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Agricultural Research

Studying the Syndrome of COLONY COLLAPSE

page 4

FORUM

Innovations for Pest Control in Produce

Postharvest control of insect pests is an integral part of maintaining safe, high-quality, abundant produce domestically and for export. Insect pests can travel with fresh produce from the field and into processing plants and storage facilities. Agricultural Research Service scientists have found many innovative and environmentally friendly methods to help meet this challenge.

ARS laboratories in Manhattan, Kansas, and Gainesville, Florida, in collaboration with others, are using unique ways to detect and disinfest stored-grain facilities of insect pests like the red flour beetle, a major pest of the flour-milling industry. Effective pest detection and monitoring are critical to pest management because they provide necessary information for timing and targeting the application of control measures.

Researchers in Gainesville have developed a new trap for monitoring the presence of red flour beetles. The trap uses ultraviolet light, a chemical attractant, and a physical configuration that guides beetles into a pit where they become trapped. This monitoring tool has promise for use in flour mills and other food-processing plants.

In Manhattan, Kansas, ARS scientists are trying to slow the growth of red flour beetle populations by altering its hormonal system. Treatment with growth regulators can kill immature insects, and survivors may have reduced fitness. When larvae were exposed to growth regulators, males were less likely to survive to the adult stage than females. Adult males that survived larval exposure to growth regulators produced fewer offspring than unexposed males. Results show that the nonlethal effects of growth regulators potentially increase the impact of insecticide treatments on population growth rates, thus making growth regulators more effective in pest management than initially estimated.

The dried fruits and tree nuts industry also requires insect pest control measures. Much of the U.S. production of dried fruits and tree nuts occurs in the Central Valley of California. To provide consumers with quality produce, processors must control postharvest insect pests. Scientists in Parlier, California, are developing nonchemical treatments to solve this issue, using temperature extremes or vacuum, as well as insect parasitoids as control agents. The target pests for most of this work include both field pests of postharvest significance and stored-product pests. Several methods are being tested, including cold storage for spotted wing *Drosophila* on grapes, radio frequency treatments for cowpea weevil in dried pulses, and low-temperature vacuum treatments for codling moth in fresh fruits.

Another insect, the Indianmeal moth, is globally the most important stored-product moth pest. ARS scientists in Parlier, along with collaborators from the University of Michigan, demonstrated that mating disruption controls Indianmeal moth in dried beans in central California. Mating disruption is safer and less disruptive than fumigants and aerosol sprays, but it is less widely used for control of Indianmeal moth. This demonstration of successful control of the Indianmeal moth will encourage adoption of mating disruption, thereby protecting food in distribution channels while reducing use of insecticides and improving worker safety.

The possible presence of the Asian citrus psyllid in loads of citrus arriving in Australia threatens California's ability to export citrus into that market. Parlier researchers found that Asian citrus psyllids are completely washed from fruits that are submerged, flooded, or sprayed at high temperatures using soak tanks and wash lines consistent with commercial practices in California. Nearly 99 percent of the insects remain trapped by the solution until they drown, and that means that Asian citrus psyllids will very likely not be present in commercially packed fruit. This research will help maintain access of California citrus to Australia, a market valued at $60 million annually.

Also on the world stage, tephritid fruit flies are serious economic pests worldwide. The larval stages feed within host fruits, making infestation difficult to detect. Fruit imported into the United States is currently checked for infestation by cutting open a small sample of fruit and looking for fly larvae. This is a time-consuming and potentially limited way to detect the insect, so more sensitive screening methods are needed. USDA-ARS scientists in Miami, Florida, in conjunction with USDA's Animal and Plant Health Inspection Service, also in Miami, conducted research to evaluate gas chromatography (GC) as a detection method. Grapefruits infested with larvae of the Caribbean fruit fly were examined to determine whether infested fruit emitted chemicals distinct from those of healthy fruit. GC analysis indicated that there were volatile chemicals indicative of citrus fruit injury and others associated with larval infestation. These "signature chemicals" were also detectable with a portable, ultra-fast GC analyzer. This is just one example of studies that hold the potential in development of a rapid screening protocol for detection of infested fruit at U.S. ports of entry.

The article on oxygenated phosphine fumigation, on page 10 of this issue, is another example of ARS research on ways to make U.S. produce as free of insect pests as possible for the consumer.

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Solving Problems for the Growing World

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(D2549-1)

The root-knot nematode can penetrate the roots of some crops and rob plant nutrients. ARS scientists and colleagues have released a resistant cotton line to help breeders fight the pest.

Story begins on page 16.

Photo by WILLIAM WERGIN and RICHARD SAYRE. Colorized by STEPHEN AUSMUS.

Cover: A honey bee being inoculated with *Nosema* to determine bee infection rates and immune responses. ARS scientists and others have been working for years to try to solve the puzzling honey bee syndrome known as "colony collapse disorder." The story that begins on page 4 examines some of the potential causes and rules out others. Photo by Stephen Ausmus. (D2484-7)

Colony Collapse Disorder

An Incomplete Puzzle

When it comes to solving the puzzling syndrome known as "colony collapse disorder" (CCD), which has been attacking honey bee colonies since 2006, the best that can be said is that there is good news and bad news. The good news is that the rate of honey bee losses seems to have leveled off rather than continuing to increase. The bad news is that the cause or causes of CCD remain unclear.

In the United States, the problem surfaced in October 2006, when an increasing number of beekeepers began reporting losses of 30 to 90 percent of the hives in their apiaries with no apparent cause. The defining characteristic of CCD is the disappearance of most, if not all, of the adult honey bees in a colony, leaving behind honey and brood but no dead bee bodies. This definition has recently been revised to include low levels of *Varroa* mite and other pathogens, such as *Nosema,* as probable contributing factors.

For the last 3 years, self-reported surveys of beekeepers have found that winter colony losses have averaged about 30 percent, with around one-third of those losses ascribed to CCD, according to Jeff Pettis, research leader of the Bee Research Laboratory in Beltsville, Maryland, who heads up the Agricultural Research Service's CCD research effort. This compares to colony losses that were averaging 15-20 percent before CCD.

"The faint good news in the survey numbers is that the CCD problem does

Entomologist Jeff Pettis inspects honey bee combs at Beltsville, Maryland, for disease. Honey bees are disappearing at an alarming rate. ARS researchers have been working diligently to solve the mysterious syndrome known as "colony collapse disorder."

STEPHEN AUSMUS (D2489-12)

not seem to be getting worse," Pettis says. "But—and this is a big 'but'—33-percent losses each year are probably not economically sustainable for commercial beekeeping operations."

While many possible causes for CCD have been proposed, reported, and discussed—both in the scientific literature and popular media—no cause has been proven. (See sidebar, page 7.)

"We know more now than we did a few years ago, but CCD has really been a 1,000-piece jigsaw puzzle, and the best I can say is that a lot of pieces have been turned over. The problem is that they have almost all been blue-sky pieces—frame but no center picture," Pettis explains.

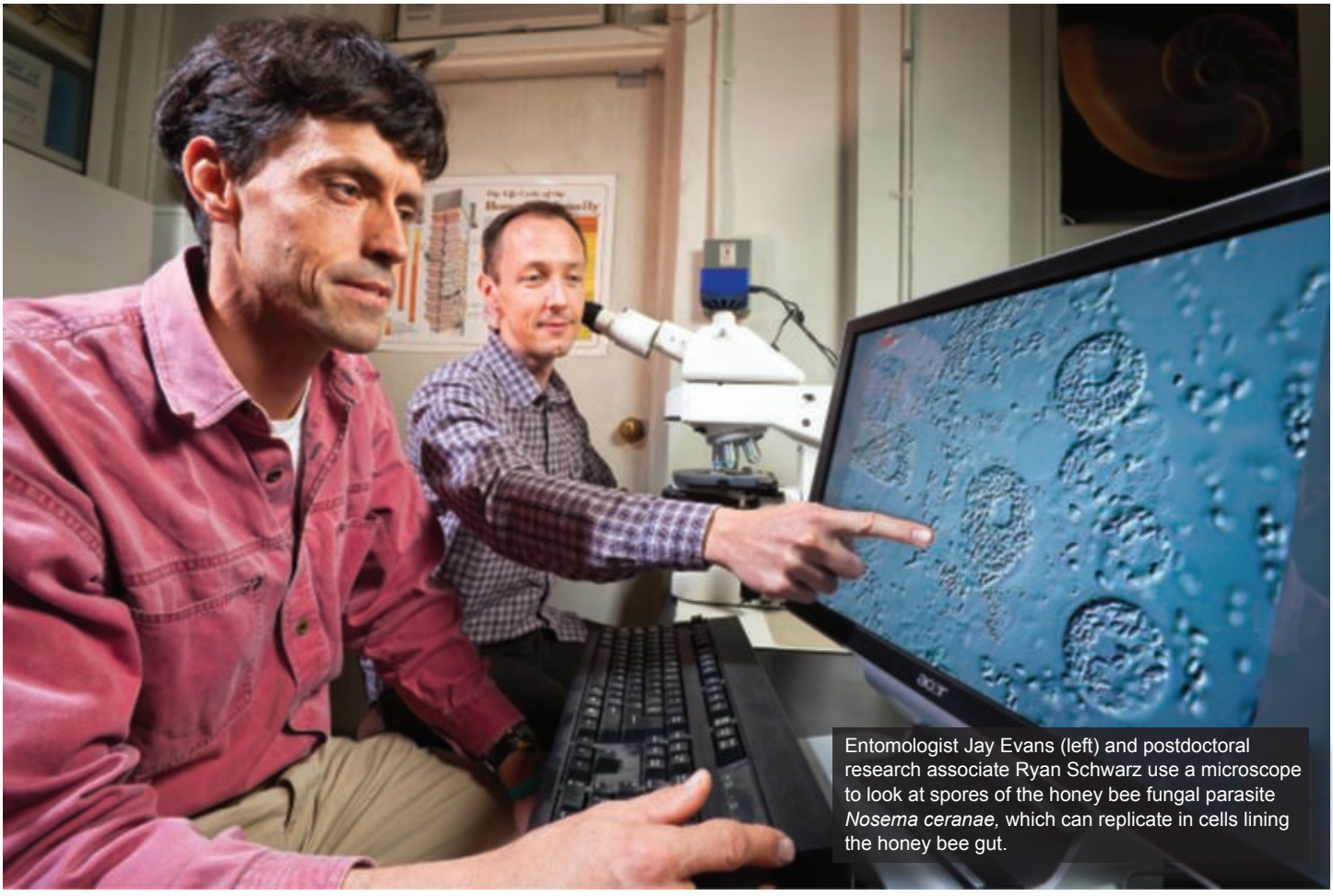
The bee lab's scientists have been looking for the cause or causes of CCD within four broad categories: pathogens; parasites, such as *Varroa* mites or *Nosema;* environmental stressors, such as pesticides or lack of nectar diversity; and management stressors. The researchers have been analyzing samples from healthy and CCD-struck colonies and applying a variety of stressors from the four groups to colonies in hopes of provoking a colony response that duplicates CCD.

