.campbell@ars.usda.gov.* ✷

KEITH WELLER (K7577-1)

Before rice is stored in granaries, it is often fumigated to control insect pests. In the future, new environmentally friendly fumigants may come from Japanese mint.

Gene Jockeys Fight *Fusarium* Head Blight

JACK DYKINGA (K5147-3)



Geneticist Ann Blechl and colleagues are the first to insert modified *Fusarium* chitinase and glucanase genes into wheat plants, which may lead to wheats that are more resistant to Fusarium head blight.

BRUCE FRITZ (K8472-2)

Lighter, discolored barley heads are infected with Fusarium head scab fungi.

In some years—no one can predict precisely which—young wheat and barley plants are beleaguered by a formidable fungal foe known as *Fusarium graminearum.* It is responsible for a disease known as wheat scab, or Fusarium head blight, which causes plump kernels to shrivel and take on an unhealthy, bleached, scabby appearance.

Right now, there's no effective control for this plant disease. Losses in any given year will vary, but can be enormous. From 1998 to 2000, for example, *Fusarium*-related losses amounted to an estimated $2.7 billion in the north-central and Great Plains states.

ARS, industry, and university scientists have joined forces to equip tomorrow's wheat and barley plants with a new weapon: genes that might provide a stronger natural defense against this longtime enemy.

The researchers are constructing some of these genes with pieces of genetic material from *Fusarium* itself. This "dirty trick" strategy may deceive the fungus and sidestep its natural defenses. What's more, this work might be applicable for fighting other major crop pests.

That's the plan of ARS geneticists Patricia A. Okubara at Pullman, Washington, and Ann E. Blechl at Albany, California. They're collaborating with Thomas M. Hohn, formerly with ARS at Peoria, Illinois, and now with Syngenta at Research Triangle Park, North Carolina, and Randy M. Berka of Novo Nordisk, in Davis, California. The scientists are seeking a patent for some of their innovative, antifungal genes.

Designer Genes May Quell Fungus

The researchers designed genes to cue wheat plants to make enzymes called chitinase and glucanase. These are the types of enzymes *Fusarium* manufactures when it needs to tear down some of its cell walls to grow and expand. Cell walls of *Fusarium* are made up, in part, of compounds called chitin and glucan, which the enzymes break down. This process occurs over and over again in the growing tip of the fungus's microscopic, rootlike structure, called a hypha.

A hypha is formed when a *Fusarium* spore—perhaps carried by wind or rain—lands on a wheat floret and germinates. The hypha's mission is to reach the developing kernel, where a storehouse of energy-rich starch compounds awaits.

In rebuilding wheat plants to make cell-wall-degrading chitinase and glucanase, the scientists intend to disrupt the hypha's orderly progress toward the food source inside the wheat plant. The strategy is to overwhelm the fungus with chitinase and glucanase that it didn't make and can't control.

Scientists have known about the role of chitinase and glucanase for years. But Okubara, Blechl, Hohn, and Berka are the first to use pieces of *Fusarium* chitinase and glucanase genes for making unique, antifungal genes. To mimic the microbe's own enzyme-making machinery as closely as possible, the researchers borrowed gene segments from a *F. graminearum* relative called *F. venenatum*. Its genes had been copied earlier into a "library" maintained by Novo Nordisk. That made this cousin a convenient source of the needed material.

Tooling Wheat To Make Crucial Enzymes

The scientists already knew that engineering a wheat plant to make chitinase or glucanase was unlikely to hurt the plant itself. That's because there's little or no chitin or glucan in wheat cell walls.

Okubara, Hohn, and Marcie Moore, who is with ARS at Peoria, have extensively rebuilt some of the *F. venenatum* genes. They have, for instance, filled in missing pieces of the original *F. venenatum* chitinase-forming gene. Okubara also outfitted the genes with a custom-built promoter. This on-off switch would enable a wheat plant to better use the newly redesigned genes.

At Albany, Blechl moved the lab-built genes into wheat embryos. She started by excising the embryos from immature wheat kernels. Then she placed the embryos into petri dishes positioned in the line of fire of a gene gun, or bioblaster. Blechl ferried the genes into the embryos on gold particles propelled by the gene gun.

