

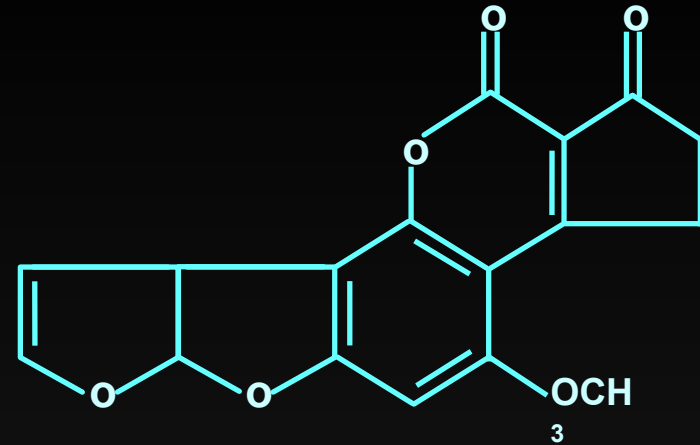
Cotton Incorporated Cotton Breeders Tour (Arizona)

Winning the War on Aflatoxin: Biological Control to Reduce Toxin Levels



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Agricultural Research Service,
United States Department of Agriculture
School of Plant Sciences, University of Arizona

September 26th, ENR2 Building, University of Arizona, Tucson



Aflatoxin B₁

Highly Toxic – high concentrations (ppm) can cause rapid death.

Human Carcinogen – very small amounts (ppb) cause cancer.

Immune Suppression – reduces defense against disease.

Stunting – influences development of humans & domestic animals.

- * Occurs in Many Crops (Corn, Peanuts, Cottonseed, Tree Nuts, Spices)
 - * Transmitted from Feed to Milk
 - * Contents in Foods & Feed Regulated Worldwide (ppb!)
 - * Influences Market Access & Crop Value

Aflatoxins in Cottonseed

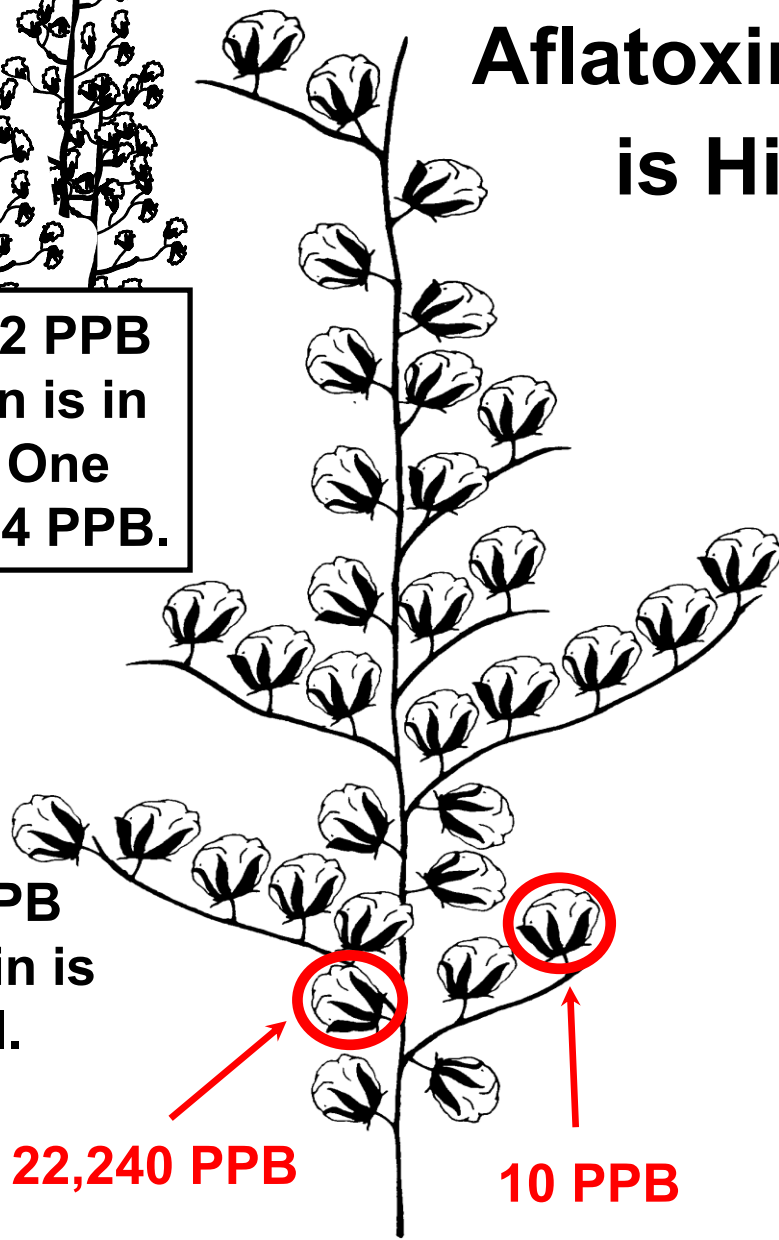
- ✓ Costs millions annually.
- ✓ Strict enforcement of federal limits.
- ✓ Cottonseed analyzed before use.
- ✓ Monitoring – Certified Samplers, Certified Laboratories.
- ✓ Arizona milk is carefully monitored.
- ✓ High cost to industry – Doing Business, Loss of Markets, Loss of Image, Litigation, Discounts.



Aflatoxin Contamination is Highly Variable



Crop average is 72 PPB but, most aflatoxin is in one in ten plants. One plant averages 654 PPB.

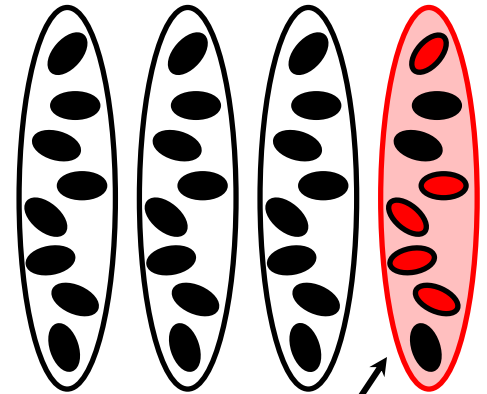


Plant has 654 PPB but, most aflatoxin is in a single boll.

22,240 PPB

10 PPB

The boll has 22,240 PPB. Most aflatoxin is in one locule which has 115,000 PPB. Some seeds have over 250,000 PPB.