STEPHEN AUSMUS (D2482-20)

In Beltsville, Maryland, ARS entomologist Jay Evans inspects a comb of honey bees for signs of mites and brood disease.

"While a number of potential causes have been championed by a variety of researchers and interest groups, none of the causes has stood up to detailed scrutiny. Every time someone has proclaimed a potential smoking gun, further investigation has not been able to make the leap from correlation to cause-and-effect for one reason or another. Other times, there hasn't even been a scientific correlation," Pettis says.

ARS's research work, along with that of university and other scientists, "pretty well supports the idea that CCD is caused by multiple factors—possibly working individually, but more likely in combination," Pettis adds. "But we still can't say whether it's the same set



Entomologist Jay Evans (left) and postdoctoral research associate Ryan Schwarz use a microscope to look at spores of the honey bee fungal parasite *Nosema ceranae,* which can replicate in cells lining the honey bee gut.

STEPHEN AUSMUS (D2483-5)

STEPHEN AUSMUS (D2487-3)

ARS scientists are studying the transfer of food or other fluids among members of a bee community through mouth-to-mouth feeding as a possible facilitator of colony collapse disorder.

of factors in every CCD incident or—if it *is* the same factors—that they are occurring in the same sequence in every case."

Pathogens continue to stand out as one of the important puzzle pieces, according to ARS entomologist Jay Evans, also with the Bee Research Laboratory. He was part of a team that used genetic analyses to look for correlations between bee health and bee pathogens and activity levels of honey bee genes.

Two picornalike viruses—acute bee paralysis virus and Kashmir bee virus—along with deformed wing virus, black queen cell virus, and two species of *Nosema,* were found to be more abundant in CCD hives. Infection by multiple picornalike viruses could result in honey bees having reduced abilities to synthesize certain proteins, the lack of which would leave the bees more vulnerable to additional stresses like pesticides, nutrition problems, or other pathogens—which sounds like a possible root cause of CCD, Evans points out.

When the researchers looked at the bees' turning on of detoxification and immune genes, which would have reflected exposure to either pesticides or disease, respectively, there was no significant difference between CCD and non-CCD colonies.

The team did find considerable differences between CCD hives on the west coast and the east coast. "Finding Kashmir bee virus in a hive was the best predictor of CCD in the western United States, while deformed wing virus, an unrelated RNA virus, was a better predictor in the East," Evans says.

Evans and Bee Research Laboratory colleague Judy Chen were also part of an international team that closely followed 29 European honey bee colonies, carefully monitoring for pathogens, parasites, and bee proteins. This study found that four factors appeared to be the best predictors so far of winter honey bee loss: presence of the microsporidian *Nosema ceranae;* levels of the protein vitellogenin, which strongly reflects the bee's protein status and plays an immune system role; *Varroa* mite infestation; and the presence of deformed wing virus, a virus often associated with *Varroa* mites.

Finding *Varroa* mites to be a good predictor of winter declines in this study was not really surprising, according to Evans, though other studies in the United States have not found as high a correlation between CCD and *Varroa* mites.

"Even if *Varroa* mites themselves do not directly cause CCD, we know they can transmit multiple viruses to honey bees," Evans says, "and higher total pathogen levels (rather than infection by any specific pathogen) have been our best correlation with CCD so far. But should the pathogens be considered primary casual agents—multiple pathogens specifically causing CCD—or are they indirectly involved because they weaken bees, making these bees more vulnerable to something else we don't know yet?"

Pesticides as Cause?

Pesticides—individually and in general—have been repeatedly nominated as a cause of CCD, often without direct scientific data to support the idea. In a pesticide survey conducted by U.S. Department of Agriculture and university scientists that analyzed wax, pollen, and

Many Suspects, But No Culprit

Colony collapse disorder (CCD) has been a subject of interest in scientific journals and the popular media since the syndrome first appeared in 2006. Despite numerous and differing claims, nothing has actually been shown to be the cause of the problem.

Pathogens

One or more pathogens remain scientists' most likely choice as the cause or partial cause. But various viruses and bacteria have had higher correlations with CCD in different parts of the United States and in different countries. Before any pathogen can be legitimately accepted as the cause, science must demonstrate that when it is introduced into a healthy colony, CCD results.

Parasites

A parasite is the other perennial suspect, either by itself or in combination with one or more pathogens. *Nosema* and *Varroa* mites remain high on the probable-cause list.

New pests or diseases

Some believe that a previously undiscovered or unidentified pest or pathogen is involved in CCD. But claims that such an agent has been identified have not held up scientifically so far.

Pesticides

There are many classes of pesticides to which honey bees can become exposed. Among those that have been stamped with a "CCD cause" label are the neonicotinoids, like imidacloprid and clothianidin. One issue with making that link is the lack of a matching pattern between neonicotinoid residues in colonies and CCD outbreaks. France, which banned imidacloprid in 1999, and Germany, which along with France banned clothianidin in 2008, still have CCD problems.

Transportation stresses from migratory beekeeping

Pollination-service beekeepers stack colonies on tractor-trailers and transport them thousands of miles during the growing season. For honey bees, orientation to their hive is vital, and being regularly relocated must be stressful. Additionally, moving hives around the country may spread diseases and pathogens as honey bees intermingle in the fields. It is possible that such stresses play into CCD, but there is no scientific evidence of it at this time.

Monoculture

Wild honey bees forage on a wide variety of nectar sources. Honey bees used for commercial pollination are mostly limited to one crop at a time, and it is possible that they may suffer nutritional deficiencies that stress their immune systems.

Genetically modified crops

Genetically modified (GM) crops, most commonly *Bt* corn, have been offered up as the cause of CCD. But there is no correlation between where GM crops are planted and the pattern of CCD incidents. Also, GM crops have been widely planted since the late 1990s, but CCD did not appear until 2006. In addition, CCD has been reported in countries that do not allow GM crops to be planted, such as Switzerland. German researchers have noted in one study a possible correlation between exposure to *Bt* pollen and compromised immunity to *Nosema*.

High-fructose corn syrup

Some researchers have attributed CCD to the practice of feeding high-fructose corn syrup (HFCS) to supplement bee colonies. But there are many reports of CCD occurring in the apiaries of beekeepers who do not feed HFCS. Others have suggested a possible connection with HFCS produced from genetically modified corn, combining two popular villains. But the simple management change of not feeding any HFCS does not stop CCD.

Global climate change

Weather changes, such as unusually warm winters, earlier springs, drought, and flooding, can lead to changes in flowering times. Plants may blossom early, limiting nectar and pollen supplies. But bees used for pollination contracts are moved to fields to coincide with flowering of crops. Still, some believe global warming is to blame, if only in part, for CCD.

Ozone

The level of the air pollutant ozone has been steadily dropping since the early 1990s. Since CCD did not appear until 2006, the timing doesn't match for ozone to be related.

Cell phones and cell phone towers

The idea of cell phones causing CCD began with the misinterpretation of a study in which a cordless home phone, not a cell phone, was shown to have some impact on honey bee navigation. The study author has repeatedly stated that the phone he tested is nothing like a cell phone and has nothing to do with CCD. But the idea remains popular. One of the most recent "proofs," (published in *Current Science* in 2010) claimed evidence suggesting "that colony collapse does occur as a result of exposure to cell phone radiations" while also reporting that the impact of cell phones in both of the test hives resulted in more bees staying in the hive longer—the exact opposite of the definition of CCD.*

bee samples for the presence of 121 different pesticides or their metabolites, the most commonly found pesticides were fluvalinate and coumaphos. While about 60 percent of the 259 wax and 350 pollen samples did show the presence of at least one systemic pesticide, almost all were found at levels well below what is considered lethal to honey bees.

There was no overall pattern of exposure among the samples for a specific pesticide or class of pesticides. The study did not look specifically at the pesticides as they might be related to CCD, but if a specific class of pesticides were involved, a pattern of residues should have been discernable, explains Pettis, who co-led the study.

Not all pesticide impact is about directly killing honey bees, however. Sublethal doses of the pesticide imidacloprid—one of the neonicotinoid group of pesticides—were found to make honey bees more susceptible to the gut parasite *Nosema,* according to a study by Pettis and University of Maryland researchers Dennis vanEngelsdorp, Josephine Johnson, and Galen Dively.

The researchers fed three generations of honey bee colonies either 5 or 20 parts per billion (ppb) of imidacloprid, which is used to protect a wide variety of crops and ornamentals from many different insects. The dosages used in the study were intentionally well below the levels that have been documented to kill honey bees after short-term exposure and reflected levels that have been measured in the environment.

After the third generation, newly emerged adult bees from these colonies were exposed to spores of *N. apis* and *N. ceranae,* gut parasites that have been a growing problem for U.S. beekeepers since the 1990s.

There was up to a fourfold increase in the levels of *Nosema* in honey bees from the imidacloprid-exposed colonies, regardless of whether 5 or 20 ppb were fed.

"While these increased *Nosema* levels were found in individual bees, there was no measurable impact at the colony level," Pettis says. "Imidacloprid was chosen for this study because of its widespread use and beekeepers' concerns about it. But it was only found in 3 percent of the pollen samples checked in the pesticide-survey study, usually at very low levels, and no connection with CCD has ever been made scientifically," he adds.