Later, Blechl tested the target embryos to see whether they had taken up the genes. If they had, she nurtured them into healthy greenhouse plants. Blechl then shipped seeds from the plants to colleague Ruth Dill-Macky in St. Paul, Minnesota, for a rigorous program of screening. Dill-Macky, a plant pathologist with the University of Minnesota, is part of a team that evaluates promising new wheat plants for resistance to Fusarium head blight. The indoor and outdoor tests pinpoint new lines that may someday replace current varieties that have been clobbered by the fungus in the past.

Pyramiding: A Strategy To Strengthen Defense

Preliminary results with the novel genes have been mixed. "But that's not unusual for biotech experiments," comments Blechl. "Our priority is to make sure the plants are using, or expressing, the new genes to the greatest extent possible. We don't know yet if we've reached that point."

In addition, bundling these genes with others is likely a key to packing a more powerful punch against Fusarium head blight—an approach known as pyramiding. To fend off *Fusarium,* plants of the future might be loaded with a multiplicity of antifungal genes, for what's called multiple-gene resistance. The plant and this pathogen have co-evolved in such a way that wheat plants probably turn on many genes to survive attack.

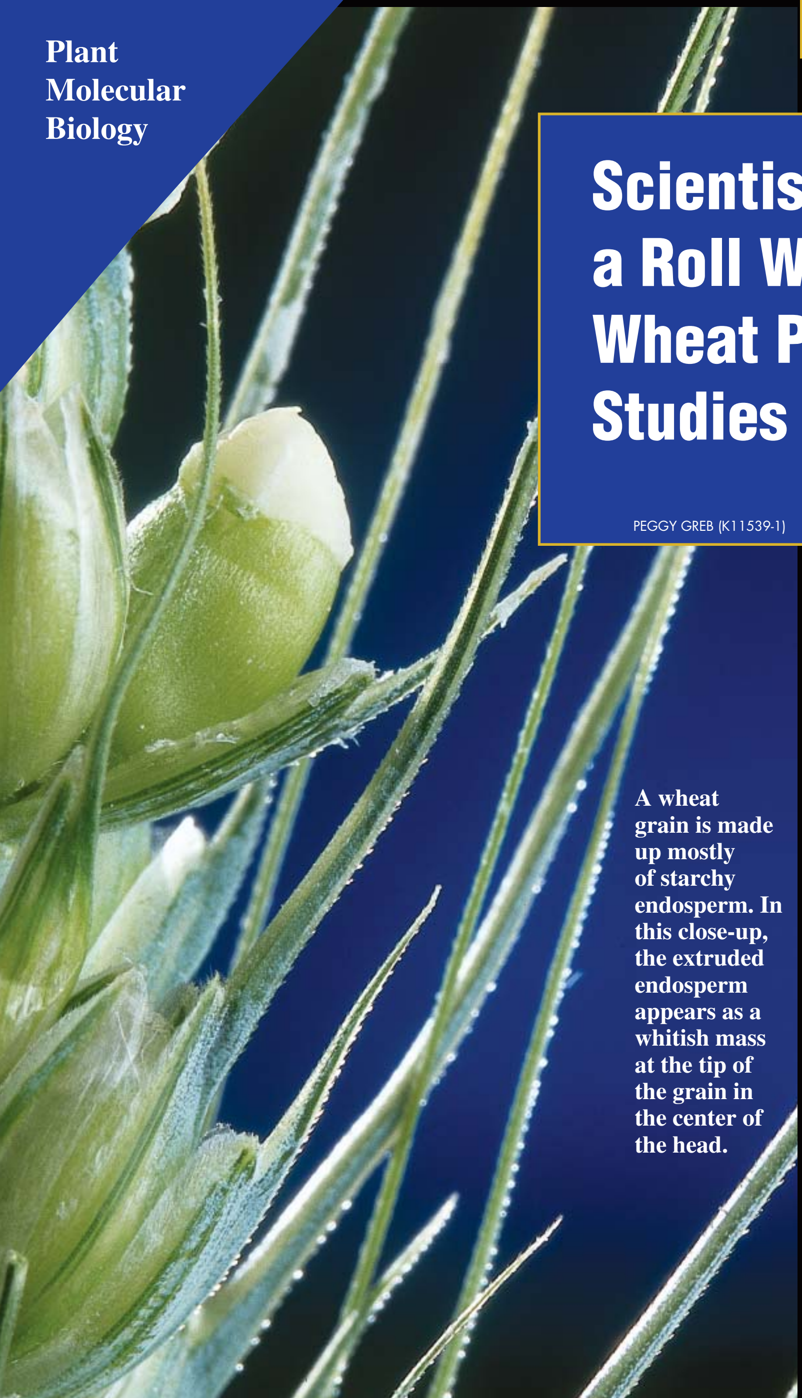
Yet wheat harbors no single gene that enables the plants to eliminate the fungus. "Following nature's lead," explains Blechl, "we're continuing to develop an array of genes. Some are from organisms other than *Fusarium.* We think that carefully developed combinations of unique genes may provide the ultimate protection against this destructive fungus."