115,000 PPB



Aflatoxin Contamination Occurs in Two Phases

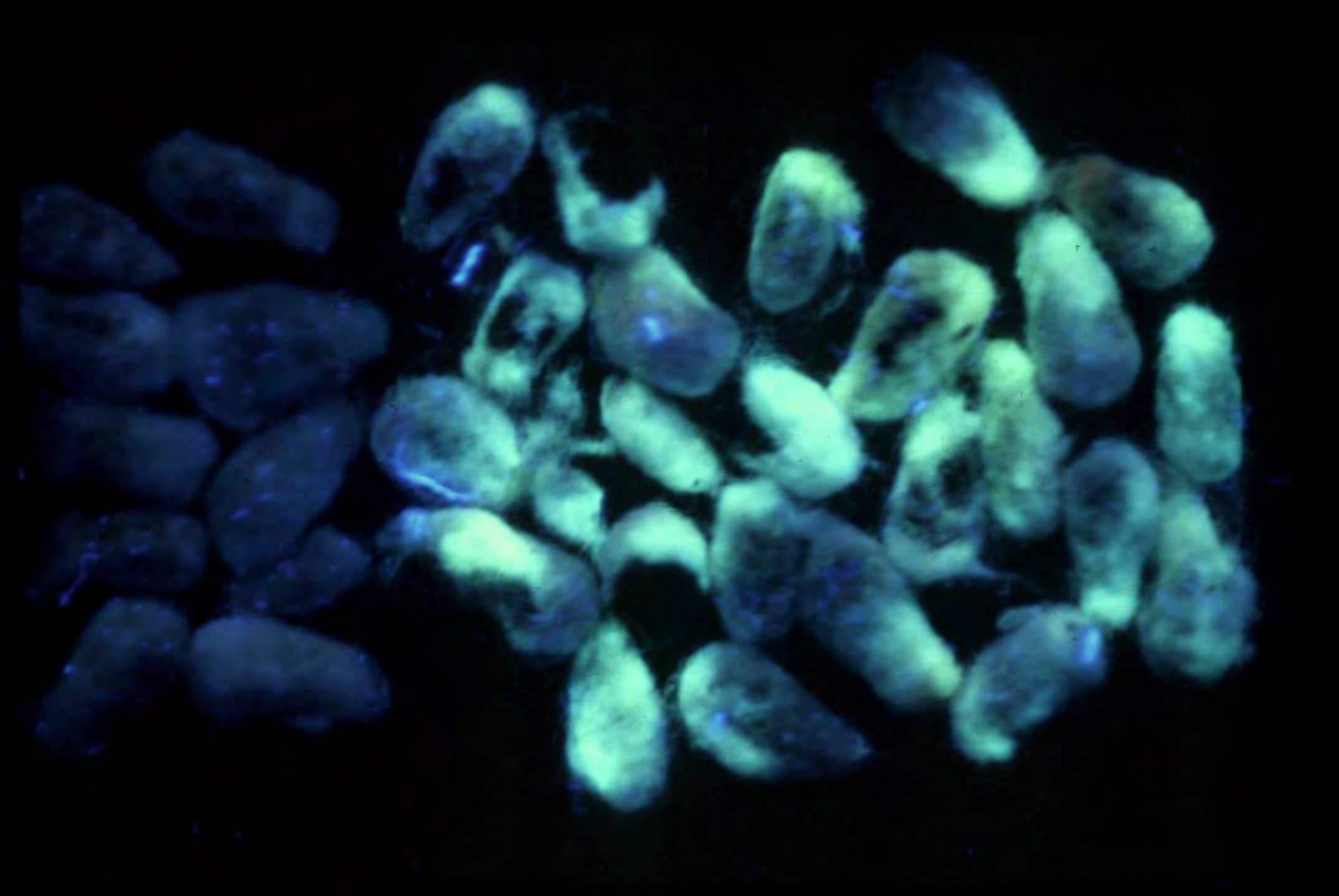
Climatic Influences Differ Between the Phases

Phase I: Before Crop Maturity

- Developing crops become infected.
- Associated with crop damage (insect, bird, stress).
- Most BGYF forms during this phase (bright-green-yellow fluorescence).
- Favored by high temperature (night), dry conditions, crop production under water deficit (drought).

Phase II: After Crop Maturity

- Aflatoxin increases in mature crop.
- May occur before or after harvest.
- Seed is vulnerable until consumed.
- Associated with high humidity in the field, & improper crop storage or transportation.
- Rain on the mature crop increases contamination.



Pink Bollworm: Repeatedly Linked to the First Phase of Contamination



Aflatoxin Content of Cottonseed from Bolls with Pink Bollworm Exit Holes and from Bolls without Exit Holes

Test	Exit Hole	With toxin	Aflatoxin (ppb)		
			Maximum	Minimum	Average
1	Yes	52%	152,000	0	16,790
1	No	8%	93	0	5
2	Yes	68%	146,000	0	17,470
2	No	8%	12	0	1
3	Yes	54%	160,000	0	8,890
3	No	0%	0	0	0

Each value is an average of 25 analyses of individual bolls.

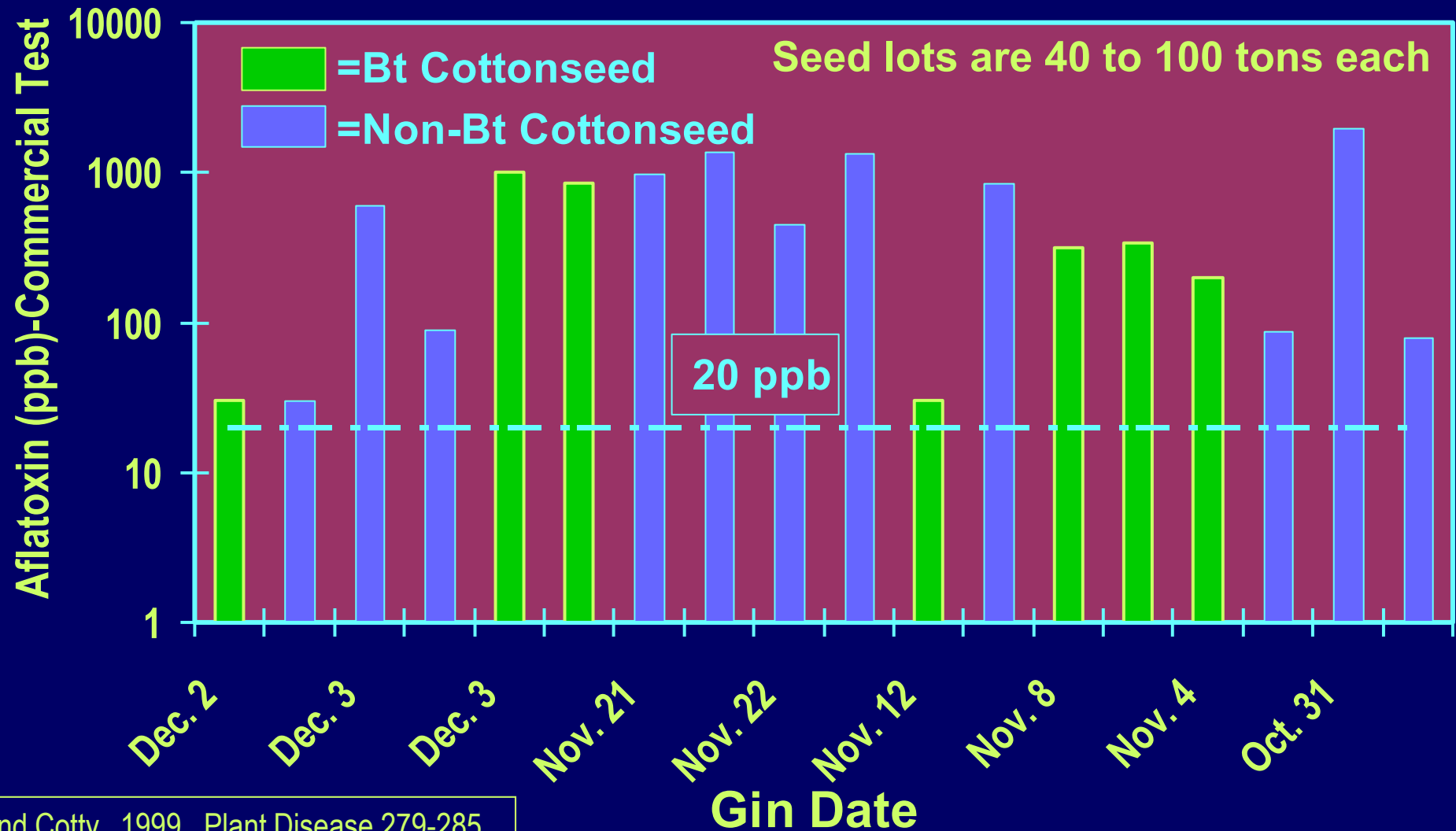
Performance of one Bt Cotton Cultivar in a Field-plot Test in the Yuma Valley of Arizona 1996

Cultivar	Seed Cotton with BGYF	Larvae dead	Aflatoxin from BGYF	Overall Aflatoxin	Yield
NUCOTN 33 ^B	0.22% ^b	100%	0 ^b	2 ^b	5,485 ^a
DPL5415	5.80% ^a	4%	61 ^a	79 ^a	3,422 ^b

Values are averages of three replicates. Values within a column not followed by a common letter differ significantly (p=0.05 by Tukey's HSD Test).

