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Screening Bee Colonies for Bacteria

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Solving Problems for the Growing World

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ZHANGUO XIN (D3377-1)

In the foreground, a newly developed sorghum on the right yields 30 to 40 percent more seeds than the elite line on the left. Read more about this versatile crop and this new sorghum, which was developed by ARS scientists. Story begins on page 4.

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Cover: ARS scientists in Beltsville and their colleagues at the Brazilian Honey Bee Laboratory in São Paulo analyzed the DNA of bees they collected in Beltsville and Brazil at different times of the year to see when two bacteria from the genus *Spiroplasma* were present in honey bee colonies. Story begins on page 3. Photo by Peggy Greb. (D3348-1)

Honey Bee Diseases Strike in All Seasons

PEGGY GREB (D3348-1)

PEGGY GREB (D3349-2)

ARS scientists in Beltsville and their colleagues in Brazil screened honey bee colonies for two species of bacteria in the genus *Spiroplasma* and found high infection rates in both locations.

Agricultural Research Service scientists have found that two pathogens causing mysterious honey bee ailments are not just a problem in the spring, but might pose a threat year round. Entomologists Ryan Schwarz and Jay Evans at the ARS Bee Research Laboratory in Beltsville, Maryland, and their colleagues have shown that two species of bacteria, *Spiroplasma melliferum* and *S. apis,* are more common than thought and infect honey bees in places as diverse as Brazil and Maryland.

The two bacteria are often lumped together, since both are in the genus *Spiroplasma,* an intriguing class of bacteria found in some insects, ticks, and plants. *S. melliferum* was discovered in the late 1970s by ARS researchers who noticed higher mortality rates in bees carrying it. French researchers discovered *S. apis* a few years later and called it "May disease," because that's the month of year when it struck. It made bees "quiver and creep," left some unable to fly, and in that instance, cut honey production by about 25 percent. Scientists, however, don't know if *S. melliferum* and *S. apis* are factors in colony collapse disorder or other major bee mortalities, and they are unsure how lethal the bacteria are to bees.

Schwarz, Evans, and their colleagues at the Brazilian Honey Bee Laboratory in São Paulo analyzed the DNA of bees they collected in Beltsville and Brazil at different times of the year between 2011 and 2013. Bees were collected from 11 states in Brazil and 2 areas in Beltsville. Schwarz had recently developed genetic markers that allow researchers to distinguish *S. apis* from other bacteria in bees. They used those markers and another recently developed set of *S. melliferum* markers to determine the year-round prevalence of the two pathogens in both locations.

As expected, the researchers found that both pathogens were prevalent in the spring. But they also found that they were common at other times of the year and that prevalence rates varied depending on the location. In Beltsville, they were more prevalent in the spring, while in Brazil they were more prevalent in the fall. They also found high infection rates: 33 percent of the U.S. colonies and 54 percent of the Brazilian colonies were infected. The results also showed that *S. melliferum* was the more prevalent of the two and that the presence of one pathogen made bees more susceptible to the other. Schwarz says the different genetics and prevalence patterns show that the two pathogens should not be lumped together.

Certain plants can act as bacteria-transmission sites, and bees pick up the pathogens when they feed on plant nectar, Schwarz says. The results add to what is known about microbe transmission between plants and pollinators and should help beekeepers and scientists monitor the health of honey bees by raising awareness about the year-round nature of any threat the pathogens may pose. With the new genetic markers now available, scientists will also be better equipped to screen bee colonies for them.—By **Dennis O'Brien,** ARS.

This research is part of ARS National Program #305, Crop Production.

Boosting Sorghum's Growing Range and Yield

BRIAN HENRY (D3379-1)

To find the optimal conditions for producing sorghum in northeastern Colorado, ARS scientists studied several factors known to affect plant maturity, such as seed density and row spacing.

When it comes to versatile crop plants, sorghum might be considered "the little engine that could."

It is drought tolerant, can thrive in poor soils, requires little or no fertilizer, and will grow in a wide range of temperatures and altitudes. Sorghum grain is used in breakfast cereals, in ethanol production, as feed for livestock, as a source of sugar for syrup and molasses, and in construction and packaging materials. It also produces large amounts of plant material, making it potentially useful for cellulosic ethanol production.