The research is funded in part by the North American Millers' Association and the U.S. Wheat and Barley Scab Initiative.—**By Marcia Wood,** ARS.

This research is part of Plant Biological and Molecular Processes, an ARS National Program (#302) described on the World Wide Web at www.nps.ars.usda.gov.

For more information on U.S. Patent Application Serial No. 09/649,747, "Nucleic Acid Sequences Encoding Cell-Wall Degrading Enzymes and Their Use To Engineer Resistance to Fusarium and Other Pathogens," contact Patricia A. Okubara, USDA-ARS Root Disease and Biological Control Research Unit, P.O. Box 646430, Washington State University, Pullman, WA 99164-6430; phone (509) 335-7824, fax (509) 335-7674, pokubara@wsu.edu, or Ann E. Blechl, USDA-ARS Crop Improvement and Utilization Research Unit, Western Regional Research Center, 800 Buchanan St., Albany, CA 94710; phone (510) 559-5716, fax (510) 559-5777, ann.blechl@ars.usda.gov. ✷

Scientists on a Roll With Wheat Protein Studies



PEGGY GREB (K11539-1)

A wheat grain is made up mostly of starchy endosperm. In this close-up, the extruded endosperm appears as a whitish mass at the tip of the grain in the center of the head.

Whether it's a fragrant slice of freshly baked artisan bread or a perfectly prepared pasta entree, most of us eat foods made from wheat flour every day. The quality of that flour is due, in large part, to the work of hundreds of different proteins that perform specialized tasks inside the wheat kernel, or grain.

Discovering more about the work of these proteins—and how they're affected by the heat, soil nutrients, and other environmental conditions in which the plant is grown—might lead to even better flours for tomorrow. A top-quality flour can make a bread or a pasta remarkable instead of just ordinary.

Gluten proteins are the most abundant and most studied. Researchers already know that these proteins have a premier role in influencing a flour's quality.

In contrast, scientists know very little about wheat kernels' so-called metabolic proteins, which occur in much smaller amounts. It is known, however, that these mostly mysterious proteins are essential to a kernel's growth. For example, wheat plants need metabolic proteins to form the gluten proteins and to make starch. Gluten and starch are the main components of flour.

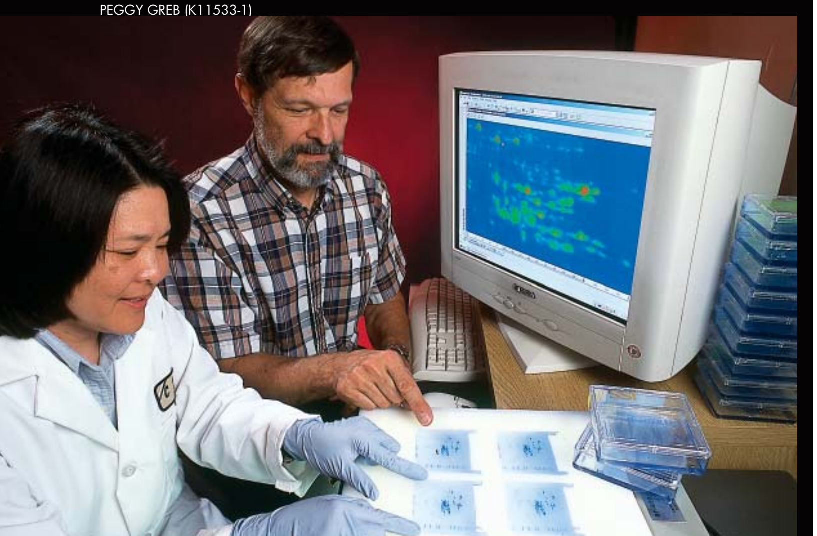
Protein Profilers Sleuth Metabolic Proteins

In Albany, California, ARS plant physiologist William J. Hurkman is working with Charlene K. Tanaka, also a plant physiologist, and chemist William H. Vensel to uncover more details about the biochemical chores carried out by these less-abundant wheat proteins. The scientists are in the ARS Western Regional Research Center's Crop Improvement and Utilization Research Unit.