Distribution of Aflatoxin Among Bt and Non-Bt Cottonseed Lots at One Gin in Western Arizona

ALL SEED LOTS GINNED BETWEEN DEC. 2 & OCT. 31 ARE SHOWN



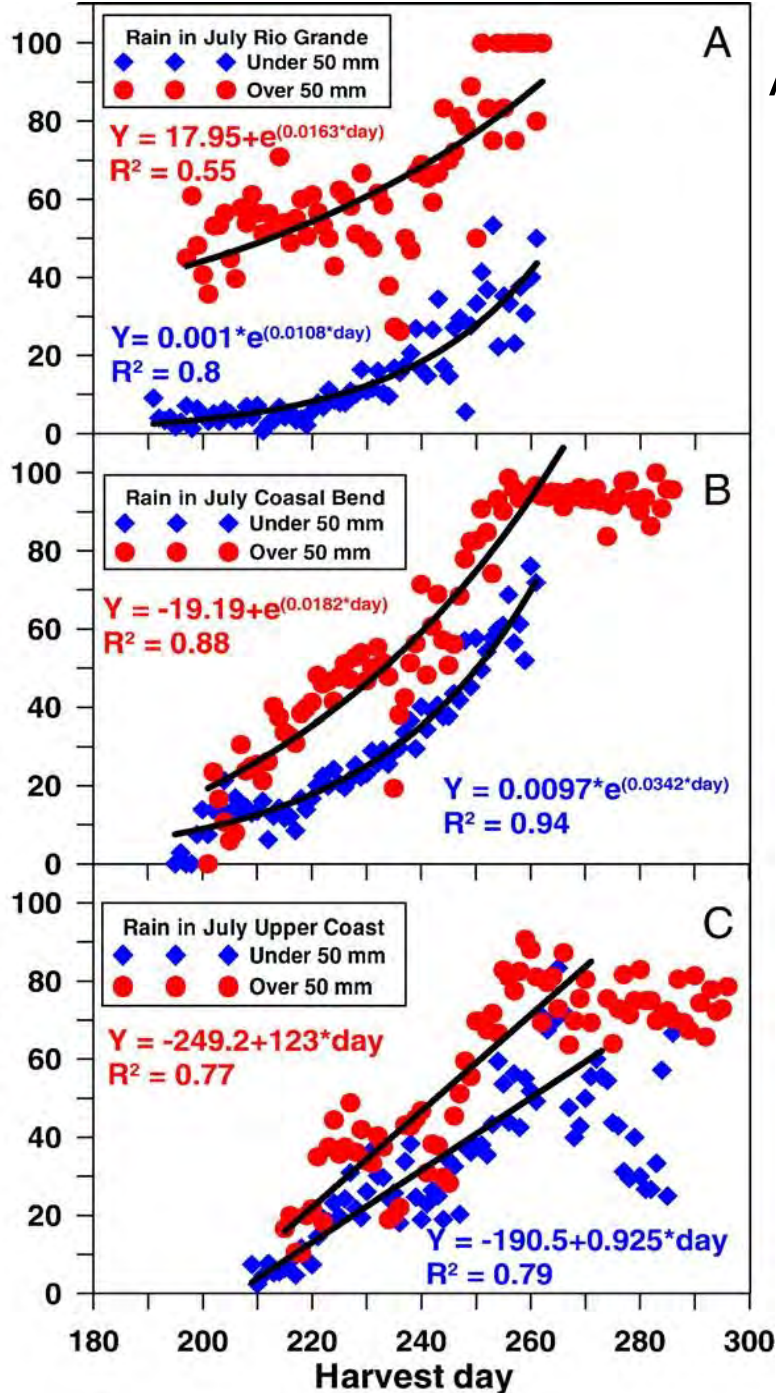
Cottonseed Sampling at the Valley Co-op Oil Mill Harlingen, TX

36,700 Data Points Collected Over 5 years
Each point represented 18 to 35 metric tons of seed.

Field Plot Studies Give Insights, but
...Commercial Data is Real



Percent of cottonseed with aflatoxin content over 20 ppb



Aflatoxin increases in the Mature Crop in the Second Phase of Contamination, Even Before Harvest

Percent of cottonseed truckloads in South Texas with aflatoxin exceeding 20 ppb as a function of gin date.

Combined data for 1997 to 2001 with precipitation in July over 50 mm (●) and under 50 mm (◆).

A: Rio Grande Valley; B: Coastal Bend, and C, the Upper Coast, of South Texas.



Texas 2010: At harvest this field's grain contained 400 to 1200 ppb aflatoxins

Aflatoxin Biocontrol in the US

- First Patent Application: 1989
- 1st Conference with U.S. Environmental Protection Agency: 1992
- **Used on commercial crops in US since 1996.**
- Three Products with Unrestricted Registrations (more coming).
- **Over 1 Million Acres Treated Annually**
- Registered Target Crops: Maize Grain & Silage, Pistachios, Cottonseed, Peanut (Almond & Fig expected in 2017).

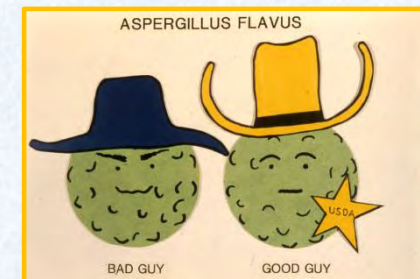


Aflatoxin Biocontrol in Africa

- Severe Human Exposure to Aflatoxins in Several Nations.
- Products registered: Nigeria, Kenya, Burkina Faso & Senegal/Gambia.
- Target Crops: Maize & Groundnut.

Aflatoxin Biocontrol in Europe

- Target Crop: Maize.
- 35,000 acres treated in 2016 – very effective.
- Maize required to be below 3 ppb for cheese industry.