"There are parts of the world where people depend on sorghum for their survival, but in the United States, it's a viable alternative crop for growers concerned about future droughts or the rising temperatures that might accompany climate change," says Merle Vigil, an Agricultural Research Service soil scientist and research leader in the Central Plains Resources Management Research Unit in Akron, Colorado.

Sorghum is also the focus of extensive research, and Vigil along with ARS researcher Greg McMaster and Colorado State University researchers Sally Sauer and Jerry Johnson, recently found a new way to exploit sorghum's potential. They found that by using certain cropping strategies, growers can produce sorghum in parts of northeastern Colorado where the growing season was previously considered too short. Meanwhile, another team of ARS scientists working in Texas has developed a new line of sorghum that could dramatically increase grain yields.

The work is considered important because of sorghum's value as a cash crop and because its drought tolerance is likely to make it a critical option in future efforts to stave off global hunger in semi-arid areas. In the United States, sorghum is grown in 14 states on 7.2 million acres, production that was valued at $1.6 billion in 2013. Most U.S. sorghum is used to feed livestock. But in Africa and Asia, it is a major part of the human diet. Worldwide, the land area dedicated to sorghum production has increased by 66 percent over the past 50 years.

Coping with Climate Change

Cool evening temperatures in the spring and cold temperatures in the early fall make the growing season in northeastern Colorado too short to raise the sorghum varieties grown in southern Colorado. Northeastern farmers plant corn rather than sorghum, often rotating it with winter wheat. But irrigation is rare in the region, and sorghum would be a good option, since it is more drought tolerant and less expensive to produce than corn.

To find the optimal conditions for producing sorghum in northeastern Colorado, Vigil and his colleagues at Colorado State University studied several factors known to affect plant maturity, including seeding density, hybrid selection, row spacing, and row orientation (a north-south or east-west orientation).

The researchers grew two early-maturing sorghum varieties and one "medium-early" maturing variety in test plots at Fort Collins, Akron, and Stratton. The three sites are up to 200 miles apart and represent a range of environments. They planted in the spring and harvested in late fall for 2 years. Based on planting dates and temperature data, they determined the number of "growing degree-days" (GDDs) needed for plants to reach maturity at each site. They also used historical weather data to calculate probabilities that the sites would experience the required number of GDDs in a growing season for plants to reach maturity.

The results showed that sorghum is a viable option for northern Colorado growers if they use an early-maturing hybrid. Yields reached acceptable levels at all three sites, but weather data showed sufficient

ZHANGUO XIN (D3377-1)

The photograph above shows sorghum growing in a breeder's field in Lubbock, Texas. The panicle on the left, with orange-yellow seeds, is an elite inbred line, while the panicle on the right is the same elite line with an induced mutation. This new sorghum variety, developed by ARS scientists, yields 30 to 40 percent more seeds.

growing seasons would be more likely in Stratton and Akron than at Fort Collins, which was the most northern and most elevated site. They also found that fields should be seeded at a rate of about 11 seeds per square meter (the mid-range of the three seeding rates evaluated). Row orientation and row spacing had inconsistent effects on maturity rates.

Higher Yielding Sorghum Plants

In other sorghum studies, molecular biologist Zhanguo Xin, plant geneticist Gloria Burow, and lab director John Burke, at the ARS Cropping Systems Research Laboratory in Lubbock, Texas, have bred a mutant sorghum plant that produces 30 to 40 percent more seeds.

The researchers developed the higher yielding sorghum by taking advantage of a plant part called a "spikelet." A spikelet is a cluster of florets found in the panicle and is a characteristic of other grasses, such as millet or rye. Sorghum produces two types of spikelets: the sessile spikelets and the pedicellate spikelets. Normally, only the sessile spikelets are fertile, but the ARS scientists developed a plant that produces seeds in both types of spikelets.

"We developed the productive sorghum line by inducing a mutation of sorghum plants that allowed infertile spikelets to grow and produce seed," says Xin. An induced mutation is produced by treatment with a mutagen, like radiation or a chemical agent such as ethyl methane sulfonate. The mutation resulted in an overall increase in size and volume (length, width, and thickness) of the sorghum panicle.