Working with kernels from wheat plants grown in their research greenhouses, the scientists separated metabolic proteins from one another with a laboratory technique called two-dimensional gel electrophoresis.

Another technology, mass spectrometry, gave them a distinct profile of each protein. Using computers, they matched many of these protein profiles to those in other plants. They located those other profiles by searching research databases posted on the World Wide Web. With this approach, the researchers identified more than 200 wheat kernel proteins and grouped them by the tasks they likely perform, ranging from storing carbohydrates to protecting the kernel against insects.

PEGGY GREB (K11533-1)



Using two-dimensional gel electrophoresis and sophisticated computer software, plant physiologists Charlene Tanaka and Bill Hurkman compare protein patterns in wheat endosperm during grain development.

PEGGY GREB (K11543-1)

Plant physiologist Bill Hurkman harvests a wheat head from plants grown in a climate-controlled greenhouse at ARS's Western Regional Research Center.

The researchers also tested kernels, from the greenhouse plants, in two different growth stages. They found changes, over time, in the relative abundance of proteins. For example, certain metabolic proteins were more abundant in the early days of kernel growth than in the final weeks. This chronology of proteins at work inside wheat kernels is the kind of detail that could lead to improved flours.

A Catalog of Kernel Proteins

The analysis of hundreds of wheat-kernel proteins is what's newly described as "proteomics," the comprehensive study of the function, structure, and location of proteins. The catalog of proteins in wheat kernels that the scientists are compiling is a proteome, just as a genome is a directory of all genetic material in a plant or animal.

Similar work has been done at other labs to identify proteins and their functions in wheat, barley, and alfalfa grains, for instance. But the California investigators are likely the first to delve this deeply into the roles and changing ratios of the lesser-known wheat-kernel proteins.

The ARS research led to new collaborations with University of California at Berkeley researcher Bob B. Buchanan and colleagues to learn more about other aspects of wheat proteins.

Tracking Proteins to Their Gene Origins

As part of uncovering even more pieces of the wheat protein puzzle, scientists are tracking the proteins' gene origins, as well as how proteins affect flours.

Whether metabolic or gluten, all wheat proteins are the product of genes. Susan B. Altenbach, a biologist with the ARS group, is studying genes that cue the wheat plant to make kernel proteins. To do this, she's using a technique called microarray analysis. This leading-edge technology makes it possible to study thousands of wheat genes, conveniently positioned on a single, 1-by-3-inch glass slide.

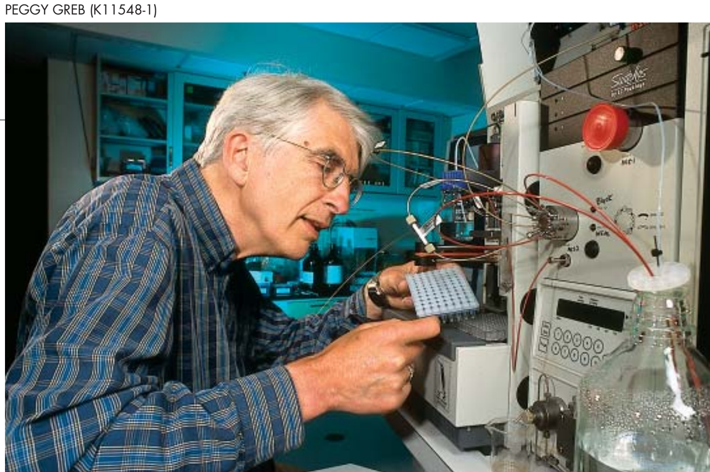
Analyzing kernel proteins in their end product—wheat flour itself—is another tactic that's revealing more about them. Plant physiologist Frances M. DuPont of the Albany team has shown that the amounts of heat and fertilizer greenhouse wheat plants were exposed to affected levels of certain kernel proteins. One outcome: lower-quality flour, resulting in doughs that were unable to withstand the rigorous mixing that's part of making bread.

The experiments highlight the importance of pinpointing kernel proteins' precise roles and using what's discovered to breed better wheat plants for the future. The superior flours these plants produce should please not only growers and millers, but also the people working in commercial or home kitchens to prepare delicious, wheat-flour-based foods for us.—By **Marcia Wood,** ARS.

This research is part of Plant Biological and Molecular Processes (#302) and Quality and Utilization of Agricultural Products (#306), two ARS National Programs described on the World Wide Web at www.nps.ars.usda.gov.