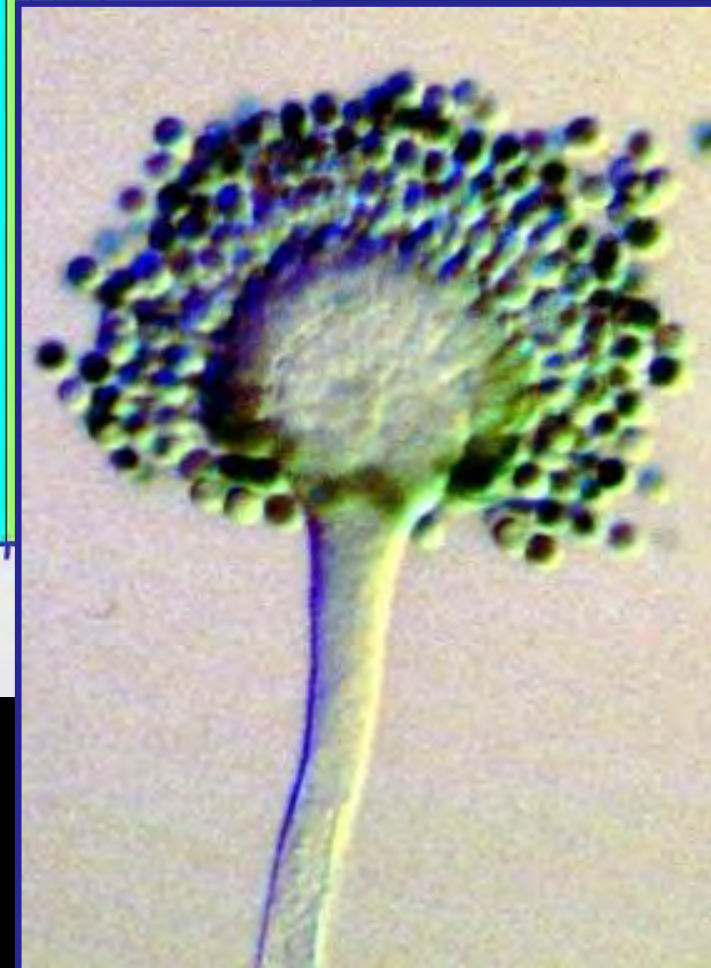
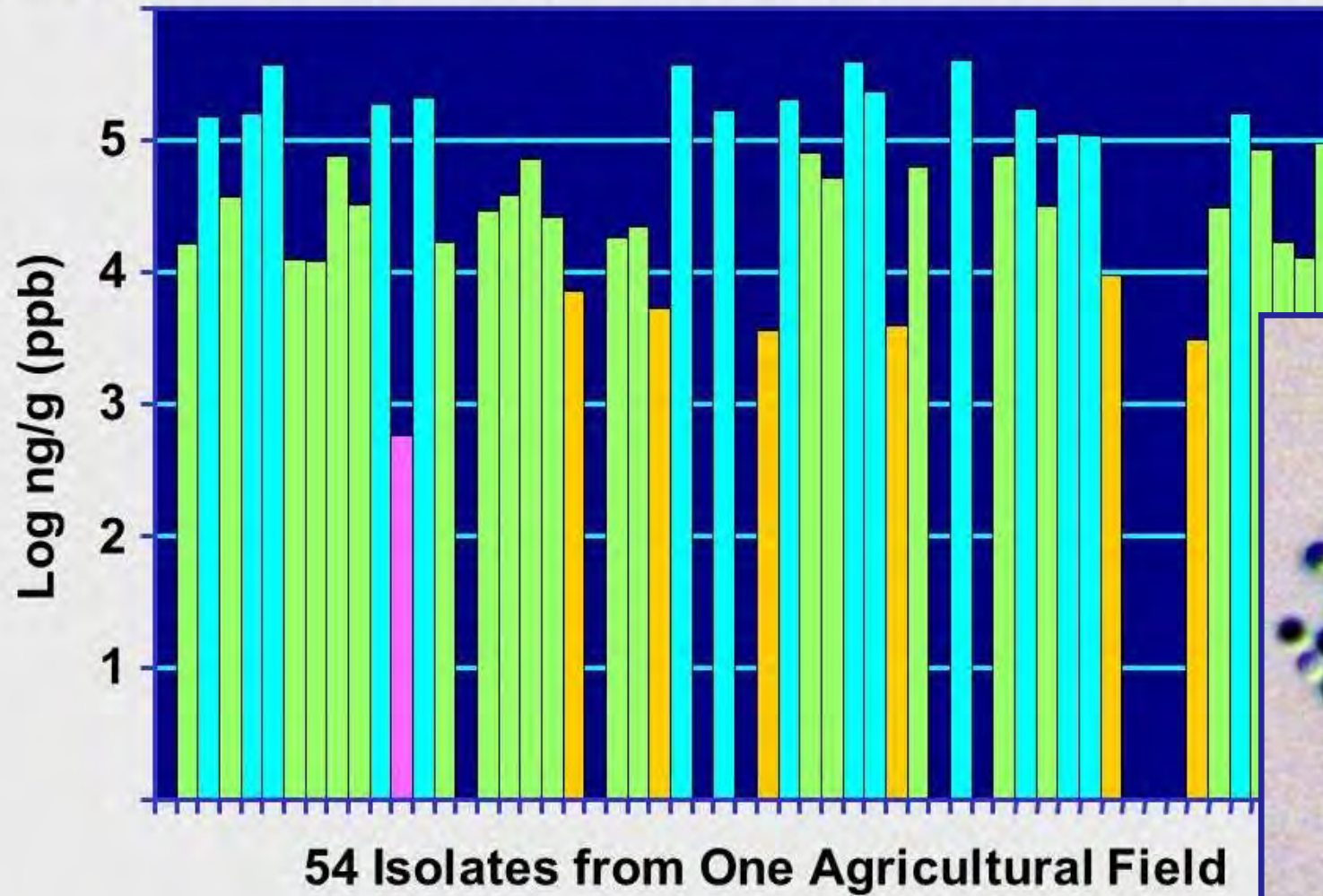


1st Biocontrol Talk 1988



1980s, Arizona
First Tests

Aflatoxin Production by Fungal Isolates in Liquid Fermentation



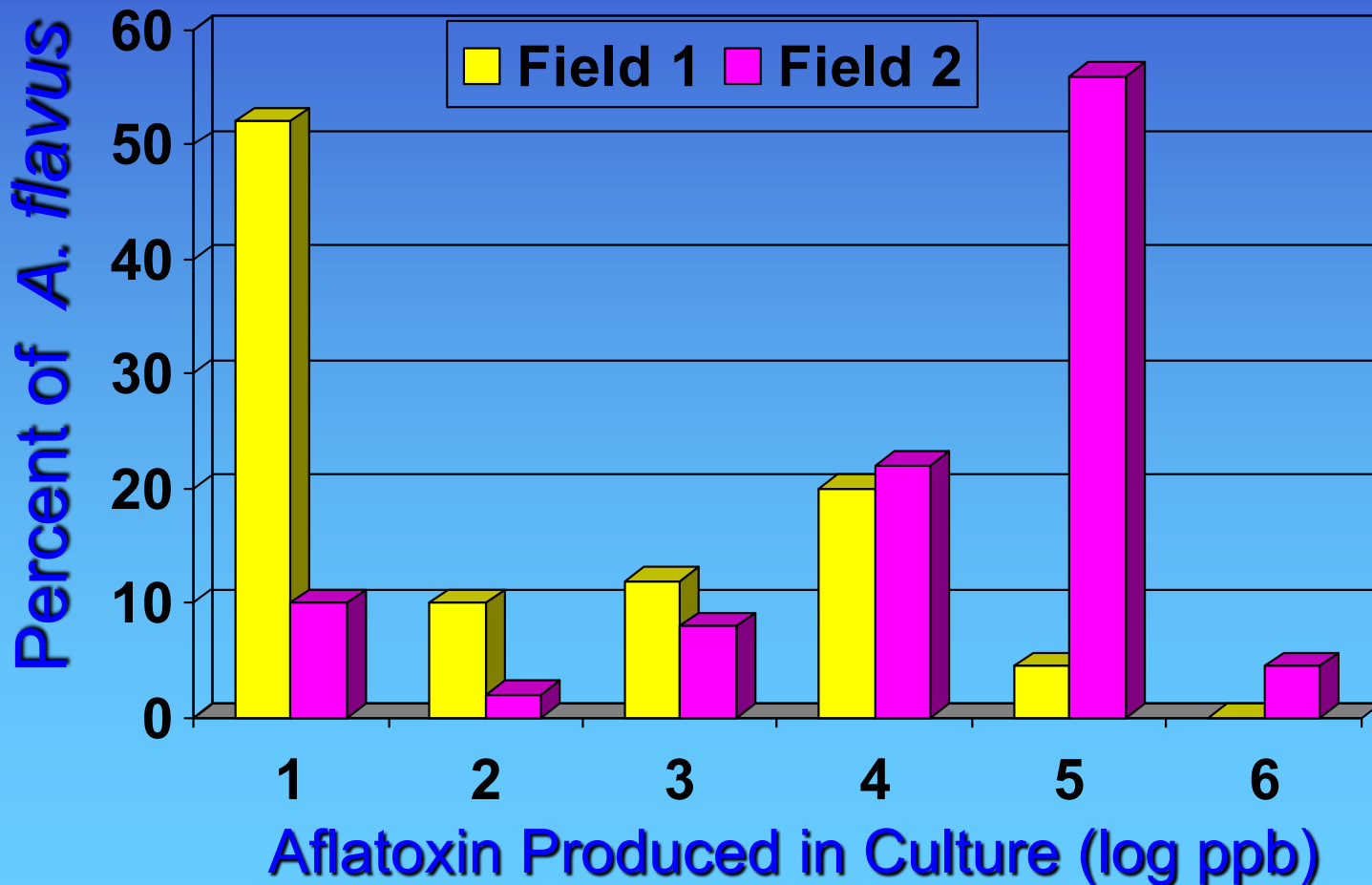
Aspergillus flavus primary causal agent– Phenotypically Variable!