"All of the spikelets of the new sorghum plant develop into flowers and produce mature seeds, thereby significantly increasing seed production and yield in comparison to conventional sorghum. The mutants may be crossed with other sorghum lines, particularly elite large-seeded lines, to improve grain yield in sorghum and other related species," says Xin. "The mutation in the sorghum line we developed is stable and can be passed on to other sorghum lines through breeding."

A sample of at least 2,500 seeds of the new multi-seeded sorghum has been deposited with the American Type Culture Collection for future research.—By **Dennis O'Brien** and **Sharon Durham,** ARS.

This research is part of two ARS National Programs, Climate Change, Soils, and Emissions (#212) and Plant Genetic Resources, Genomics, and Genetic Improvement (#301).

Trapping Stable Flies at Zoos

PEGGY GREB (D3374-1)

Zebras graze in the Smithsonian's National Zoological Park in Washington, D.C. Along with farm animals, they are vulnerable to stable flies.

Humans aren't the only ones visiting zoos nowadays. The stable fly, typically a pest of farm animals, also pesters tigers, foxes, and other exotic species in zoos. A biting insect that feeds on blood, the stable fly, *Stomoxys calcitrans,* can create open lesions on the animals.

Scientists at the Smithsonian's National Zoological Park in Washington, D.C., and the Agricultural Research Service's Center for Medical, Agricultural, and Veterinary Entomology (CMAVE) in Gainesville, Florida, are testing the effectiveness of traps in capturing and killing flies at zoos.

"The more adult flies trapped, the fewer are available to bite and feed on exhibit animals," says Smithsonian entomologist Gregory Ose. "Pesticides are effective, but it is not always possible to use them on zoo animals."

Some zoo animals do not tolerate pesticides applied directly to their bodies, he says. In addition, animals often quickly remove treatments by rubbing, rolling, or licking them off.

Stable flies are not reproducing at the zoos, says entomologist Jerome Hogsette, who works in CMAVE's Mosquito and Fly Research Unit. These pests—which also bite humans and dogs—prefer a habitat of decaying fibrous plant materials, such as hay, silage, or grass clippings.

"The National Zoo in Washington, D.C., one of the places where we set traps, is very clean and does not have any accumulation of waste that these flies might utilize," Hogsette says. "We canvassed the whole zoo and never found signs of stable fly development."

The nearest site of agricultural production is about 60 miles west of the National Zoo, which leads researchers to believe that flies are arriving with prevailing winds. "Stable flies can move with weather systems, traveling much farther than they normally do," Hogsette says. "We also believe that similar weather-related movements are bringing stable flies to zoos in Chicago and Virginia."

Ose and Hogsette evaluated the effectiveness of blue-black cloth targets covered with clear sticky wraps to capture stable flies. Scientists think that the blue-black color contrast may mimic natural forest edges where stable flies alight to rest and digest their food.

Target traps were compared with Alsynite fiberglass adhesive traps, the standard trap used for years to capture and monitor stable fly populations. Traps were placed at 10 sites for 15 weeks at a zoological park in Reston, Virginia. The modified traps captured fewer stable flies than the Alsynite traps. Only 20 percent of the approximately 12,550 stable flies captured were trapped on the modified traps.

PEGGY GREB (D3371-1)

ARS entomologist Jerry Hogsette examines a standard fiberglass sticky trap used for catching stable flies near a cheetah cage at the Smithsonian's National Zoological Park in Washington, D.C. He is testing the effectiveness of this trap compared to new blue/black cloth targets he developed with Smithsonian entomologist Greg Ose.

Results suggest that covering the blue-black cloth targets with sticky wraps may block the wavelengths of light that attract the flies, says Hogsette. However, the research provided data on stable fly distribution and seasonality, which could make it easier to manage this pest at zoos by predicting the best times to set out traps.

Scientists are continuing their research to help monitor and manage stable flies at zoos. They have gathered 3 years of population data from the National Zoo and are testing new traps that have just come on the market.—By **Sandra Avant,** ARS.