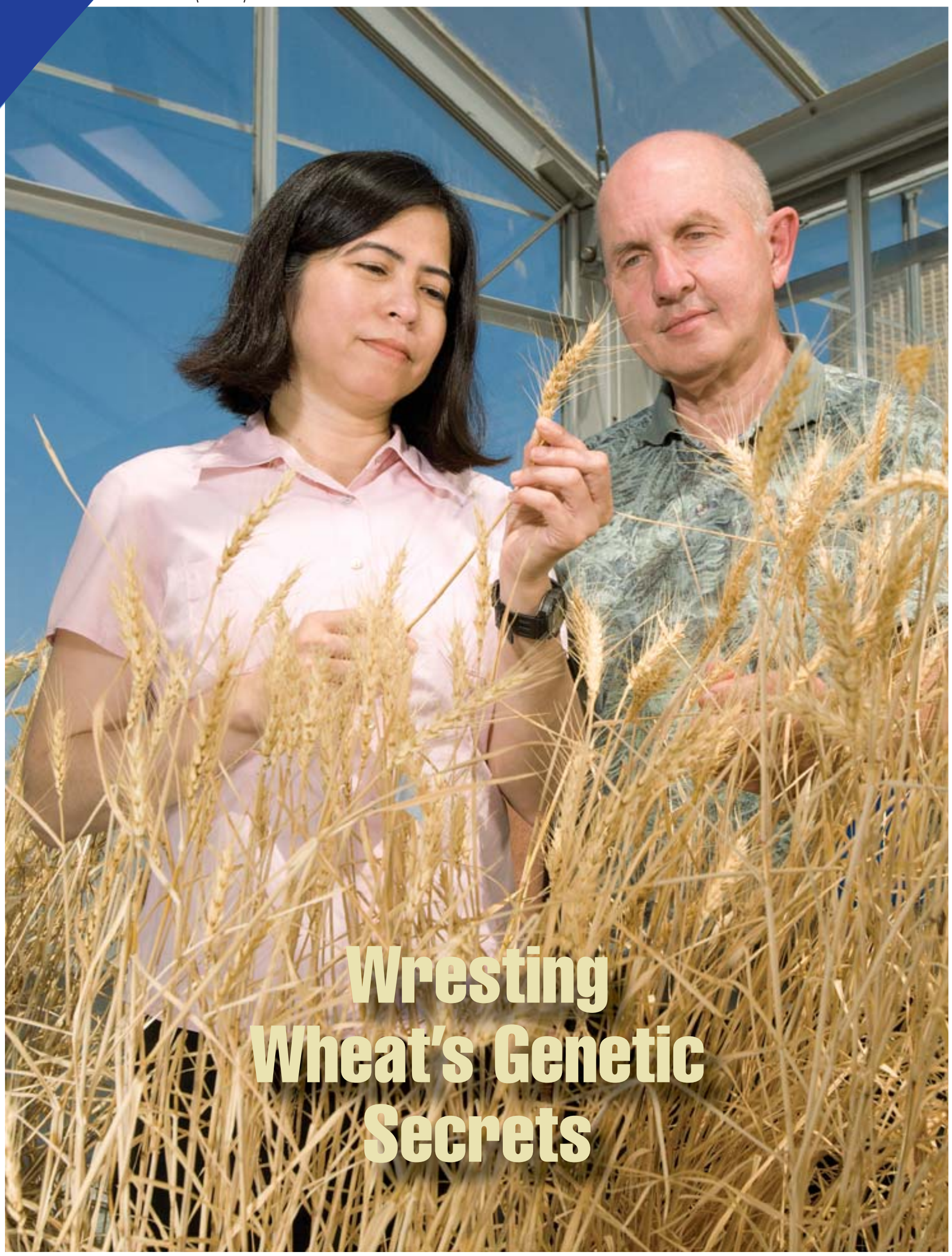
William J. Hurkman, Charlene K. Tanaka, William H. Vensel, Susan Altenbach, and Frances M. DuPont are in the USDA-ARS Crop Improvement and Utilization Research Unit, Western Regional Research Center, 800 Buchanan St., Albany, CA 94710; phone (510) 559-5750, fax (510) 559-5818, e-mail william.hurkman@ars.usda.gov, charlene.tanaka@ars.usda.gov, william.vensel@ars.usda.gov, susan.altenbach@ars.usda.gov, frances.dupont@ars.usda.gov. ✷

PEGGY GREB (K11548-1)



Chemist William Vensel loads a plate of wheat endosperm protein digests into an autosampler for protein analysis.

