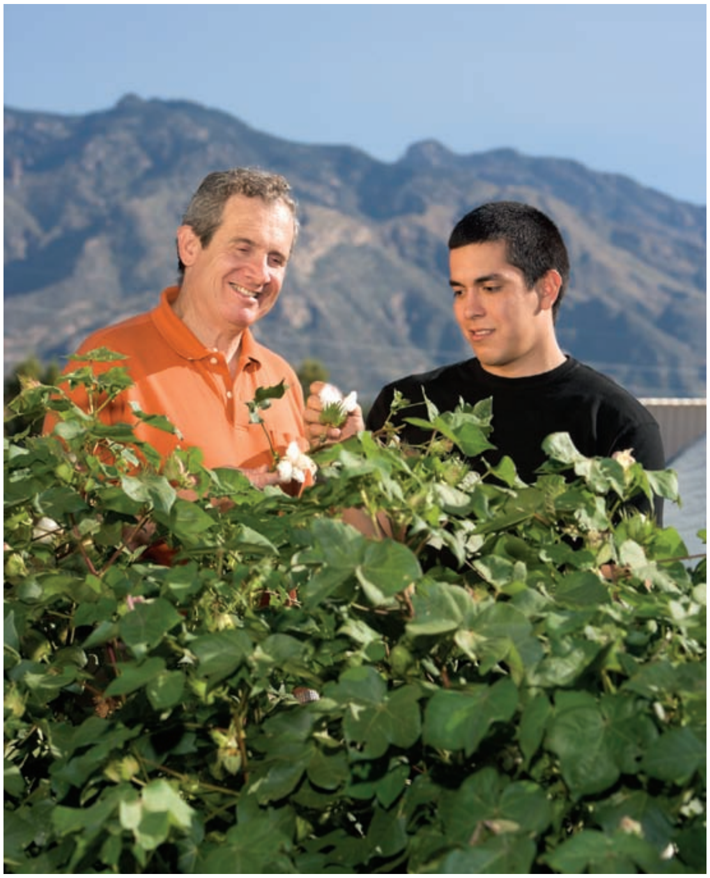
A Fungal Fight in the Desert

A little competition among fungi could help save the Southwest's cotton crop.

PEGGY GREB (D790-1)



Plant pathologist Peter Cotty (left) examines cotton in an Arizona research field with University of Arizona graduate student Alejandro Ortega-Beltran.

Plant pathologist Peter Cotty watches a dust cloud billow up over the baking Tucson, Arizona, desert. Oddly enough, it's got him thinking about one thing: fungi.

Despite the parched air and blazing temperatures, microscopic communities of fungi, including *Aspergillus* species, are thriving all around there.

"*A. flavus* fungi are found throughout the Southwest," says Cotty (who is part of ARS's Food & Feed Safety Research Unit in New Orleans, but is based at University of Arizona-Tucson). "They live in agricultural soils and desert soils, on crops and native plants—even in the dust and air."

The problem with some *A. flavus* fungi is that, as they invade agricultural fields, they can produce potent poisons. The carcinogenic compounds they make, a type of mycotoxin called "aflatoxin," are a major concern for U.S. cotton growers. That's because cottonseed is an important feed source of the nation's dairy herds. Toxins in contaminated cottonseed transfer to the animals' milk.

To ensure that aflatoxin never makes its way into milk or other foods, the Food and Drug Administration has established a stringent 20-parts-per-billion limit on these mycotoxins in cottonseed and in other crops vulnerable to toxic molds—including corn, peanuts, pistachios, almonds, walnuts, and figs.

Because of these troubling toxins, farmers lose profits and export opportunities. And natural resources, like irrigation water and fertilizer, are inefficiently used. Every year in the Southwest, toxin-producing fungi ruin $3 to $8 million worth of cottonseed.

They're Not All the Same

But not all *A. flavus* fungi have a bad reputation. In fact, there's much diversity among the *A. flavus* bunch. For instance, some strains, Cotty says, like the S strain, can pump out incredibly high levels of aflatoxin. "It's not unusual," he says, "for an S strain isolate to produce more than 1 million parts per billion of aflatoxin in the lab."

In contrast, many *A. flavus* strains are essentially harmless. They lack the genetic equipment needed to churn out poisonous aflatoxins. And it's one particular nontoxic strain that Cotty is banking his hopes on.