There are Many Atoxigenic Strains

Fungi Vary Across Areas in Aflatoxin-Producing Ability

Farmers apply atoxigenic strains to reduce the average aflatoxin-producing potential of fungi on farms & thus the vulnerability of crops to aflatoxin contamination

Aflatoxin Production by *A. flavus* from Two Fields



Aflatoxin-Producing Potential

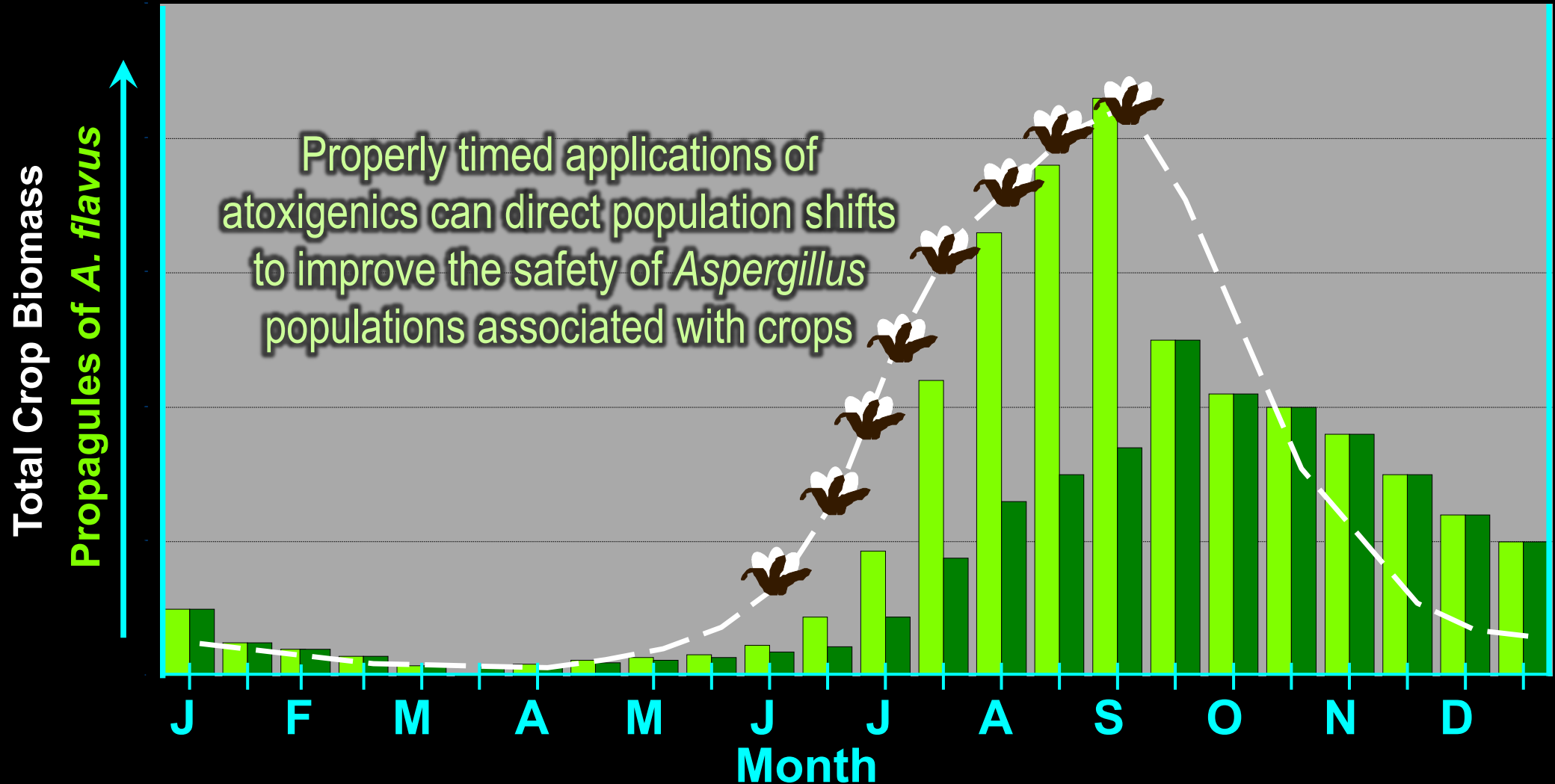
Field 1 = Low,
3,400 ppb

Field 2 = High,
54,000 ppb

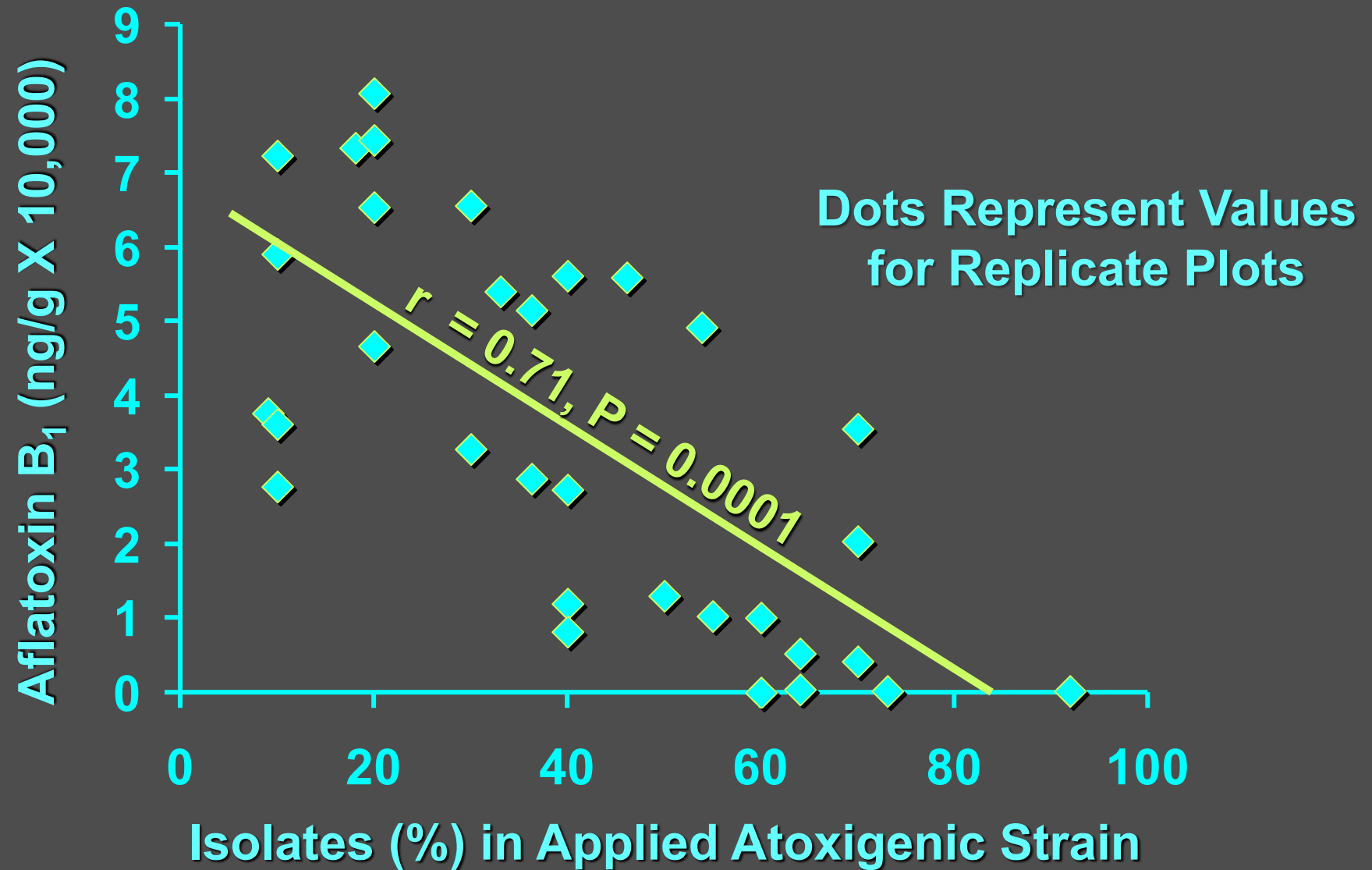
Aspergillus Populations Rapidly Increase with Crop Biomass

Founder Effects Drive Shifts in Population Structure.

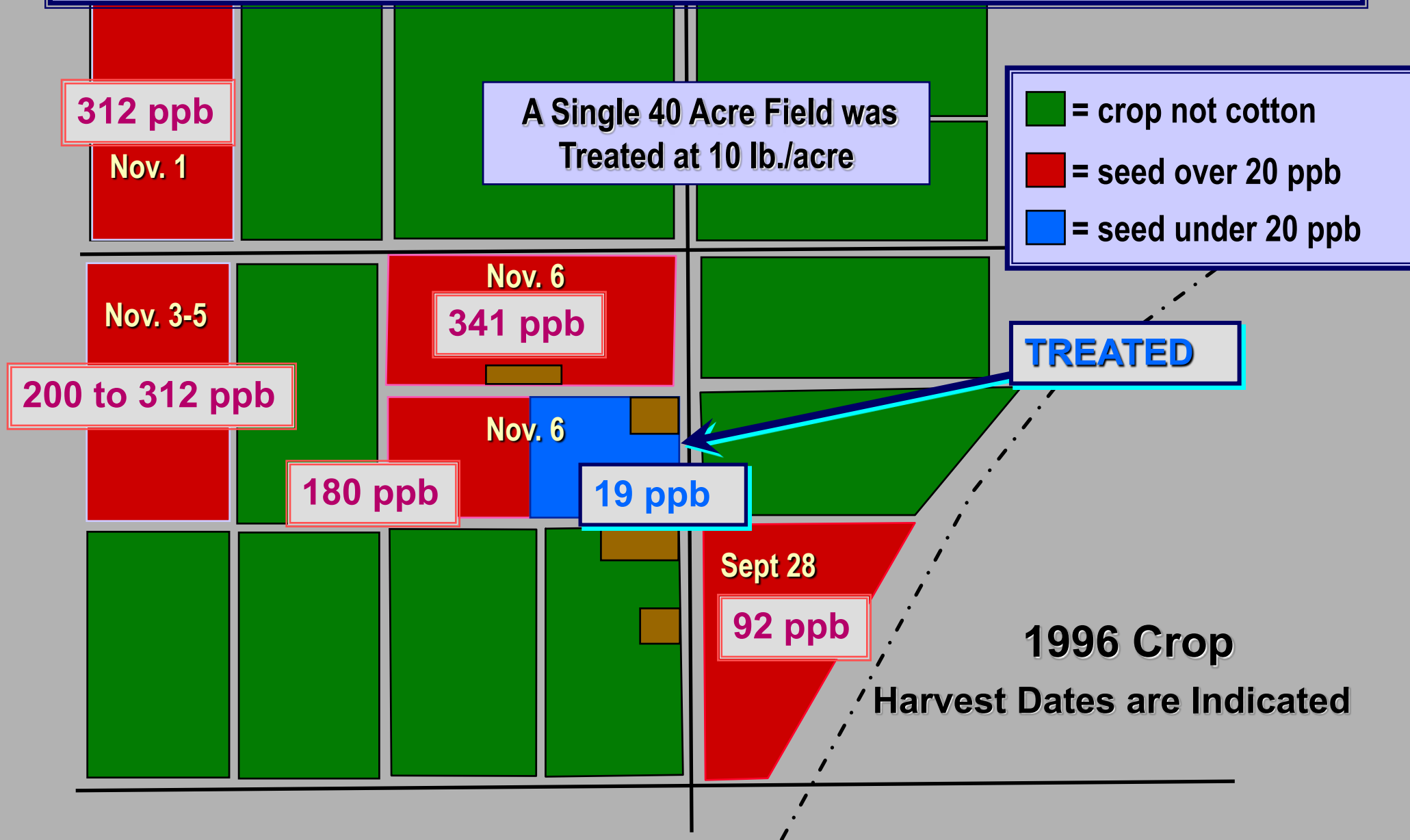
■ =soil *A. flavus* ■ =total *A. flavus* — =total crop biomass