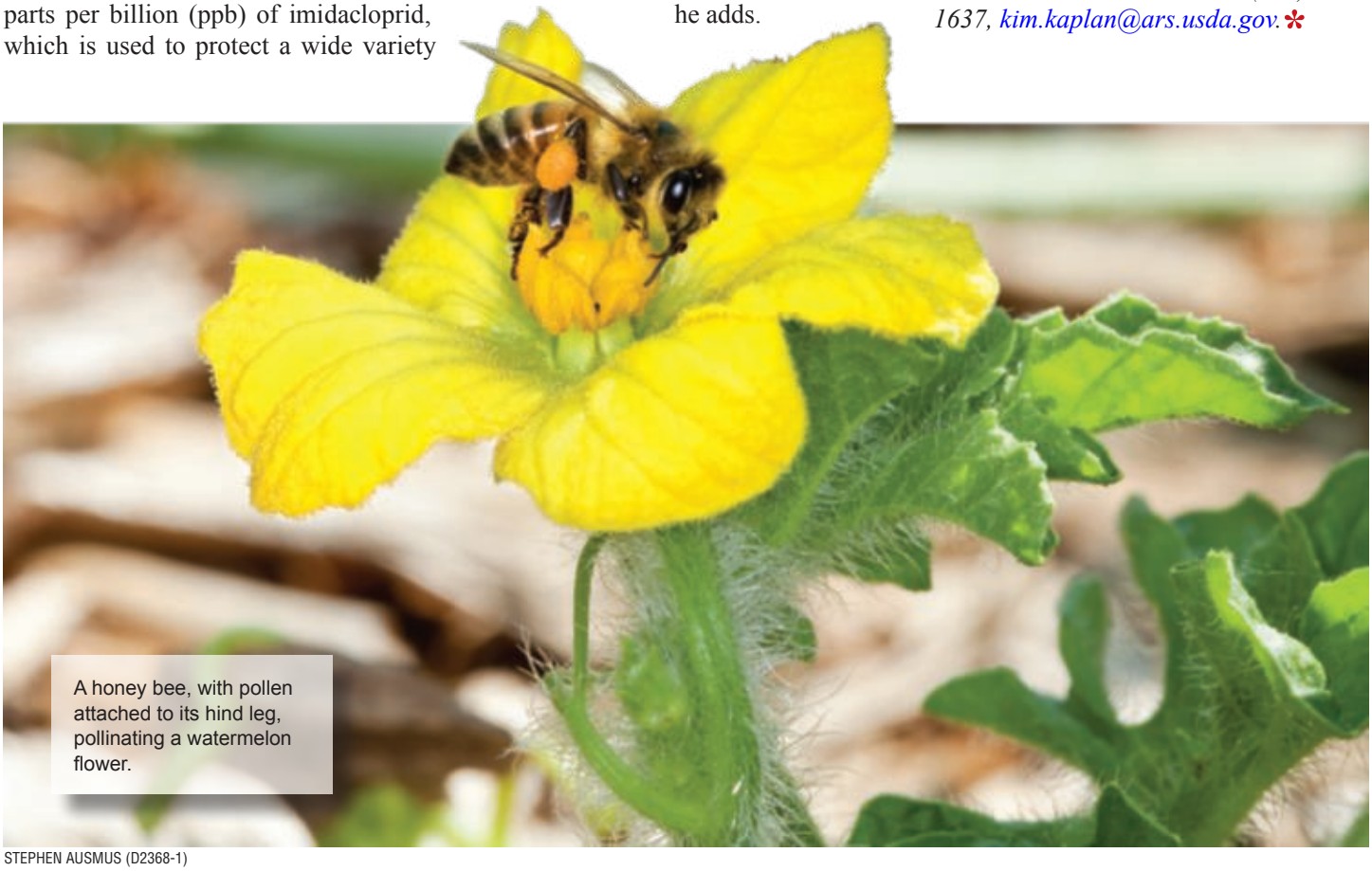
To better account for such sublethal impacts, Pettis is working as part of an international group of scientists and regulators to help the U.S. Environmental Protection Agency (EPA) develop recommendations for pesticide-testing guidelines that factor sublethal effects into test protocols. ARS scientists have already developed a sublethal-impact cage assay that has been provided to EPA.

Could imidacloprid and *Nosema* together be the cause of CCD, as some claim?

"This study did not look for nor establish any connection between either imidacloprid or *Nosema* and CCD," Pettis explains. "But the effect of the combination of imidacloprid and *Nosema* demonstrates that there are many complex interactions between stress factors that need to be considered in looking for a cause of CCD and high honey bee mortality in general."—By **J. Kim Kaplan,** ARS.

This research is part of Crop Production, an ARS national program (#305) described at www.nps.ars.usda.gov.

To reach scientists mentioned in this article, contact Kim Kaplan, USDA-ARS Information Staff, 5601 Sunnyside Ave., Beltsville, MD 20705-5128; (301) 504-1637, kim.kaplan@ars.usda.gov.



A honey bee, with pollen attached to its hind leg, pollinating a watermelon flower.

STEPHEN AUSMUS (D2368-1)

Cracking Down on Poultry Disease with Egg Yolk

It's not mother's milk, but egg yolk may be the closest remedy for boosting the immune system of newly hatched chickens against infectious diseases such as coccidiosis.

A major disease of chickens, coccidiosis is caused by intestinal parasites—single-celled protozoans in the genus *Eimeria*. Disease-affected birds are unable to absorb feed or gain weight, costing the poultry industry more than $600 million annually in the United States and $3 billion worldwide.

Scientists at the Agricultural Research Service Henry A. Wallace Beltsville [Maryland] Agricultural Research Center (BARC) and collaborators from different universities and the Mexican company IASA (Investigacíon Aplicada, S.A.) have developed a novel, antibiotic-free method that uses hyperimmune egg yolk antibodies to control intestinal poultry diseases.

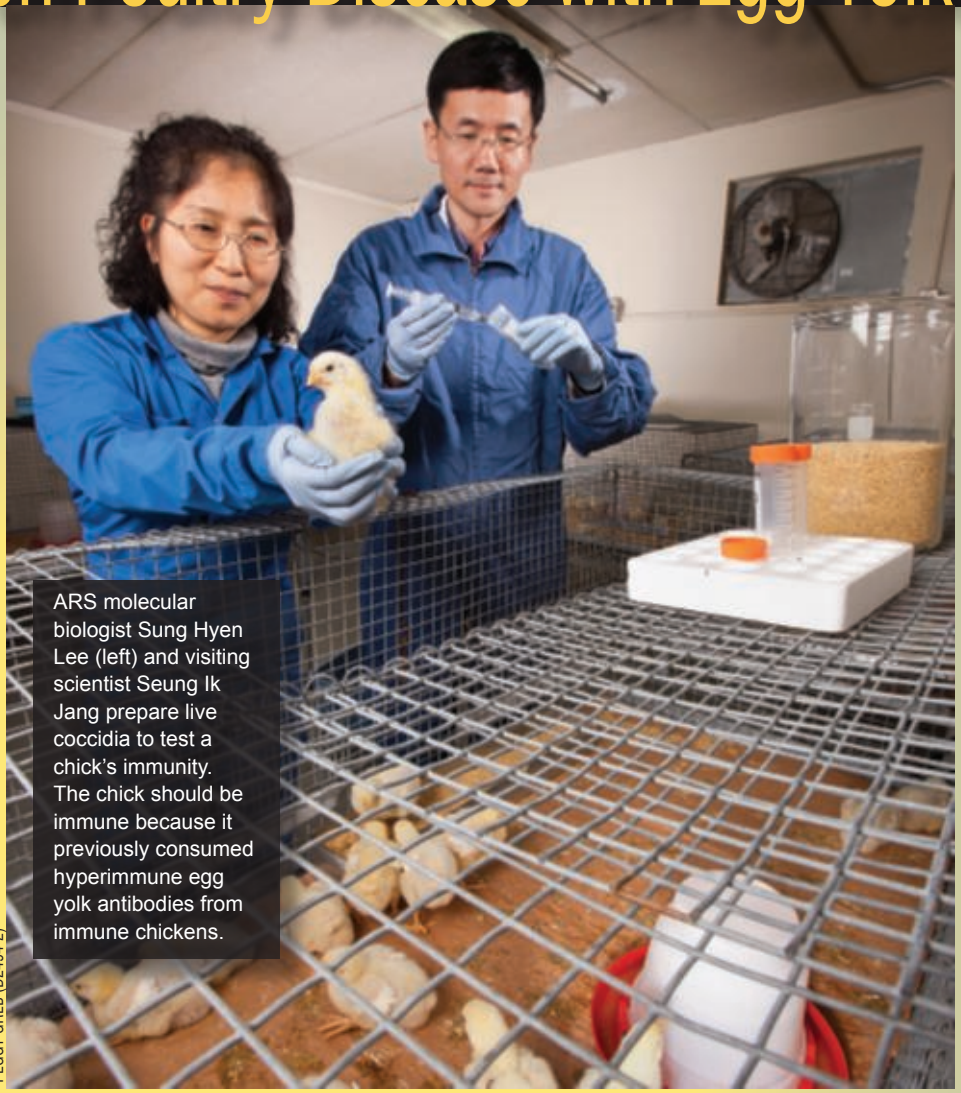
"Coccidiosis is associated with other pathogens, such as the one that causes necrotic enteritis—a prevalent gut disease of poultry," says avian immunologist Hyun Lillehoj, who works in BARC's Animal Parasitic Diseases Laboratory. "By controlling one, you're also reducing the impact of the other."

Good management practices and live vaccinations reduce the spread of coccidiosis, but alternative strategies are needed to help control drug-resistant strains and to enhance organic farming for the poultry industry.

Generally, a host can develop two types of immunity—active and passive—to resist infection. Passive immunity allows immune molecules that are already formed to be transferred from the hen, via the yolk, to the chick. Active immunity relies on vaccines to build immunity in the birds.

"When chicks hatch, they have no immunity to this pathogen. But if we give preformed immune proteins to 1-day-old progeny, they are ready to fight infection," she says. "It's similar to how immunity is passed to newborns through milk."

The method involves extracting antibodies from yolks of eggs from pathogen-free birds that have been hyperimmunized, meaning they possess greater-than-normal immunity due to an abundance of antibodies against the disease. Egg yolk is spray dried, mixed with feed, and given to chicks that have no immune protection right after hatching.



