



Larry Antilla, left, and Leighton Liesner of the Arizona Cotton Research and Protection Council with a bag of Aspergillus flavus AF36 Prevail at the group's Phoenix, Ariz. headquarters.

AF36 battles aflatoxin with 'good guys vs. bad guys'

Biological pesticide has helped growers reduce aflatoxin levels in western cotton, corn, pistachios, figs - plus almonds starting in 2018

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As veteran Arizona cotton growers can attest around 1970 was one of the worst times in the state's fiber industry as news reports printed in The Arizona Republic (the Phoenix area's largest newspaper) showed photos of semi-trucks dumping cow's milk into the almost always dry Salt River bed near the downtown area.

Some of the milk contained dangerous and potentially deadly levels of aflatoxin traced to back cottonseed fed to milk cows. According to the federal government, cottonseed with more than 20 parts per billion of aflatoxin cannot be fed to dairy cows.

Aflatoxins are toxic chemicals produced by certain strains of fungi during crop infection, compounds that can also cause acute or chronic toxic effects in humans. Aflatoxin-producing fungi are found naturally in the soil.

Searching for answers

With this black eye on Arizona cotton, grower leadership of the Arizona Cotton Growers Association (ACGA), led then by President Ron Rayner of Goodyear, took the serious issue by the horns. Their tremendous challenge was to find a way to reduce overall aflatoxin levels in cottonseed to ensure that milk from dairy cows and other products were safe for human consumption.

Major players in the ACGA's early aflatoxin effort included the group's executive vice-president Rick Lavis (deceased), plus growers Ted Pierce, Bruce Heiden, Rayner, and others.

The non-profit ACGA faced two options: One, ask the farm chemical industry to develop an aflatoxin-reduction product; or provide the same marching orders to the cotton grower-led Arizona Cotton Research and Protection Council Program (ACRPC) at Phoenix.

Marching orders

The expectation for the group chosen was to develop an effective atoxigenic product and bring it to the commercial market; plus making the product affordable for cotton growers to apply to cotton stands.

ACGA chose ACRPC, a non-profit organization formed by statute in 1984 to serve cotton growers through research and applied technology to solve industry challenges. ACRPC is funded by an assessment on every bale of Arizona-grown cotton. In essence, growers would help fund finding the aflatoxin answer.

The ACRPC invested \$7 million of growers' money into the development of the anti-aflatoxin technology.

ACRPC Director Leighton Liesner (2011 to present) and former Director Larry Antilla (1992-2011) sat down with Western Farm Press at their headquarters to discuss the road toward product development, the product status, and its expansion into other crops including tree nuts.

Peter Cotty delivers

ACRPC turned to scientist Dr. Peter Cotty of USDA's Agriculture Research Center (ARS) to search for a way to reduce aflatoxin levels in cotton. Today, Cotty is a U of A adjunct professor and ARS research plant pathologist associated with the university's campus at Tucson.

Over the years, Cotty's lab has sought ways to reduce aflatoxin contamination caused by strains of the common fungus *Aspergillus flavus*, according to the ACRPC website.

In the laboratory, Cotty tried various methods. The process that worked the best was displacing toxin-producing group S strains with non-toxigenic strains from the L strain group. Cotty tested this by isolating non-toxigenic strains of *Aspergillus flavus*. He coated those on wheat seeds, placed them inside a pillowcase, and dried the product in a pizza oven.

It worked as the number of highly toxic strains greatly decreased while the non-toxigenic strains increased dramatically.

With success at the lab level, Cotty took his research to Lyredale Farms at Roll, Ariz. where owner-operators Clyde and David Sharp grew cotton. Through field tests, Cotty's new product was expanded, producing enough non-toxigenic *Aspergillus flavus* units to apply the product over several hundred acres of cotton to further test Cotty's invention.

Good guys win over bad guys

In four years of cotton field trials near Yuma, Antilla says the amount of bad strains fell from 82 percent to 4 percent while the good strain soared from 5 percent to 51 percent.

"The results were pretty remarkable," said Antilla at the ACRPC helm at the time. "Not only did Dr. Cotty prove that the use of non-toxigenic fungi applied in the field could greatly lower overall aflatoxin levels it also had a halo effect on the fields around it."

In essence, "We reversed the ration of 'good guys and bad guys' in the field. Somehow, the product out competed the highly toxic S strains."

ACRPC's new aflatoxin lowering product was born and named *Aspergillus flavus* AF36.

Antilla added, "If we can consistently treat acre after acre with AF36 over long periods of time we can change the fungal makeup of the soil community. There are still a few bad guys out there but a whole lot less than there would have been naturally."