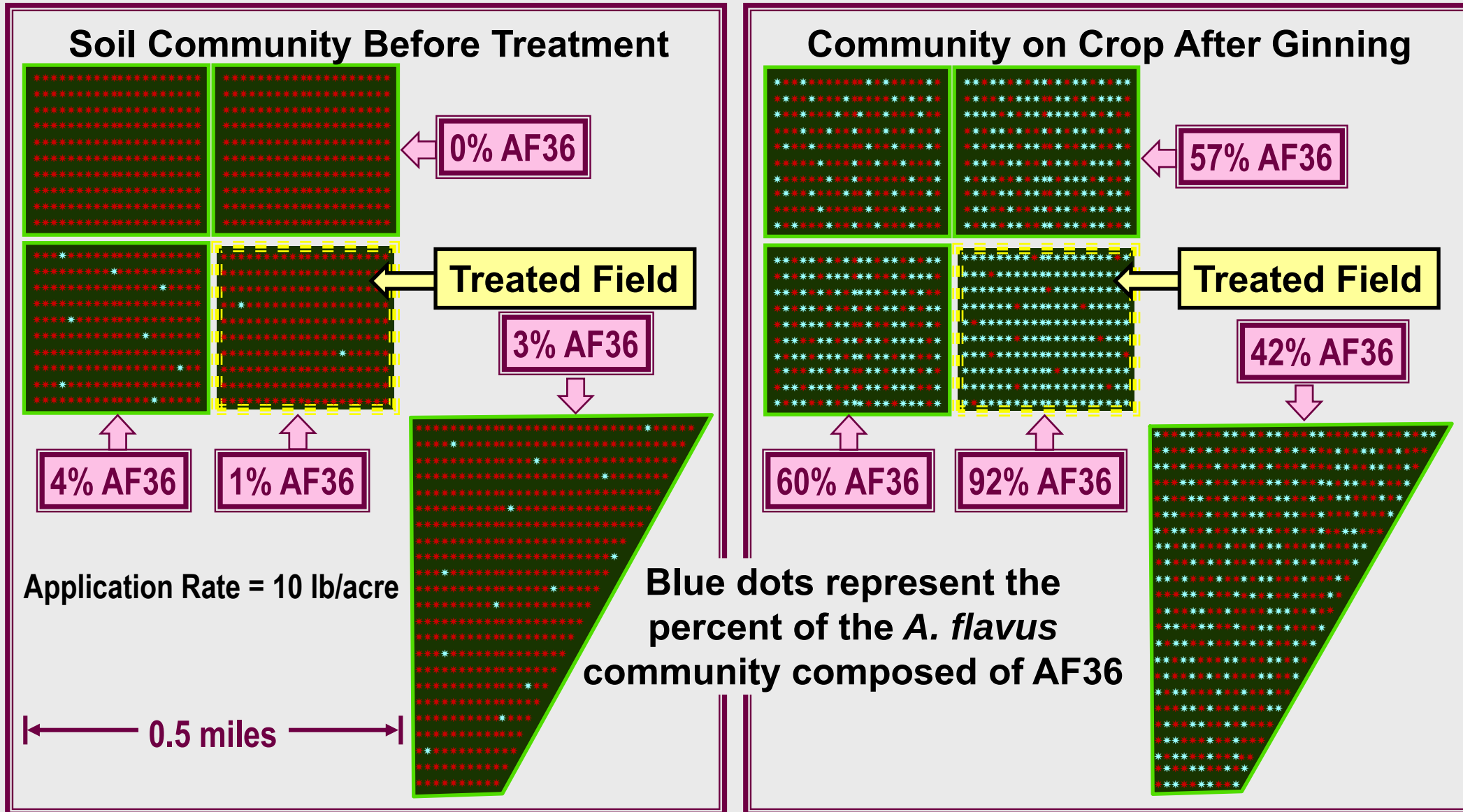
Aflatoxin in Crop versus Atoxigenic Incidence



Mohawk Valley, Arizona: First Commercial Field Test of the Use of an Atoxigenic Strain to Prevent Aflatoxin Contamination



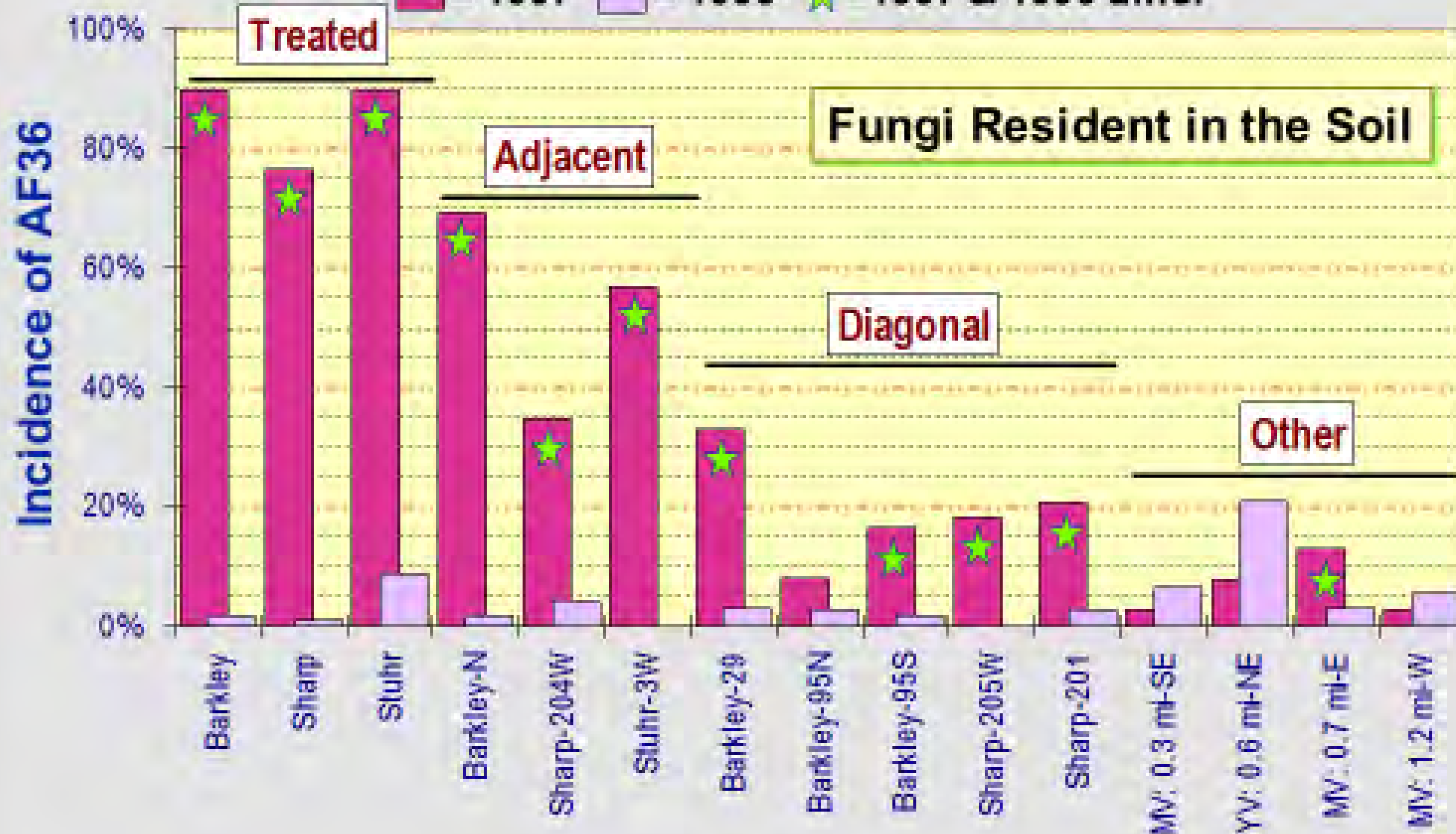
Application of Atoxigenic Strain AF36 in Commercial Cotton Influences the Composition of Fungi on Crops in both Treated and Nearby Fields

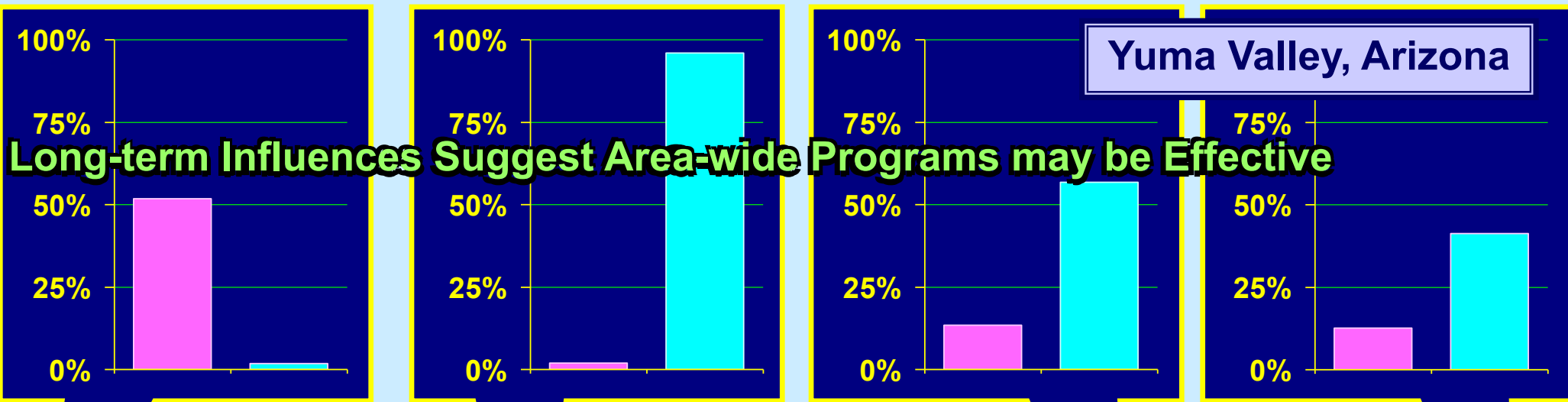


Data from 564 vegetative compatibility analyses.