PEGGY GREB (D869-1)



Wresting Wheat's Genetic Secrets

In a greenhouse, molecular biologist Debbie Laudencua-Chingcuanco and geneticist Olin Anderson examine wheats containing a high level of high-molecular-weight glutenin protein, which controls dough elasticity and wheat quality.

In those early morning hours when most of us are still sleeping, bakers from coast to coast are busy making fresh, fragrant loaves of bread for us to enjoy. In fact, an estimated 50 million loaves of bread are baked daily in this country.

And wheat flour is, of course, a star ingredient.

Solving some of the mysteries about wheat's genetic makeup may help streamline the work of tomorrow's millers and bakers, enabling them to provide or bake with flour that's consistent and predictable in its properties. Superior flours for breadmaking, for example, would consistently make doughs that have the optimal balance of strength and elasticity. That would happen without having to blend various different flours—a costly and sometimes frustrating task for today's millers.

STEPHEN AUSMUS (D001-1)

ARS scientists in Albany, California, are studying wheat genes that may improve quality of wheat flour and bread.

A Trio of Genomes

Scientists at the ARS Western Regional Research Center in Albany, California, are wresting secrets about bread-quality genes—and other genes—from wheat's remarkably complicated, mostly unexplored genetic makeup, or genome. "Wheat is a complex union of three ancestral grass genomes that together make the wheat genome 10 times the size of the human genome," says ARS plant geneticist Olin D. Anderson. He leads the Albany center's Genomics and Gene Discovery Research Unit.

Unraveling the makeup of wheat's gene pool poses a formidable challenge. It's more daunting than that of deciphering the structure and function of all the genes in, for example, rice—a sister grain. "Wheat's genome is about 40 times bigger," notes colleague Yong Q. Gu, a geneticist with Anderson's team.

Right now, Gu, Anderson, and colleagues with the Albany research team are comparing and contrasting genetic material of Chinese Spring wheat with that of several other wheats. They are hunting for naturally occurring differences in the order of appearance, or sequence, of the infinitesimally small units—called "nucleotides"—that make up genes. Differences of a single nucleotide are known as "single nucleotide polymorphisms," or "SNPs" (pronounced "snips") for short.

Small Unit Can Make Big Difference

What difference could the whereabouts of a single nucleotide—among the millions in a wheat plant—possibly make?

A big one.

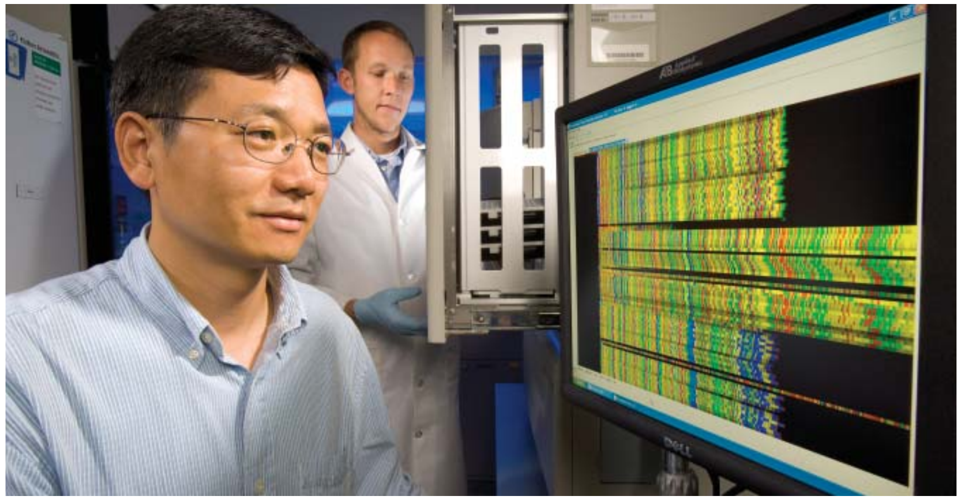
In humans, a SNP's presence or absence at a specific location could, for example, "mean the difference between having or not having sickle cell anemia," explains Gu.

In wheat plants, a SNP "might mean the difference between having high amounts of a protein important in breadmaking or very low amounts of it."

But there's much more to the gene search than better bread for making your morning toast. Single-nucleotide variations could affect genes for many other key traits, such as the ability to resist attack by insects or diseases or to survive drought.