ARS molecular biologist Sung Hyen Lee (left) and visiting scientist Seung Ik Jang prepare live coccidia to test a chick's immunity. The chick should be immune because it previously consumed hyperimmune egg yolk antibodies from immune chickens.

PEGGY GREB (D2494-2)

Lillehoj teamed up with ARS visiting scientist Sung Hyen Lee from the Rural Development Administration in South Korea, IASA scientist Eduardo Lucio, and other researchers to conduct different experiments to demonstrate the efficacy of inducing passive immunity against coccidiosis.

One-day-old broiler chickens were continuously fed a standard diet containing a commercially available egg yolk powder prepared from hens hyperimmunized with multiple species of *Eimeria*. They were then given a challenge infection with live coccidia. Body weight gain between days 0 and 10 and fecal shedding between days 5 and 10 postinfection were analyzed. Chickens given 0.5 percent or less of the hyperimmune egg yolk antibodies had a significant increase in body weight gain, reduced fecal *Eimeria* shedding, and fewer gut lesions compared to control birds fed a nonsupplemented diet.

"It's very simple technology, and it works," Lillehoj says.

Based on these results, one company has developed a commercial product that can be fed to chickens to control coccidiosis. Similar technology may be used in the future to guard against other devastating poultry diseases.—By **Sandra Avant,** ARS.

This research is part of Animal Health, an ARS national program (#103) described at www.nps.ars.usda.gov.

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Oxygenated Phosphine Fumigation for Pest Control on Harvested Fruits and Vegetables

Shipments of U.S. fresh fruits and vegetables can face obstacles in export to overseas markets if they harbor unwanted pests. There are few workable options to kill the pests, and methyl bromide fumigation is the most common one. But methyl bromide destroys atmospheric ozone, and its production is being phased out globally. Therefore, methyl bromide fumigation is unsustainable—as well as expensive. In addition, methyl bromide fumigation injures some fresh produce, such as lettuce. Scientists are searching for alternative ways to solve postharvest pest problems on exported fresh products.

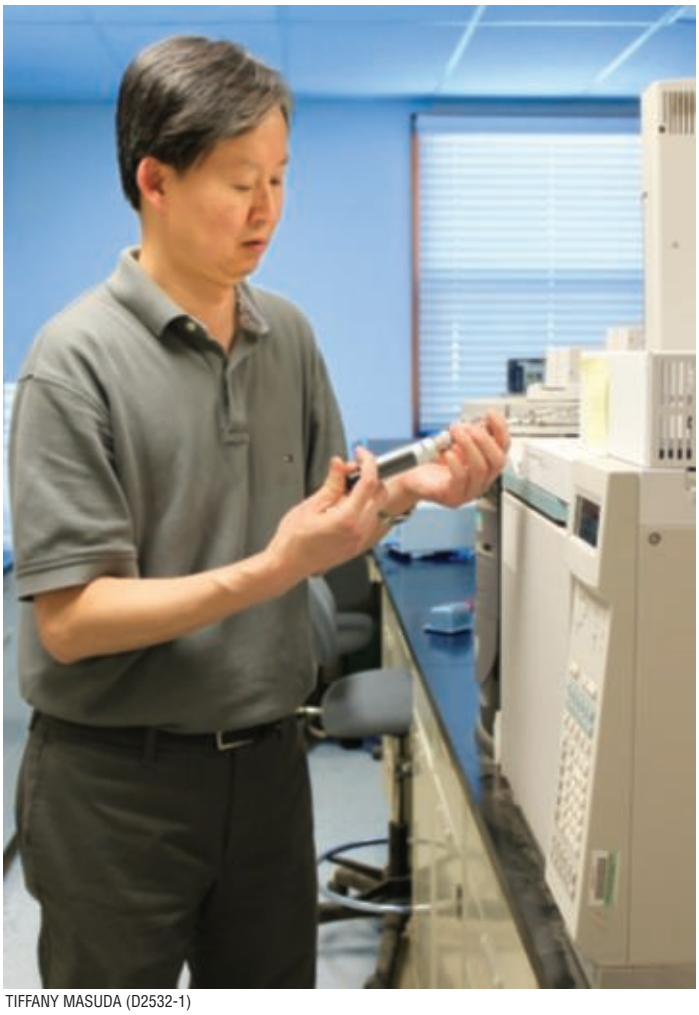
Entomologist Yong-Biao Liu, at the U.S. Agricultural Research Station in Salinas, California, is working on low-temperature fumigation with phosphine as an alternative to methyl bromide for control of pests on harvested fresh fruits and vegetables.

Phosphine has been used for more than 80 years as a fumigant to control stored-product pests. It acts slowly against insects. Many insects, especially at egg and pupal stages, are very tolerant of phosphine, and it may take more than 10 days of fumigation treatment to control them. In working on fumigation with pure phosphine at low temperatures, Liu found that oxygen enhances phosphine toxicity against insects. Liu calls the treatment "oxygenated phosphine fumigation."

In a series of experiments, Liu tested phosphine fumigation under high levels of oxygen against four insects and at different life stages: western flower thrips adults and larvae, leafminer pupae, grape mealybug eggs, and Indianmeal moth eggs and pupae. Liu says that the four species represent a range of insect types and life stages for which quarantine treatments are needed.

"It is important to test the fumigation on eggs and pupae because, in general, eggs and pupae are more tolerant of

At the Crop Improvement and Protection Research Unit in Salinas, California, entomologist Yong-Biao Liu injects fumigant samples into a gas chromatograph to determine phosphine concentrations.



TIFFANY MASUDA (D2532-1)

Directly below: Lettuce aphids, *Nasonovia ribisnigri,* infesting a lettuce leaf. Bottom: Close-up of a lettuce aphid. This pest (about 1-3 millimeters long) appeared in California's Salinas Valley in 1998 and is now found in all lettuce-production areas of that state and Arizona.

YONG-BIAO LIU (D2534-1)

STEPHEN AUSMUS (D689-7)

phosphine than larvae and adults," says Liu. "If we can control insects at the egg and immature stages, we can be sure adults will also be controlled. These insects eat, and thus damage, produce while they are in the larval stage, but all stages require quarantine treatment."

The small-scale study demonstrated that in 5-hour fumigations with 1,000 ppm (parts per million) phosphine at 5˚C, control of western flower thrips on lettuce increased from 80 percent to 98 percent when oxygen was increased from 21 percent to 40 percent. When the oxygen level was increased to 80 percent, mortality of western flower thrips reached 99 percent.

Western flower thrips are a common pest of fruits and vegetables in the United States and are often found on fresh products exported to Taiwan, where it is a quarantined pest. Currently, fresh fruits and vegetables exported to Taiwan are fumigated with methyl bromide to control western flower thrips.

Leafminer pupae control was also improved when oxygen was introduced: At normal oxygen levels, more than 70 percent survived, but only 16 percent were still alive at 40 percent oxygen, and just 1 percent survived at 80 percent oxygen. This fumigation treatment was conducted over 24 hours and contained 500 ppm of phosphine at 5˚C. Complete control of leafminer pupae was achieved in a 24-hour fumigation with 1,000 ppm of phosphine at 5˚C under 60 percent oxygen.

"Increased oxygen levels also resulted in significantly lower survival rates of Indianmeal moth pupae in response to 24-hour fumigations with 500 and 1,000 ppm phosphine at 10˚C, and complete control was achieved at 1,000 ppm phosphine at 60 percent oxygen," says Liu.

"Oxygen enhanced the phosphine toxicity significantly for all life stages tested. This has not been reported previously, and the findings have potential to be used to develop more effective phosphine fumigation treatments for pest control. The magnitude of toxicity enhancement will likely vary depending on the insect species and their life stage."

In a follow-up study for control of lettuce aphid on lettuce, Liu demonstrated that oxygenated phosphine fumigation is not only more effective against the pest but also less toxic to plants than regular phosphine fumigation. At a low temperature of 3°C, regular phosphine fumigation took 3 days at more than 2,000 ppm concentration to control lettuce aphid, and the treatment resulted in significantly higher percentages of both romaine and head lettuce having injuries than the 2-day oxygenated phosphine fumigation at 1,000 ppm concentration under 60 percent oxygen, which also controlled lettuce aphid. Liu says that the shorter treatment combined with lower phosphine levels in oxygenated phosphine fumigation makes the treatment less phytotoxic than regular phosphine fumigation.

Fumigating under high oxygen is also safer to conduct than regular phosphine fumigation. Used alone, phosphine will burn in the air if its concentration exceeds 1.8 percent. Past research showed that oxygen will suppress phosphine ignition and thus reduce risk of fire. The shorter treatment time and lower phosphine concentration used in oxygenated phosphine fumigation also reduce fire risk. Liu says that this is particularly true for fresh products as they are fumigated under low temperature and high humidity conditions.

Even though oxygenated phosphine fumigation is more complex and expensive than regular phosphine fumigation, Liu is optimistic that its benefits in terms of reduced treatment time and enhanced ability to control tolerant pests will more than compensate for the added costs and complexity. Liu expects that the oxygenated phosphine fumigation he developed will have an impact on the phosphine fumigation industry, because many insects that cannot be controlled by regular phosphine fumigation in a reasonable time frame can now be controlled effectively with the new method.

The research studies described in this story were published between 2008 and 2012 in the *Journal of Asia-Pacific Entomology* and the *Journal of Economic Entomology*.—By **Sharon Durham,** ARS.

This research is part of Crop Protection and Quarantine (#304) and Methyl Bromide Alternatives (#308), two ARS national programs described at www.nps.ars.usda.gov.

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An insulated, covered pallet used in fumigation trials to control western flower thrips on head lettuce. The trials were conducted to test phosphine fumigation under high levels of oxygen against insects at different life stages.

YONG-BIAO LIU (D2533-1)

Getting Your Kids To Eat More V

Maybe you're one of those lucky parents whose kids already love vegetables.

If you're not, you're among legions of moms, dads, grandparents, and others who know that some of the youngsters in their lives should eat more of these good-for-you foods, but don't.

Help may be on the way.

And it's coming from a perhaps unlikely source: Your smart phone.