The number 36 in the product name was Cotty's 36th isolate tested that achieved the desired results.

ACRPC and Syngenta

ACRPC holds the registration for the AF36 technology but doesn't have exclusive rights to it. The chemical company Syngenta has a similar biological control agent on the commercial market, also based on Cotty's research, called Afla-Guard GR designed for aflatoxin control in corn and peanuts.

Syngenta says Afla-Guard's natural biocontrol agent "significantly reduces aflatoxin contamination levels to protect the marketability and value of your crop."

The Syngenta and ACRPC products are both atoxigenics.

At ACRPC, Antilla proceeded to bring AF36 to commercialization. The group's initial meeting with the U.S. Environmental Protection Agency (EPA) was in 1992 followed by the first experimental use permit granted for 1996-1998. By 2002, EPA granted the Section 3 label for AF36 for cotton including 20,000 acres treated in Arizona cotton and 2,000 acres treated in Texas.

Trying to gain faster federal approval, ACRPC filed the EPA labeling request for *Aspergillus flavus* AF36 as a biological pesticide.

"Dr. Cotty is the progenitor of this technology," Antilla said. "Dr. Cotty discovered that even though *Aspergillus flavus* is cosmopolitan there are certain strains that produce a lot of bad toxins, strains which produce intermediate amounts of toxins, and some strains which produce no toxin. The latter one became AF36.

Next step - production

Rolling out AF36 on a commercial scale was a daunting task for ACRPC. It meant taking the Cotty process and inventing large equipment for commercialization.

According to Liesner, the ACRPC team developed steam sterilization of wheat seed used as a nutrient medium to prevent germination so it wouldn't grow in the field, and to kill any existing bacteria (fungi). Then the sterilized, devitalized wheat seed was inoculated AF36 to make the fungus stable on wheat, and then packaged so the product could be successfully used at a later date in fields.

AF36 and tree nuts

Antilla said AF 36 has greatly benefitted cotton growers in Arizona, and corn producers in Texas, and the 2,000,000 pounds of AF36 produced for these markets almost maxed out the ACRPC's production ability.

As the possible use of AF36 neared in pistachio and almond orchards, Liesner and Antilla worked quickly and efficiently in 2013 to re-tool the current AF36 production facility. With Cotty's assistance, they created new production equipment which produces up to 5 tons per hours, compared to 3 tons per day using the original process.

With the new process and formulation, the name of the product was changed from *Aspergillus flavus* AF36 to *Aspergillus flavus* AF36 Prevail.

"With Dr. Cotty's leadership, we developed a new process that's faster and more efficient to achieve the same end result with the same number of spores in the field to gain the results we want," Antilla said.

Collaborators

During the ramping up process, EPA registration was granted for product use in pistachio in California, Arizona, New Mexico, and Texas. Among the California collaborators with Liesner and Antilla for the little-green nut were Bob Klein, manager of the California Pistachio Research Board and the Administrative Committee for Pistachios; plus Dr. Themis Michailides, University of California plant pathologist. The Section 3 label for the Prevail formulation was granted for cotton, corn, and pistachio in March 2016.

Since the supply for pistachios was initially limited to half the label rate at 5 pounds per acre, the expanded supply meant that pistachio growers could buy AF36 Prevail at the maximum label rate of 10 pounds/acre. This is also the same label amount allowed for cotton and corn.

In 2018, California almond and fig growers can use the product at the same 10-pounds-per-acre rate in orchards. Antilla said the best time to apply Prevail via granular spreader in tree nuts is from mid-May through mid-July. ACRPC has worked with Gabrielle Ludwig and Bob Curtis of the Almond Board of California on Prevail use in almonds, and Karla Stockli of California Figs.

Antilla noted, "This technology works best when used area-wide in cooperation with neighboring growers as the fungus moves easily in the air. If only several individual fields are treated, a resurgence of toxin-producing strains of the fungi is likely."

Dr. Michael Braverman and the staff of the IR4 project at Rutgers University have provided important support for the registration of AF36 products, and have "continually championed the technology from the earliest stages of development," noted Liesner and Antilla.

Summary

"This is a completely natural product from the soil – an endemic strain," said Director Liesner. "It's a strain of fungus already in the soil and we're not adding anything to it."

The moto on the Prevail bag says, "For growers by growers."

Liesner says the Council's two main goals from Day One have been – 1 - "Stewardship of the technology – making sure it doesn't get compromised; 2 - provide it for commodities at an economical price."

The distributor of AF36 Prevail in California is Western Milling, the ACRPC in Arizona, and Scott Averhoff of Double CT in Texas.

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