PEGGY GREB (D3370-3)

At the National Zoological Park in Washington, D.C., entomologists Greg Ose (left) and Jerry Hogsette attach the blue/black cloth test targets to a fence near an exhibit of rare wild horses.

This research is part of ARS National Program #104, Veterinary, Medical, and Urban Entomology.

No Harm to Soils When Cattle Graze Cover Crops

STEVEN KNAPP (D3382-1)

A cow and calf grazing on a summer cover crop of pearl millet. ARS scientists conducted studies on cover crops and cattle grazing and found that moderate grazing does not significantly affect the amount of organic matter in the soil.

For years, some growers in the Southeast have used cover crops to reduce soil erosion, boost organic matter, and keep more moisture in soil. Combined with no-till production, cover crops are credited with sequestering more carbon in soil so that less of it is released as a greenhouse gas.

But more growers could be using cover crops.

Alan Franzluebbers, an ecologist in the Agricultural Research Service's Plant Science Research Unit in Raleigh, North Carolina, wanted to see if the use of cover crops could be encouraged by allowing cattle to graze cover crops. Conventional wisdom holds that grazing would remove the nitrogen and carbon otherwise left on the soil in the cover crop plant residue. Allowing cattle to tread on the soil could also compact it, preventing air and water from passing through the soil to reach plant roots. But if grazing wouldn't harm the soil, it might encourage more growers to try using cover crops.

Franzluebbers and his colleagues conducted a 7-year study to assess whether grazing cover crops at a site near Watkinsville, Georgia, affects the health of soils typical of the Piedmont region of the Southeast. They looked at growing winter and summer grains, with cover crops planted in the off-season for each grain crop. They compared no-till versus tilling, and grazing versus no grazing. Cow/calf pairs were allowed to graze at a rate of one animal per 4 acres. The scientists took periodic samples of the soil to a depth of 1 foot.

The results showed that the relatively low rate of grazing did not significantly affect the amount of organic matter in soil and did not cause soil compaction. Additional studies should be conducted to determine a stocking threshold that increases compaction, Franzluebbers says. The findings also showed that cover crops make for high-quality forage. Organic matter lost by allowing cattle to graze on cover crops is likely made up in the organic input from manure. As in previous studies, the team found that using no-till generally keeps more carbon and nitrogen in soil than using conventional tillage.

The study was the first in the region to analyze these practices for such an extensive period, and that makes it important, Franzluebbers says. "Soil conditions can fluctuate year to year, and when it comes to something as lasting and significant as the health of the soil, it's important for us to have a comprehensive picture of the effects of these practices over an extended period," he says.—By **Dennis O'Brien,** ARS.

This research is part of ARS National Program #212, Climate Change, Soils, and Emissions.

Disinfectant Wipes Out Fungus on Fish Eggs

CINDY LEDBETTER (D3380-1)

To control fungus, ARS toxicologist Dave Straus treats channel catfish eggs with peracetic acid.

DAVE STRAUS (D1936-1)

Fungus on untreated channel catfish eggs.

DAVE STRAUS (D3381-1)

Healthy, fungus-free channel catfish eggs, less than 24 hours old.

A product used as a disinfectant in agriculture, food preparation, and medical facilities also kills a fungus that causes the disease saprolegniasis on catfish eggs, and it has the potential to treat harmful parasites.

At the Agricultural Research Service's Harry K. Dupree Stuttgart [Arkansas] National Aquaculture Research Center, toxicologist David Straus and his colleagues took a close look at the use of peracetic acid—a stabilized mixture of acetic acid (concentrated vinegar) and hydrogen peroxide—for treating catfish eggs. They first determined how much peracetic acid was toxic to hatching catfish fry. The toxicity study indicated that a safe treatment rate was 2.2 parts per million (ppm) peracetic acid for 2-day-old yolk-sac fry and 1.3 ppm for 7-day-old swim-up fry.

"The advantage of using peracetic acid is that it does not produce any residues that would harm fry or the environment," Straus says. "It is safe and effective to use on fish at a low dose, breaks down rapidly into harmless residues, and does not leave an environmental footprint like other chemicals."