"We're creating a fun, science-based video game that gives parents of preschoolers a quick, easy way to learn some of the best approaches for getting their kids to eat more veggies," says Tom Baranowski, a psychologist at the Agricultural Research Service's Children's Nutrition Research Center (CNRC) in Houston, Texas, and a professor of pediatrics at Baylor College of Medicine, also in Houston. The college operates the nutrition center in cooperation with ARS.

"Kiddio: Food Fight!"—the lively, upbeat video game that Baranowski's team is creating, will offer users a series of short, interactive episodes that they can play on their smart phone. The engaging, fast-paced game features "Kiddio," an appealing preschooler who doesn't like vegetables.

Each episode will give users several choices of what to do to improve the balky youngster's eating behaviors. Importantly, parents can customize the game so that Kiddio's temperament matches that of their child. "That way, what parents learn can help them reshape their own child's eating habits," says Baranowski. "We want the game to be relevant to the real-world food-choice issues of their household."

Parents who want their kids to eat more fruits and vegetables may involve the youngsters in helping to select items from the supermarket produce section.

USDA-FNS (D2535-1)

In the course of each episode, parents will be able to select—with a quick touch on the smartphone screen—multiple options for influencing Kiddio. For example, after deciding whether to offer Kiddio a serving of broccoli, carrots, corn, or peas, players next select what to say to him to increase the chances that he will at least taste the veggie.

(D2538-1)

"Kiddio," an appealing character who doesn't like vegetable parents learn some of the best approaches for getting their whether to take a bite, or say something like "Yuk!" are bas to parental tactics. Images courtesy of ARCHIMAGE, Inc.

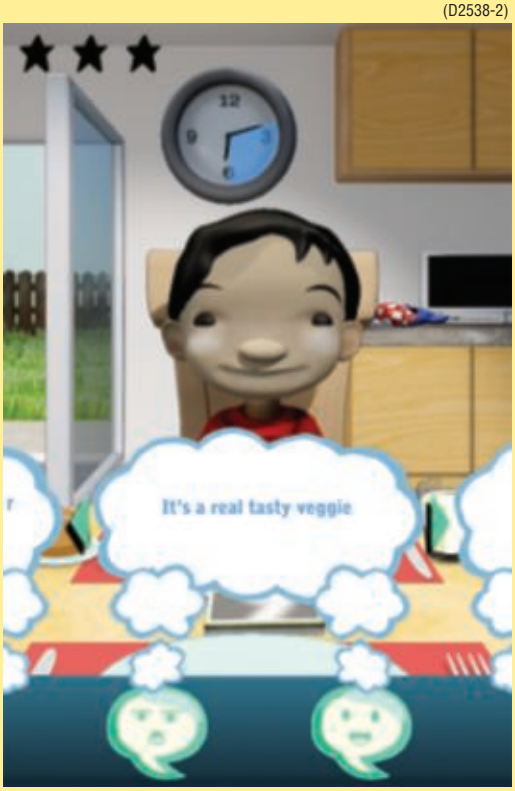
Some of these options, says Baranowski, "create effective, 'teachable moments,' such as when the parent says, 'That's a really tasty veggie.' Other options may express a perhaps-ineffective, 'firm discipline' approach in which the parent tells Kiddio, 'You will taste it before you leave the table!'

"Each of the options is based on a parenting practice that we've studied in our research. And Kiddio's responses to these options—whether to take a bite or to say something like 'Yuk!'—are based on what we've learned so far about kids' reactions to these parental tactics."

By working their way through the various options, "parents can learn which tactics

Vegetables Scientists Scrutinize "Parenting Practices"

(D2538-2)



es, stars in a fun, science-based video game that helps preschool kids to eat more veggies. Kiddio's responses—ed on what researchers have learned about kids' reactions

succeed," says Baranowski. "The point is to give them a safe, low-risk, nonthreatening way to sharpen their parenting skills and to boost their confidence in their decisions.

"We plan to make the episodes increasingly difficult, so players won't become bored or complacent. We hope parents will want to play each episode several times, and that they'll learn something new every time."

Baranowski says that by limiting each episode to just a few minutes, the team will "make it convenient for on-the-go parents to play and learn in spare moments, such as when they're waiting for their kids at the dentist or at soccer practice."

The video-game project, funded by ARS and a grant from the National Institute of Child Health and Human Development, will draw upon five studies that the Houston scientists have conducted over the past decade. These investigations, involving thousands of parents, kids, and nutrition-related professionals, are examples of "behavioral nutrition," a comparatively new scientific discipline that has roots in both psychology and nutrition.

The field is "all about exploring—and explaining—the internal and external factors that influence our food choices," says Baranowski. His work, and that of his Houston coinvestigators, has helped make the CNRC an international leader in behavioral-nutrition research geared to understanding—and helping solve—the most urgent nutrition-related problems of America's children and adolescents.

How do veggies fit into this picture?

Increased vegetable consumption helps kids get the recommended amounts of several vitamins and minerals and is thought by some experts to help reduce the risk of chronic diseases such as diabetes, heart disease, and some cancers.

Peer-reviewed articles by Baranowski and colleagues about the use of video games to improve kids' eating habits have been published in the *American Journal of Preventive Medicine* and the *Journal of Diabetes Science and Technology*.

Survey Reveals Some Parenting Practices

Among the studies that are helping shape the new "Kiddio" series is an investigation that drew upon the real-life experiences of more than 700 Alabama and Texas parents and their preschool-aged children. "Kiddio" collaborator Teresia O'Connor, M.D., an assistant professor of pediatrics at the CNRC and at Baylor College of Medicine, led this study, analyzing—from a different perspective—data collected as part of an earlier, larger investigation headed by CNRC colleague Theresa Nicklas.

Unlike some previous studies, this one didn't focus on just one category of

Proactive actions, such as creating a home environment where kids are likely to see and be served fruits and vegetables and to see a parent enjoying eating fruits and vegetables, are believed to be more effective ways to get children to eat these healthful foods.

USDA-FNS (D2537-1)

parenting practices. Instead, O'Connor's team looked at an array of categories and at combinations of specific tactics from within each category.

"Parents don't do just one thing when trying to influence their child's eating behaviors," says O'Connor. "Rather, they do a combination of things. So, we attempted to investigate this by looking at data pertaining to five different types of behaviors that parents in our study reported using when trying to get a child to eat a veggie or a fruit.

"These categories were: 'teachable moments,' such as telling your son or daughter to try a couple of bites of a vegetable or a fruit, but that he or she doesn't have to eat all of it; 'practical methods,' such as adding something to make a veggie or fruit taste better to the child; 'firm discipline,' like preventing your child from having sweets if he or she doesn't eat the veggie or fruit; 'restriction of junk foods,' such as not keeping any junk foods in the house; and 'enhanced availability and accessibility,' such as keeping a container of ready-to-eat carrots on a lower shelf of your fridge that your preschooler can easily reach.

"We then grouped parents into three clusters according to their use of tactics that are within these general categories of practices," O'Connor says. "On average, no matter what group their parents were in, kids ate less than the recommended number of daily servings of veggies and fruits. But children of the parents who used less of the reactive 'firm discipline' tactics and showed a preference for the proactive 'teachable moments' and 'enhanced availability and accessibility' approaches ate slightly more veggies and fruits than children whose parents were in the other two groups. The finding was statistically significant."

Using combinations of proactive practices "appears to be more effective than using combinations of other parenting tactics," she says. "So, we now want to determine which specific combinations give the best results."

This study was "one of the first to look at how parents use combinations of parenting practices and how these combinations are related to children's vegetable and fruit intake," O'Connor notes. She plans to use this research as the starting point for a longer study. "We looked at one time period—essentially, 3 days in the lives of our volunteers. Now we want to look at how parenting practices influence children's intake of vegetables and fruits over a longer period of time, such as 1 or 2 years."

The findings were documented in a 2009 issue of *Public Health Nutrition*, a peer-reviewed journal.

Lessons From Home and Abroad: Pros Share Their Insights

Other parent-and-kid-focused research led by O'Connor has yielded a globe-spanning glimpse of parenting practices pertaining to fruits and veggies. Her Houston team, and several university researchers based in the United States and abroad, designed, conducted, and analyzed results of an Internet survey that tapped the expertise of nearly 900 doctors, nurse practitioners, registered dietitians, and other healthcare specialists, mostly in Australia, Chile, Mexico, Spain, and the United States.

USDA-FNS (D2536-1)

Many U.S. kids eat more servings of fruits than vegetables, but most eat less of each than they should.

Survey participants were asked to rate the long-term effectiveness of nearly 40 different parenting practices. "The people who took part in this survey have firsthand experience counseling parents about their preschoolers' eating habits," says O'Connor.

"In general, those surveyed agreed that it's more helpful for parents to be proactive than reactive in getting children to eat fruits and vegetables. Proactive actions, such as creating a home environment in which kids are likely to see and be served fruits and vegetables, to see their parent enjoying eating fruits and vegetables, and to have the chance to help a parent select and prepare fruits or veggies, were believed to be more effective techniques, in the long term, for getting children to eat these foods."

On the other hand, being reactive by pressuring, scolding, or punishing the child who's not eating fruit or vegetables was believed to be ineffective—or even counterproductive—in the long run."

According to O'Connor, these consensus opinions "can be useful for parents who are trying to find new ways to encourage their child to eat more fruits and vegetables, and also for public health and healthcare specialists who are developing strategies to promote increased fruit and vegetable intake among young children.

"At Houston, we're using what we learned from this study, and others, to develop food-based strategies for doctors and other clinicians to use as a first-line treatment of obesity among their younger patients."

O'Connor and colleagues reported their findings in a peer-reviewed article published in 2010 in the *Journal of the American Dietetic Association.*

"Today, most kids in this country eat less than the recommended amounts of veggies and fruits," O'Connor says. "We hope that findings from our studies will help change this for the better."—By **Marcia Wood,** ARS.

This research supports the USDA priority of improving children's health and nutrition and is part of Human Nutrition, an ARS national program (#107) described at www.nps.ars.usda.gov.