Eighteen years ago, Cotty discovered a strain of *A. flavus*, called AF36, that not only lacks the ability to produce toxins, but can also outcompete and outlive fungi that do.

In 1996, after many laboratory and field studies, ARS was awarded approval from the U.S. Environmental Protection Agency (EPA) to test the biocontrol fungus in commercial fields in Arizona. When tests under an experimental-use permit were successful, EPA awarded a Section-3 pesticide registration for the fungus, allowing treatments of unlimited acreage in Arizona and Texas. California was added to the label in 2005.

Ten years ago, only 120 acres of commercial cotton were treated with AF36. Since that time, AF36 has been sprinkled, sprayed, and dropped onto well over 100,000 experimental acres of southwestern cotton. And it's making a serious dent in the populations of toxic *A. flavus* fungi present in those fields.

"We routinely observe more than 80-percent reduction in aflatoxin-producing fungi in cottonfields in Arizona and Texas after treatment with AF36," says Cotty.

A Field Guide to AF36

To optimize the biocontrol's chances for success, Cotty is drafting cultural recommendations that he can pass on to growers interested in using AF36. So far, after multiyear field studies in both Arizona and Texas, he's found that both soil type and crop rotation type influence fungal community structure.

"High-clay soils and cotton rotations," Cotty says, "favor the incidence of the S strain." He and Ramon Jaime-Garcia of the University of Arizona have linked this particular strain to some of the most severe aflatoxin outbreaks in cottonfields in southern Texas. With this information, growers now know they should target their control efforts on this especially potent strain.

Cotty and Jaime-Garcia have also found that corn-cotton rotations growing in southern Texas and treated with AF36 need prompt harvest. Leftover corncobs can serve as "oases" for poison-producing *A. flavus* fungi, providing them a critical food source and refuge through the winter season.

When Defective Is Desirable

Cotty is also addressing concerns that AF36 could evolve in the field over time, somehow gaining the ability to make toxins. To help assuage such worries, Cotty needed proof that his AF36 strain is inherently nontoxic.

Now, he's got that proof. Last year, Cotty and colleague Ken Ehrlich of ARS's Food and Feed Safety Research Unit in New Orleans, Louisiana, confirmed that it simply isn't in AF36's genes to produce aflatoxins. In fact, according to the scientists scrutinizing its genetic material, the fungus possesses defective genes. Without normal versions of such genes, AF36 cannot create the gene products needed for making aflatoxin.

Furthermore, Cotty and Ehrlich defined the specific genetic kink that makes AF36 so different from its *A. flavus* cousins. This finding means that AF36 can be monitored easily and rapidly in the field.

A Mass-Production Line

As with most beneficial microbes, the AF36 fungus has little practical value until it can be mass-produced. On this front, Cotty has succeeded, too. Along with the grower-run Arizona Cotton Research and Protection Council (ACRPC), Cotty has helped develop a commercial-scale process for making large quantities of AF36.

A facility in Phoenix has been up and running for 7 years and now produces 2,700 kilograms of AF36 product every day. Cotty and ACRPC collaborators continue to scale up the process, improving formulations and making more AF36 more efficiently. This should further reduce the already affordable price of the biocontrol. Right now, ACRPC provides it to producers for $5 an acre.

In this southwestern corner of the United States, the future is bright for AF36. In the last 10 years, it's helped reduce aflatoxin levels by up to 90 percent. And Cotty expects that its use will spread. "We know that pistachio growers in California and corn growers in the Southwest are also interested in tapping AF36's potential," he says. "I'm hopeful they'll get the chance."—By **Erin Peabody,** ARS.

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

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PEGGY GREB (D788-1)

University of Arizona graduate student Claudia Probst examines plates of *Aspergillus flavus* isolates to determine which strains produce the most aflatoxin.

PEGGY GREB (D789-1)

ARS biological science aid Alix McCloskey and University of Arizona scientist Ramon Jaime-Garcia examine wheat kernels containing a strain of *Aspergillus flavus* that acts as a biocontrol agent against strains of *A. flavus* that produce aflatoxin. The kernels are sterile, so they won't germinate when applied in a field; instead, they will be a food source for the biocontrol agent to establish itself.