Peracetic acid is approved by the U.S. Environmental Protection Agency for many uses in the United States, including disinfecting wastewater and sterilizing items for the food industry and hospitals. It is not approved for aquaculture use in the United States, but it is in Europe. "In Europe, the compound is considered a safe and effective replacement for banned chemicals and antibiotics," Straus adds.

"My colleagues in Germany and Denmark are using it to control fungus on adult fish, and we have also found it very effective for certain parasites," Straus says. "We are working together on acute toxicity studies in different species of fish, because this information is lacking. You need to know how safe it is for fish before you can treat them."

In a separate study, Straus looked at the effectiveness of different concentrations of peracetic acid in killing fungus on eggs. His experiments included five peracetic acid concentrations (2.5, 5, 10, 15, and 20 ppm) and an untreated control in a flow-through system. Eggs were treated twice daily until the embryos developed eyes. Hatching was complete in 9 days and the fry were counted to determine the survival rate in each treatment.

Fungal growth was severe in the controls, resulting in 11 percent survival compared to 60 percent in the 2.5-ppm group. Very little fungus was present in treatments of 2.5 ppm or higher. However, treatments of 15 and 20 ppm were toxic to eggs. "Although the highest survival rate of hatched fry was at 5 ppm, we found 2.5 ppm to be a safe treatment," Straus says. "This rate is very low, and peracetic acid is flushed out of the troughs quickly."

Fungal infections such as saprolegniasis in hatchery-reared catfish eggs can cause serious losses of young fish, Straus says. This research provides significant information that could help aquaculture producers deal with fungus problems on eggs.—By **Sandra Avant,** ARS.

This research is part of ARS National Program #106, Aquaculture.

OLé: A New Spanish Peanut High in Oleic Acid

PEGGY GREB (D3383-1)

Above is a new Spanish peanut variety (newly picked/raw) called OLé, developed by ARS scientists and collaborators. It is high in oleic acid, which makes its oil heart healthy for consumers.

A new Spanish peanut variety that packs high levels of healthful oleic acid has been released by Agricultural Research Service scientists and university cooperators. The new variety, called OLé, could provide producers and consumers with a peanut that has a longer shelf life and heart-healthy qualities.

ARS biologist Kelly Chamberlin, in the Wheat, Peanut, and Other Field Crops Research Unit in Stillwater, Oklahoma, and cooperators at Oklahoma State University developed OLé.

Oleic acid is a beneficial monounsaturated fatty acid, and the high oleic acid levels in the peanut make its oil a heart-healthy one for consumers. Growers like the new variety because of its disease resistance and potential for high yield and grade.

"This is an important variety that will have a lot of impact on the peanut industry as a whole," says Chamberlin. Plus OLé has resistance to *Sclerotinia* blight, a fungal disease that can cause yield loss and is a particular problem for peanut growers in Oklahoma, Texas, and the Virginia-North Carolina region. Depending upon the severity of field infestation, yield losses due to such soilborne diseases may be as high as 50 percent.

Cultivated peanut is an economically important crop throughout the world. Peanut is susceptible to many pathogens, with most damage being caused by fungi. Soilborne fungi cause diseases that adversely affect peanut health and productivity throughout the growing areas of the United States.

"Sustainable peanut production in the southwestern United States demands that cultivars grown there possess certain characteristics, including a high oleic/linoleic acid ratio, which increases peanut product shelf life, and resistance to multiple diseases," says Chamberlin.

In tests at three locations in Oklahoma, Chamberlin and her colleagues found that growing OLé reduced *Sclerotinia* blight infestation. This can save growers approximately $100 per acre in fungicide costs for *Sclerotinia* blight alone. The variety also has good resistance to pod rot.

"The first high-oleic Spanish cultivar released, called OLin, was the result of the collaborative efforts of ARS, Texas AgriLife Research, and Oklahoma State University. It was released in 2002," says Chamberlin. OLé produces higher peanut yields than OLin.

The OLé variety is now being grown for foundation seed before being made available commercially.—By **Sharon Durham,** ARS.

This research is part of ARS National Program #301, Plant Genetic Resources, Genomics, and Genetic Improvement.