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STEPHEN AUSMUS (D2505-5)

Beneficial Yeast

A New Weapon for Keeping Tree Nuts Safe To Eat

Tomorrow's orchards of almonds, pistachios, and walnuts might be sprayed with fine mists of a beneficial yeast, *Pichia anomala*. Studies led by Agricultural Research Service plant physiologist Sui-Sheng T. (Sylvia) Hua have shown that this yeast can undermine a troublesome mold, *Aspergillus flavus.* The mold is of concern because it produces aflatoxin, a natural carcinogen.

Federal food safety standards and quality-control procedures at U.S. packinghouses help ensure that these crunchy, healthful tree nuts remain safe to eat. Nonetheless, growers and processors have a continuing interest in new, environmentally friendly ways to combat the mold.

Hua is one of several scientists at ARS's Western Regional Research Center in Albany, California, who are investigating new strategies for thwarting *A. flavus*.

The idea of developing a practical, affordable way for growers to use a yeast to fight a mold isn't new. But Hua's tree-nut-focused investigations of *P. anomala* may be among the most extensive of their kind to date.

Her research has included exploring the yeast's talents as a biocontrol candidate in a series of laboratory tests at Albany and in a field test at a California pistachio orchard. The orchard study, documented in a patent issued to Hua in 2009, indicated that the yeast was responsible for a 96-percent reduction in the number of mold spores.

For ongoing laboratory research, Hua has selected, refined, and applied several analytical procedures to discover precisely how the yeast disables the mold. "If we understand the underlying mechanisms," she says, "we may be able to use that knowledge to increase the yeast's effectiveness."

In a collaborative experiment with Albany coinvestigators Bradley J. Hernlem, a chemical engineer; and Maria T. Brandl, a microbiologist, the mold was exposed to the yeast and later to several different compounds that fluoresce red or green when evidence of specific changes in the mold's cells is detected.

Results of these assays, documented in a peer-reviewed article in the scientific journal *Mycopathologia,* suggest that the yeast interfered with the mold's energy-generating ATP (adenosine triphosphate) system, vital for the mold's survival. The findings also suggest that the yeast damaged mold cell walls and cell membranes. Walls and membranes perform the essential role of protecting cell contents.

The team used a different analytical procedure—quantitative reverse transcriptase PCR (polymerase chain reaction) assays—to analyze the activity of certain *P. anomala* genes in the presence of the mold. Preliminary findings, which Hua reported at the annual national meeting of the American Society for Microbiology in 2010, suggest that exposing the yeast to the mold may have triggered the yeast to turn on genes that code for production of two enzymes—PaEXG1 and PaEXG2.

"These enzymes are capable of degrading the mold's cell walls and causing damage to membranes," Hua notes.

Though further studies are needed, Hua says these early, PCR-based findings point to "gene-controlled mechanisms that may be involved in the cell wall and cell membrane damage observed in the fluorescence assays."—By **Marcia Wood,** ARS.

This research supports the USDA priority of ensuring food safety and is part of Food Safety, an ARS national program (#108) described at www.nps.ars.usda.gov.

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Above: Plant physiologist Sylvia Hua (right) and technician Siov Sarreal display petri dishes showing the effectiveness of a biocontrol yeast against *Aspergillus flavus.* On the left, a mutant *A. flavus* turns the agar orange, signifying aflatoxin production. On the right, when the same *A. flavus* was inoculated between two streaks of yeast, growth was inhibited and no aflatoxin was produced.

The Search for Nematode-Resistant Cotton

Agricultural Research Service scientists in Georgia and Mississippi are helping cotton growers deal with the double-barreled threat posed by two nematode species that lurk in their fields. The root-knot nematode *(Meloidogyne incognita)* thrives in the sandy soils throughout much of the southern United States and can cause crop losses of up to 10 percent worldwide. The reniform nematode *(Rotylenchulus reniformis)* is limited to warmer regions of the Cotton Belt, but its range is expanding. It causes an estimated $130 million in losses each year to the U.S. cotton industry. In some areas, crop losses caused by the reniform nematode are as high as 75 percent, depending on weather conditions. Losses are greatest under drought stress that typically occurs from midsummer to early fall.

Plant breeders have struggled to develop resistant lines in part because cotton has a diverse and complicated genome—some plants have two sets of chromosomes and some have four—making it difficult to cross "wild" resistant germplasm with commercial cultivars and come up with a hybrid that will produce seed. Developing lines resistant to root-knot nematode has been particularly challenging because resistance is a multi-gene trait, and that makes developing a resistant cultivar time consuming and extremely expensive.

ARS efforts have attracted support from cotton growers looking for environmentally friendly ways to repel soil pests. "Our best hope for future management of nematodes is to achieve through plant breeding much of what we are now doing with chemical treatments," says Robert Nichols, senior director for Cotton Incorporated, which is funding much of the work.

The research has taken on a sense of urgency because a pesticide widely used to control nematodes in cotton fields, Temik, is in short supply and is scheduled to be discontinued in the years ahead because of health and environmental concerns. The phase-out of the pesticide, also known as "aldicarb," is "prodding everyone working in this area to step lively," Nichols says.

Eliminating undesirable traits in cotton is a team effort in which researchers

Plant pathologist Sally Stetina (left) and technician Kristi Jordan examine cotton roots with a microscope to determine the level of infection by reniform nematode. By comparing infection levels in resistant test lines to those in susceptible controls, they can identify lines with the most resistance.

STEPHEN AUSMUS (D2513-2)

STEPHEN AUSMUS (D2511-15)

Geneticist John Erpelding cross-pollinates *Gossypium* cotton flowers to develop new populations.

essentially "pass the baton" to plant breeders to develop commercial varieties. Scientists use molecular tools to link nematode resistance with certain patterns in the plant's DNA, and those patterns are referred to as "markers." Researchers provide new cotton lines with those markers to plant breeders and they use them to screen for resistance based on the markers, crossing plants that have them with adapted commercial lines. This process eventually leads to lines with both resistance and the desirable traits inherited from commercial varieties.

ARS researchers are making it easier for breeders to develop commercially acceptable materials by transferring resistance genes from wild plants into cotton cultivars and releasing the resulting lines as breeding tools. They are also developing molecular markers to speed up identification of key nematode-resistance genes. Much of the research is focused on upland cotton *(Gossypium hirsutum),* which is native to Mexico and Central America and is one of two principal types of cotton, making up more than 95 percent of U.S. production.

"Finding genetic markers is critical if we want cotton breeders and private companies to get involved and begin developing commercial varieties with nematode resistance," says Richard Davis, an ARS plant pathologist at the Coastal Plain Experiment Station in Tifton, Georgia.

Davis and colleagues at the University of Georgia have released a root-knot-nematode-resistant line for breeders to work with, and they are hunting for additional genetic markers that will open pathways toward development of commercially viable resistant lines.

Davis has been focused on combating nematode resistance for years. In 2006 he and Peng Chee, his University of Georgia partner, published a paper that identified areas of the cotton genome where root-knot resistance genes are likely to reside. They have since refined the search by mapping portions of the chromosome where the resistance genes are located and identifying "flanking markers" that lie on either side of the genes themselves. These results, published in *Theoretical Applied Genetics,* will be critical in the search for the specific genes that confer resistance to nematodes.

Their new line is the result of several years of field trials where researchers evaluated crosses among cotton plants, some raised in fields inoculated with the nematode and others raised in fields free of it. The new line is susceptible to the reniform nematode and is not intended as a commercial cultivar. But it is an excellent tool for breeders and provides a source of resistance to root-knot nematode, along with yields higher than and quality superior to a breeding line released in 1989 and still used in many field trials as a research standard. Davis released the new line in a recent report in the *Journal of Plant Registrations*.

"What makes this release significant is that it has extremely good fiber quality, it resists the root-knot nematode, and it can grow all over the southeastern United States," Davis says.

Developing Cotton That Resists Both Nematodes

At the Crop Genetics Research Unit in Stoneville, Mississippi, ARS plant pathologist Sally Stetina and plant geneticist John Erpelding are conducting a program to insert genes for reniform nematode resistance into cultivated upland cotton varieties. Those resistance genes will come from several distant relatives: *G. aridum, G. arboreum, G. herbaceum,* and *G. barbadense.*

But crossing cultivated cotton with its distant cousins isn't easy, mainly because of chromosomal incompatibilities.

"Upland cotton is tetraploid—meaning it has four sets of chromosomes—and most of the related species with reniform nematode resistance are diploid, having two sets of chromosomes," explains Stetina.

Agronomist Jack McCarty (left) and geneticist Johnie Jenkins study one of the cotton lines that resist root-knot nematode. In ongoing studies, this resistant line is being crossed with other cotton plants to transfer resistance.

RUSS HAYES (D2518-1)

"When you cross these directly, you get a triploid hybrid, a plant with three sets of chromosomes that is sterile; it will not set seed, and the resistance you moved in will never be passed to the next generation."

The researchers' solution was to create an intermediary cotton strain, known as a "bridging line," using a series of complicated procedures, including embryo rescue and chromosome doubling. Its express purpose is to serve as a bridge between species so that genes for reniform nematode resistance can be passed from cotton's distant relatives into cultivated varieties or germplasm lines used to breed them. However, says Stetina, "When you bring in resistance from the related species, you can introduce undesirable traits such as smaller bolls, limited flowering, poor fiber quality, and poor performance under typical U.S. crop conditions. That's why additional crosses with adapted lines that have desirable agronomic traits are critical to getting the right combination of resistance and crop performance."

Markers provide an important tool to track resistance over multiple generations of crossing to ensure successful transfer, Erpelding adds.

Erpelding and Stetina aim to develop markers associated with reniform nematode resistance in *G. arboreum* and *G. herbaceum* and make them available to breeders. Markers are already available for reniform nematode resistance from *G. longicalyx, G. aridum,* and *G. barbadense* sources. These were developed by teams of researchers from ARS, Texas A&M University, Mississippi State University (MSU), Cotton Incorporated, and Monsanto Company, Stetina says.

Depending on the field in which it is grown, cotton can be attacked by many different nematodes, so varieties with resistance to two or more nematode species can be beneficial. In Mississippi, reniform nematode and root-knot nematode are the biggest challenges to profitable cotton production.

Stetina and Erpelding have teamed with MSU researchers Peggy Thaxton and Ted Wallace to develop cotton varieties with resistance to the two nematode species by using marker-assisted selection. Offspring from crosses are first selected based on the presence of markers for resistance. Plants that are found to have multiple sources of resistance are directly challenged with the nematodes to confirm the resistance. Advanced lines of upland cotton that resist one or both of the nematode pests may be ready for release in 2 to 4 years.