The Albany scientists are collaborating with investigators from six labs around the United States in their ambitious pursuit of wheat SNPs. The work is the basis of the fourth such wheat-genome-based, multimillion-dollar investigation that the Albany team and coinvestigators nationwide have undertaken with the help of funding from the National Science Foundation (NSF).

PEGGY GREB (D870-1)

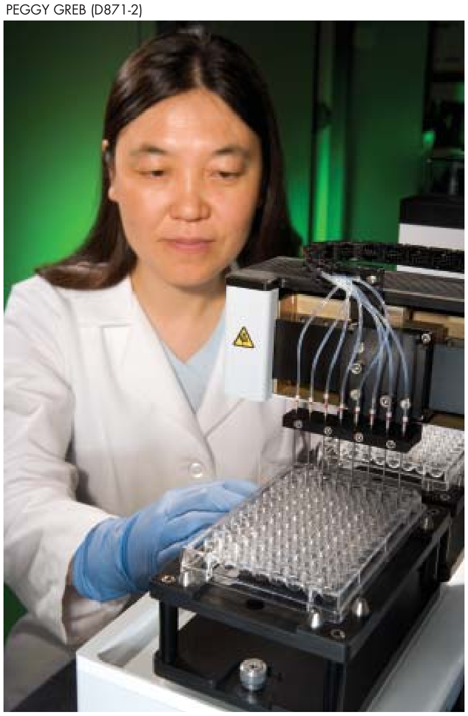


Geneticist Yong Gu (left) and technician Devin Coleman-Derr generate DNA sequences to identify breadmaking-quality genes in wheat.

Track Record of Discoveries

The SNPs project was an outgrowth of earlier, NSF-funded work on larger expanses of genetic material that, like SNPs, can also serve as reliable signposts for scientists looking for interesting genes. Anderson and others singled out more than 100,000 stretches of these so-called ESTs, short for "expressed sequence tags."

PEGGY GREB (D871-2)



Molecular biologist Naxin Huo uses a high-throughput robot to set up reactions for wheat genetic mapping.

"ESTs are reliable identifiers of genes that were active, or expressed, during the wheat plant's various stages of growth," says Anderson. He collaborated in the work with ARS plant geneticist Gerard R. Lazo and other Albany colleagues, ARS plant geneticist J. Perry Gustafson at Columbia, Missouri, and university researchers.

When the EST quest began in 2001, "there were only nine publicly available EST markers for wheat genes on the World Wide Web," Anderson points out. Now, thanks to the 10-laboratory search for wheat ESTs, more than 120,000 ESTs were posted to the freely accessible database at www.ncbi.nlm.nih.gov.

In all, the work represents the first significant collection of wheat ESTs available for all to use.

Other labs worldwide followed suit, contributing their discoveries of even more of these handy markers of wheat genes. That led to today's impressive total of more than 800,000 ESTs now displayed on the Web.

Scientists around the globe can use the markers—and soon the SNPs emerging from the newer work—in finding and studying previously unknown wheat genes that breeders might bring to stardom in superb new wheat plants. Such global access is vital, given that wheat is still the world's most widely planted crop.

Everyone benefits, from those of us who look forward to munching a perfectly toasted slice of whole-wheat bread at breakfast to the growers who—thanks in large part to this pioneering research—will be able to farm superior wheat plants.

The USDA national nutrition guidelines recommend eating at least three servings of whole-grain foods a day for optimal health. Tomorrow, thanks to ARS gene discoveries, the plentiful, varied, and tasty breads, crackers, and other wheat-flour-based products that help us meet that nutrition quota should be better than ever.—By **Marcia Wood,** ARS.

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

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Plant Molecular Biology

Wild Potato's Genes May Blunt Late Blight

What's a potato's number-one enemy?

Anywhere on our planet the likely answer is: late blight. But tomorrow's tubers may be safeguarded against this disease, thanks to research by ARS scientists from coast to coast. The researchers intend to develop hardy, highly productive potato plants that not only produce top-quality potatoes but also shrug off attack by *Phytophthora infestans,* the fungus-like microbe that causes late blight. The disease, which led to the Irish potato famine of the 1840s, costs about $400 million in losses each year in the United States, where potatoes are our favorite veggie.

An ARS team at the Western Regional Research Center in Albany, California, has found and copied a gene that may work in concert with other genes to fend off late blight.

They've named the gene *Sbul,* for *Solanum bulbocastenum,* a late-blight-resistant species of wild potato that grows in Mexico.