A Pest for the Past 100 Years

At the ARS Genetics and Precision Agricultural Research Unit in Mississippi State, Mississippi, geneticist Johnie Jenkins and his colleagues have also made significant strides in coming up with nematode-resistant cotton lines.

(D2549-1)

A juvenile root-knot nematode, *Meloidogyne incognita,* penetrates a tomato root. Once inside, the juvenile, which also attacks cotton roots, causes a gall to form and robs the plant of nutrients. Photo by William Wergin and Richard Sayre. Colorized by Stephen Ausmus.

Root-knot nematode has been recognized as a cotton pest for the past 100 years, according to Jenkins. "Since the 1930s, scientists have been looking for resistance to nematodes. In the 1960s, ARS started research in root-knot nematode resistance in cotton," says Jenkins. Raymond Shepherd, a retired ARS scientist, was instrumental in using root-knot nematode resistance in a line of wild cotton from Mexico to develop resistant germplasm, he says.

Jenkins and his colleagues found patterns of DNA associated with root-knot nematode resistance and key genetic underpinnings that confer resistance to reniform nematode. The markers they developed for resistance to root-knot nematode in upland cotton—found on chromosomes 11 and 14—should be useful in selecting plants with resistance. They also found that resistance to reniform nematode in a wild *G. barbadense* line is governed by more than one gene, and they have identified markers linked to these genes on chromosomes 21 and 18. They published separate articles on the root-knot nematode work and the reniform nematode work in *Theoretical and Applied Genetics.*

Commercial breeders had steered away from efforts to breed root-knot resistance into upland cotton lines over the years because it was governed by more than one gene and seemed so costly and time-consuming, says ARS agronomist Jack McCarty. But the research contributions from Jenkins and his colleagues may change that due to the use of marker-assisted selection.

"This research has sparked interest from some plant breeding companies in trying to develop high levels of resistance to root-knot and reniform nematode in upland cotton," he says.—By **Dennis O'Brien, Jan Suszkiw,** and **Sharon Durham,** ARS.

This research is part of Plant Diseases (#303) and Plant Genetic Resources, Genomics, and Genetic Improvement (#301), two ARS national programs described at www.nps.ars.usda.gov.

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No-Till Crops Can Improve Air Quality in the Pacific Northwest

For more than 100 years, farmers in the Pacific Northwest interior have favored winter wheat/summer fallow production systems. Since rain typically falls during the mild winters, this schedule gives the growing crops the water they need when they need it. But this rotation can require up to eight tillage passes during the fallow season to control weeds and conserve soil water. Tillage also creates a dry, loose bed of fine soil particles that is easily carried away by the strong summer winds.

"In the inland Pacific Northwest, when atmospheric levels of PM10—particulate matter that is 10 microns or less in diameter—exceed federal limits, it's usually because of erosion from farm lands," says Agricultural Research Service scientist Brenton Sharratt. He is the research leader in the ARS Land Management and Water Conservation Research Unit in Pullman, Washington. "Since the U.S. Environmental Protection Agency regulates air quality, farmers in this area are looking for ways to reduce erosion from their fields and assist communities in complying with regulations."

To help address these concerns, Sharratt, ARS agronomist Frank Young, and Washington State University research associate Gary Feng conducted an 11-year study that evaluated whether no-till spring cereal rotations could help mitigate wind erosion. The systems they studied included the typical winter wheat/summer fallow rotation, a no-till spring barley/spring wheat rotation, and a no-till spring wheat/chemical fallow rotation. During the study, they tracked several soil characteristics, including aggregation, moisture, roughness, crusting, and crop residue cover.

Soil properties were measured twice during the study: once after sowing spring wheat and once after sowing winter wheat. Soils are most exposed after sowing, and sowing schedules coincide with the seasons when high winds prevail in the Pacific Northwest.

The scientists found that in the spring, soils in spring barley and spring wheat rotations were wetter than soils in traditional winter wheat systems. In late summer, the no-till spring barley rotation also had more standing stubble than the other two rotations. Stubble helps keep soil on the ground and out of the air.

Results from the study highlighted other soil-quality payoffs from using spring wheat/spring barley rotations. Soils had larger and more continuous pore space, higher water-infiltration rates, higher saturated hydraulic conductivity, and higher drainage rates.

Given these findings, the team concluded that annual no-till spring cereal crops could significantly improve water infiltration and retention and help retain crop surface residue in the late summer. Farmers could benefit from improved soil quality—and a reduced risk of wind erosion.

"One major windstorm can generate enough airborne dust to exceed air quality standards for PM10," Sharratt says. "But annual no-till cereal crops appear to be a viable strategy that farmers can use to control erosion and meet air quality regulations in the Pacific Northwest." The next challenge is finding ways to make annual no-till crop systems as profitable as the current winter wheat/fallow system.—By **Ann Perry,** ARS.

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

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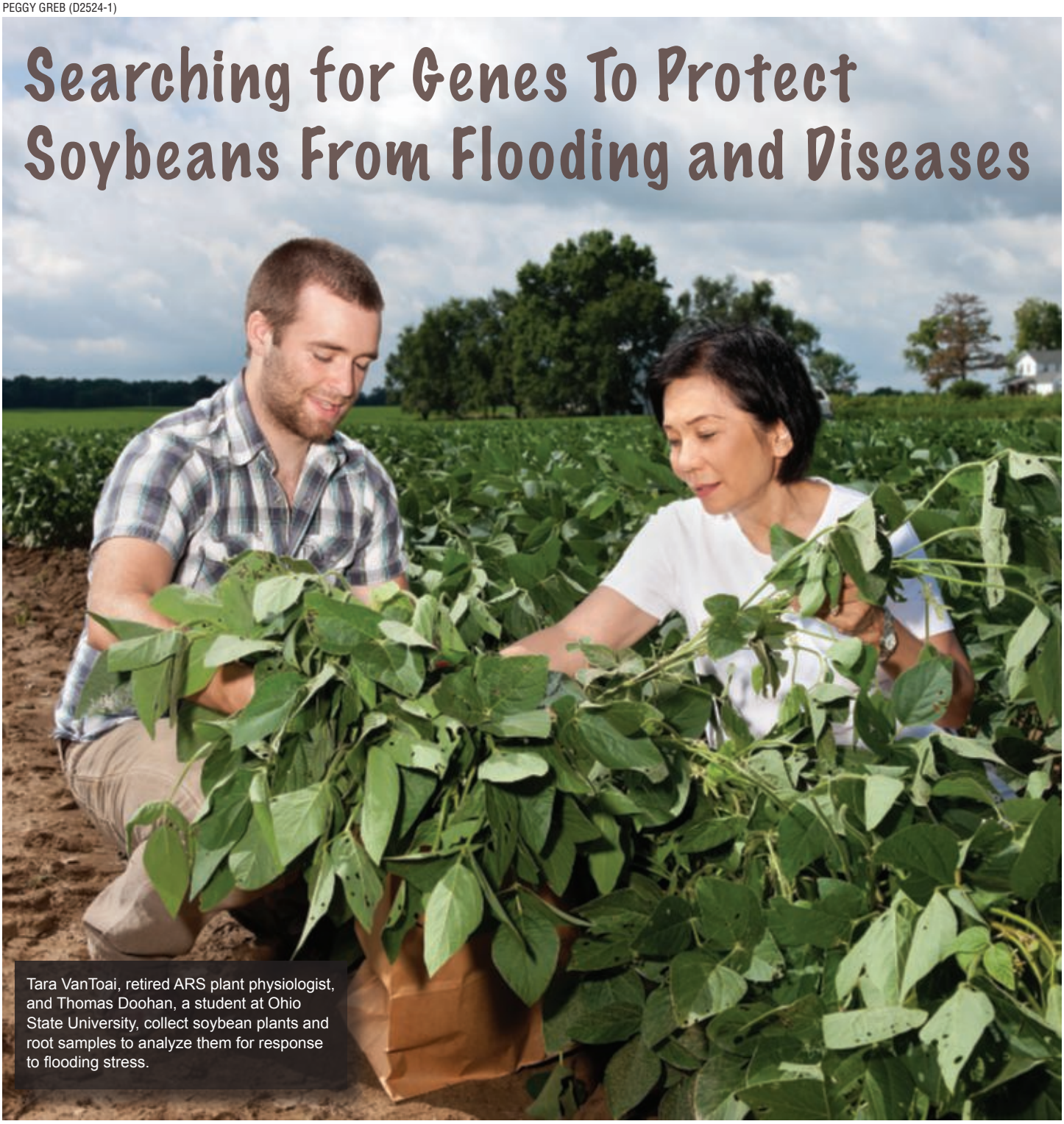
Near Ritzville, Washington, scientists use a wind tunnel to measure wind erosion of soil particles from a recently planted wheat field.

BRENTON SHARRATT (D2539-1)

PEGGY GREB (D2524-1)

Searching for Genes To Protect Soybeans From Flooding and Diseases



Tara VanToai, retired ARS plant physiologist, and Thomas Doohan, a student at Ohio State University, collect soybean plants and root samples to analyze them for response to flooding stress.

Soybean varieties that grow in rice paddies in Southeast Asia could provide the United States with much-needed genes for developing soybeans tolerant to flooding—as well as to root rot and other plant diseases found in waterlogged soils.

Tara VanToai pursued the genes for these traits. She recently retired from the Agricultural Research Service's Soil Drainage Research Unit in Columbus, Ohio.

VanToai worked with plant pathologist Anne Dorrance and soybean breeders Grover Shannon and Henry Nguyen in the search for genes that protect against both flooding and plant diseases. Dorrance is at Ohio State University's Ohio Agricultural Research and Development Center in Wooster; Shannon and Nguyen are at the University of Missouri, in Portageville and Columbia, respectively.

The team works on multiple fronts, including molecular plant breeding, with the help of DNA markers, genetic transformation, and soil management—all in an effort to protect soybeans growing on wet soils.

Flooding—Vietnam to Ohio

Growing up in the Mekong Delta of Vietnam, and then working in Ohio, VanToai has experienced firsthand the harm flooding does to crop yields. VanToai has

been studying flood tolerance in soybeans for more than two decades, in greenhouse, lab, and growth-chamber conditions as well as in experimental and farm fields in Ohio and in Missouri. She has also studied and collected soybean lines in Vietnam and China, including backyard soybean plants that survived China's 1991 flood-of-the-century. The series of research studies is being funded by ARS, the U.S. Department of Agriculture's Foreign Agricultural Service, the United Soybean Board, and the North Central Soybean Research Program.

She has also collaborated with scientists from Brazil, China, France, and Hungary, as well as Vietnam.

U.S. Soybeans Need New Genes

VanToai and colleagues need to incorporate genes from plants native to other countries to supplement the narrow genetic base of U.S. soybeans and to improve tolerance to wet soil and associated diseases.

The varieties of soybeans used by most American farmers are damaged by even short periods in waterlogged soil. Yield losses as high as 25 percent are estimated in the Mississippi Delta region, Asia, and other regions of the world where soybean crops are rotated with paddy rice. The losses are from injuries due to flooding and flood irrigation.

Tests of 21 soybean varieties in flooded experimental fields at Can Tho, Vietnam, revealed three lines of soybean—VND2, Nam Vang, and ATF15-1—with superior flood tolerance.

VanToai and the team did this research with Tran Thi Cuc Hoa and Nguyen Thi Ngoc Hue—both with the Mekong Delta Rice Research Institute, where the experiments were done.

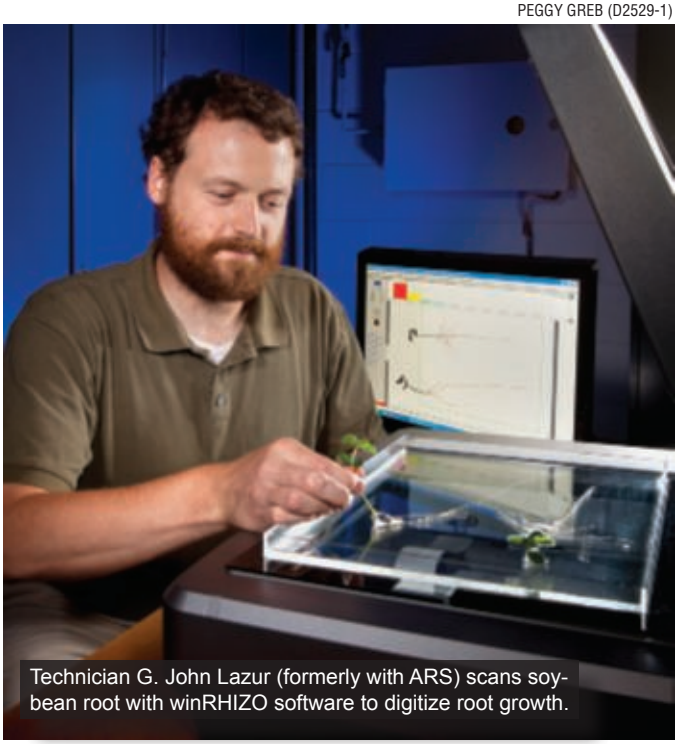
Soybean Plants with Superior Flood Tolerance

Plants from the three flood-tolerant lines grew tallest and had the biggest seeds and the highest yields.

The 21 lines tested included plants native to Vietnam and Cambodia and those developed through selection by farmers and gardeners. They also included lines developed by modern breeding practices and imported from Australia, China, Japan, and Taiwan.

Nam Vang is native to Cambodia, while VND2 and ATF15-1 are from China and Australia, respectively. The initial experiments were done in outdoor "screenhouses," which are greenhouses with screens instead of glass. VanToai found that the screenhouses, while not completely duplicating field results, allowed for accurate predictions of flood tolerance in soybeans.

PEGGY GREB (D2529-1)



Technician G. John Lazur (formerly with ARS) scans soybean root with winRHIZO software to digitize root growth.

Flood-tolerance is defined as the ability of a plant to survive 10 days of steady flooding during the plant's critical flowering stage.

For the screenhouse tests, the plants were grown in pots. When each plant was in full bloom, it was placed for 2 weeks in a bucket of water so that the water level was 2 inches above the soil surface.

In later field tests, the plants were subjected for 2 weeks to water 4 to 6 inches above the soil surface.

The screenhouse tests killed up to 100 percent of susceptible varieties. The test accurately identified the top three flood-tolerant lines, later borne out by the flooded-field tests.

Soybean Secrets

VanToai has also analyzed flood-tolerant eastern gamagrass in the United States for its secrets, one of which turned out to be aerenchyma. This is tissue with air channels that enable roots of plants—rice, for example—to grow under water. These open channels allow flooded roots to snorkel air from the above-water parts of the plants. ARS scientists and university breeders have introduced aerenchyma into wheat and corn experimentally.

VanToai found that soybean plants that survive flooding use some of the same mechanisms as rice growing in paddies.

The two standbys that rice roots rely on for its ability to grow in water are adventitious roots and aerenchyma. Adventitious roots grow out of the plant's stem near the soil surface. They work in tandem with aerenchyma to provide even more oxygen to flooded roots.

Ohio State University scientist Liming Chen examines flooded soybean plants.

ANN HOUSER (D2531-1)

PEGGY GREB (D2527-1)

PEGGY GREB (D2528-1)

Far left: Soybean plants after 2 weeks of flooding stress. Left: Soybean plants that were not flooded.

Genes for Flood Survival

The scientists are looking for genes that trigger the development of those two standbys, as well as other flood-tolerant aids.

They found that flood-tolerant soybean lines develop more adventitious roots and more aerenchyma than flood-susceptible lines. Some soybean lines developed adventitious roots as early as 2 days after being flooded. They discovered that a flood-tolerant South Korean variety, PI 408105A, showed only a 30-percent yield reduction after 10 days of flooding during the critical flowering stage, while the susceptible U.S. variety S99-2281 lost more than 80 percent of its yield. The scientists crossed PI 408105A with S99-2281 to study and transfer the resistance genes.

They evaluated 200 lines from these crosses in fields in Ohio and Missouri for 2 years and identified several lines with a consistent tolerance to flooding.

DNA markers were found that could effectively identify plants that carried the resistance genes. Clear genetic differences were found between the roots of flood-tolerant and flood-susceptible plants.

Because the parents PI 408105A and S99-2281 also show differences in resistance to soybean root and stem rot diseases, Dorrance used the same 200 lines of the population to look for genes for root rot resistance.

Are Flood- and Root Rot-Resistance Genes the Same?

"Now we're wondering whether root rot was contributing to the yield reductions we saw in susceptible plants," VanToai says. "Did the tolerant plants do so much better because they were tolerant not only of flooding but also of root rot? Are some of the same genes involved?" VanToai wonders.

She and colleagues have mapped the genes and found that there is an overlap of genes for resistance to flooding and wet-soil-borne diseases, which indicates some genes are involved in both, while others are not.

Several of the plant lines VanToai and colleagues have developed over the years are included in the ARS Soybean Germplasm Collection in Urbana, Illinois.—By **Don Comis,** formerly with ARS.

This research is part of Water Availability and Watershed Management (#211), Plant Genetic Resources, Genomics, and Genetic Improvement (#301), and Plant Biological and Molecular Processes (#302), three ARS national programs described at www.nps.ars.usda.gov.

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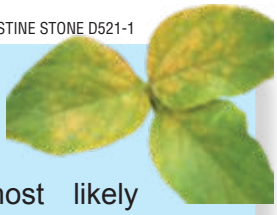
CHRISTINE STONE D521-1



Resistance to Soybean Rust Asian soybean rust, a major disease of soybeans worldwide, was found in soybean fields in South America in 2000. It was first found in the continental United States 4 years later—most likely its spores delivered by winds of an unusually busy hurricane season.

From 2006 to 2009, ARS plant pathologists Tara VanToai (retired), at Columbus, Ohio, and Glen Hartman, at Urbana, Illinois, and other colleagues at the University of Illinois at Urbana and the University of Missouri at Columbia collaborated with scientists at the Plant Protection Research Institute and the Thai Nguyen Education University in Hanoi, Vietnam, to test 65 soybean varieties for resistance to soybean rust. These varieties included rust-resistant accessions from the USDA Soybean Germplasm Collection identified in earlier research, resistant varieties from the Vietnam collection, and susceptible checks. In all, she and colleagues found that the Vietnamese soybean variety DT 2000 showed the strongest resistance in all five experiments. Response of varieties with known resistance genes varied from experiment to experiment.

Asian soybean rust is constantly forming new strains. "This means that we need to develop standardized 'tester' strains so that we can compare results across studies," VanToai says.

The next step will be to identify and map the resistance genes present in the resistant variety found in the Vietnam study and determine their effectiveness against specific soybean rust strains.

VanToai says that the rust-resistant soybean varieties from Vietnam may have genes that could be helpful wherever rust strains have a similar virulence to those in Vietnam.—By **Don Comis,** formerly with ARS.

The Agricultural Research Service has labs all over the country.

Locations Featured in This Magazine Issue

Locations listed west to east below.

Western Regional Research Center, Albany, California
8 research units ■ 225 employees

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

San Joaquin Valley Agricultural Sciences Center, Parlier, California
3 research units ■ 125 employees

Pullman, Washington
6 research units ■ 136 employees

Center for Grain and Animal Health Research, Manhattan, Kansas
5 research units ■ 129 employees

Children's Nutrition Research Center, Houston, Texas
1 research unit ■ 7 employees

Jamie Whitten Delta States Research Center, Stoneville, Mississippi
7 research units ■ 323 employees

Urbana, Illinois
2 research units ■ 42 employees

Mississippi State, Mississippi
2 research units ■ 71 employees

Columbus, Ohio
1 research unit ■ 15 employees

Tifton, Georgia
3 research units ■ 118 employees

Center for Medical, Agricultural, and Veterinary Entomology, Gainesville, Florida
4 research units ■ 144 employees

Subtropical Horticulture Research Station, Miami, Florida
1 research unit ■ 45 employees

Henry A. Wallace Beltsville Agricultural Research Center, Beltsville, Maryland
27 research units ■ 890 employees

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

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