This discovery followed up on pioneering research over the past decade by plant physiologist John P. Helgeson, formerly with ARS in Madison, Wisconsin. He fused *S. bulbocastenum* with the familiar domesticated potato, *S. tuberosum,* creating unique hybrids that he offered to researchers such as pathologist Dennis L. Corsini and geneticist Joseph J. Pavek.

Corsini and Pavek, then with ARS in Idaho and now retired, crossed the Wisconsin hybrids with other potatoes, then provided samples—varying in their resistance to late blight—to other investigators. Each cross had lessened the amount of genetic material from the wild potato. This narrowed the California team's search for the resistance gene and proved faster than trying to tease out the gene directly from the wild potato.

Plant molecular biologist Teruko Oosumi, working with a group led by ARS plant physiologist William R. Belknap at Albany, isolated and cloned the *Sbul1* gene from one of the Aberdeen hybrids. Then, Belknap, microbiologist David R. Rockhold, and plant molecular biologist Malendia Maccree moved the gene into domesticated *S. tuberosum* potatoes for tests in the specialized greenhouses of plant pathologist Kenneth L. Deahl in Beltsville, Maryland.

The California group also determined the blueprint, or structure, of the *Sbul1* gene and pinned down its location within the wild potato's genome. To do that, Belknap used a portable, compact "library" of all the genes in *S. bulbocastenum,* provided by Jiming Jiang, professor of horticulture at the University of Wisconsin, Madison.

In Deahl's greenhouse research, the test tubers from Albany that had the newly added *Sbul1* gene showed resistance to the disease. Now, additional tests, conducted in Michigan by David Douches, plant and soil sciences professor at Michigan State University, East Lansing, will reveal how well *Sbul1*-equipped potatoes perform outdoors when exposed to the microbe. These field experiments should bring scientists a step closer to determining whether genes from a wild, south-of-the-border potato can protect its northern cousins from being battered by late blight.—By **Marcia Wood,** ARS.

This research is part of Plant, Microbial, and Insect Genetic Resources, Genomics, and Genetic Improvement, an ARS National Program (#301) described on the World Wide Web at www.nps.ars.usda.gov.

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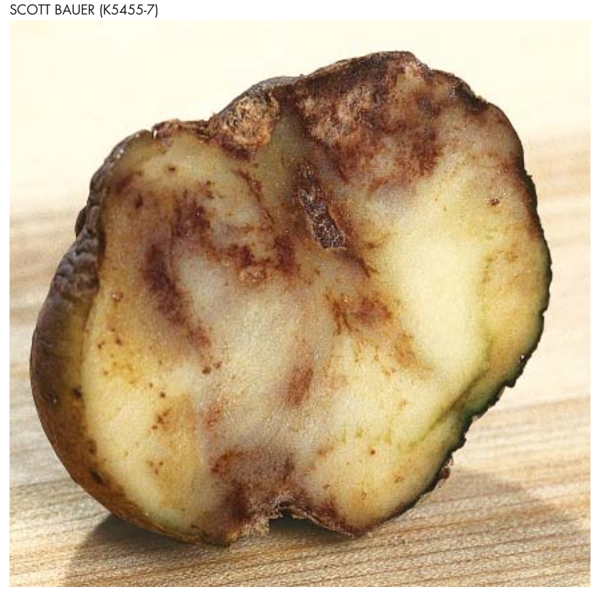
SCOTT BAUER (K5455-7)



Potatoes infected with late blight are shrunken on the outside, corky and rotted inside.

Western Regional Research Center Wins National Historic Chemical Landmark Award

Prestigious award presented by the American Chemical Society on December 11, 2002

TIME-TEMPERATURE TOLERANCE STUDIES

United States Department of Agriculture
Agricultural Research Service
Western Regional Research Center
Albany, California
1948-1965

After World War II, the staff of the Western Regional Research Center conducted complex and comprehensive investigations of frozen foods, focusing on how time and temperature affected their stability and quality. The discovery of the chemical change occurring as frozen food went from the farm to the dinner table provided much of the scientific basis needed for the future success of the fledgling frozen food industry. The freezing protocols, analytical techniques, and food handling and food storage recommendations from the Western Regional Research Center studies led to superior flavor, texture, and appearance of today's frozen food. These pioneering Time-Temperature Tolerance Studies were honored as a National Historic Chemical Landmark by the American Chemical Society on December 11, 2002.