Incidence of AF36 within *Aspergillus flavus* Communities Prior to and One Year After Application

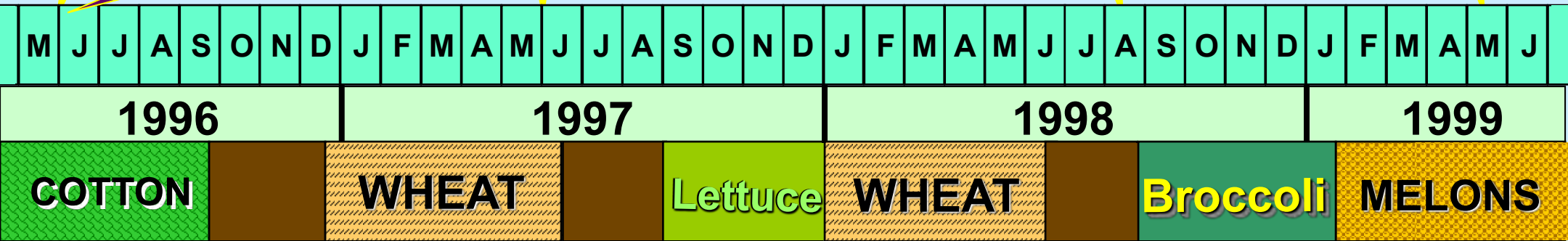
■ = 1997 ■ = 1996 ★ = 1997 & 1996 differ





Percent of the *A. flavus* Soil Community Composed of the Applied Atoxigenic Strain & the Highly Toxigenic S Strain

Application



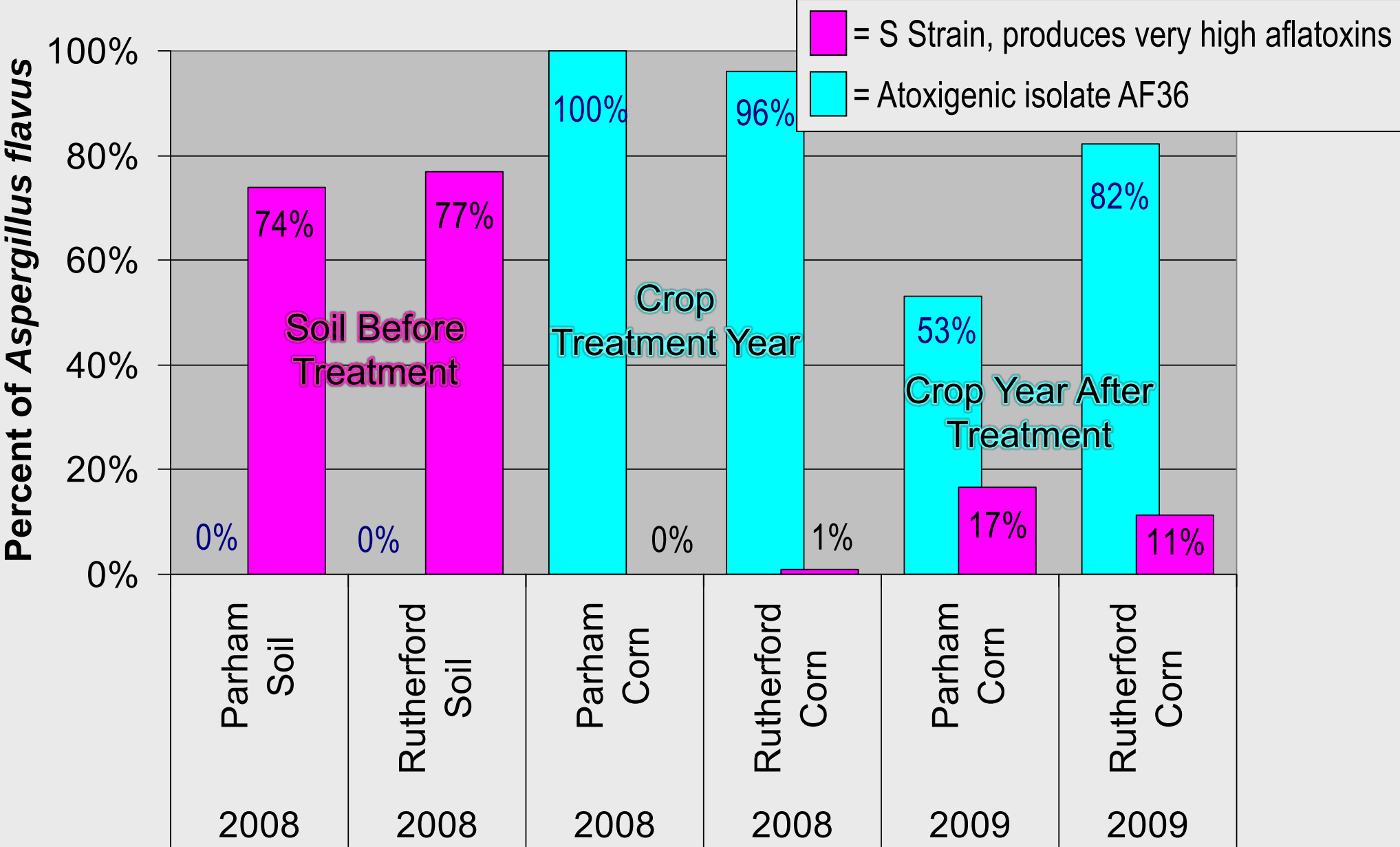
■ = % Atoxigenic Strain ■ = % S strain

Commercial Maize: North Central Texas 2008

Area	Samples (#)	AF36 (%)	Aflatoxin (ppb)	
			Mean	Range
Grayson North	17	96 a	12 a	0 to 48
Grayson South	16	98 a	15 a	0 to 38
Grayson Control	8	24 b	230 b	5 to 530

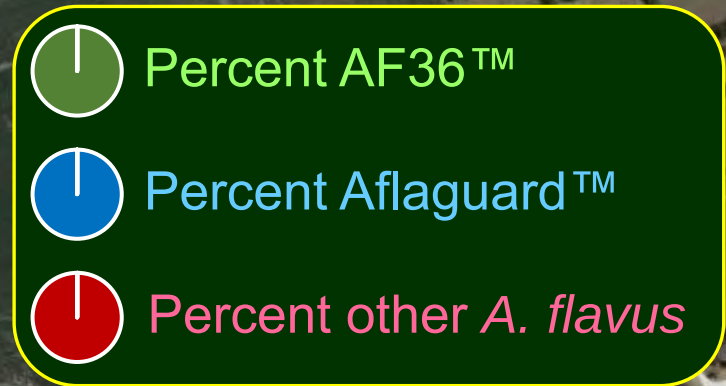
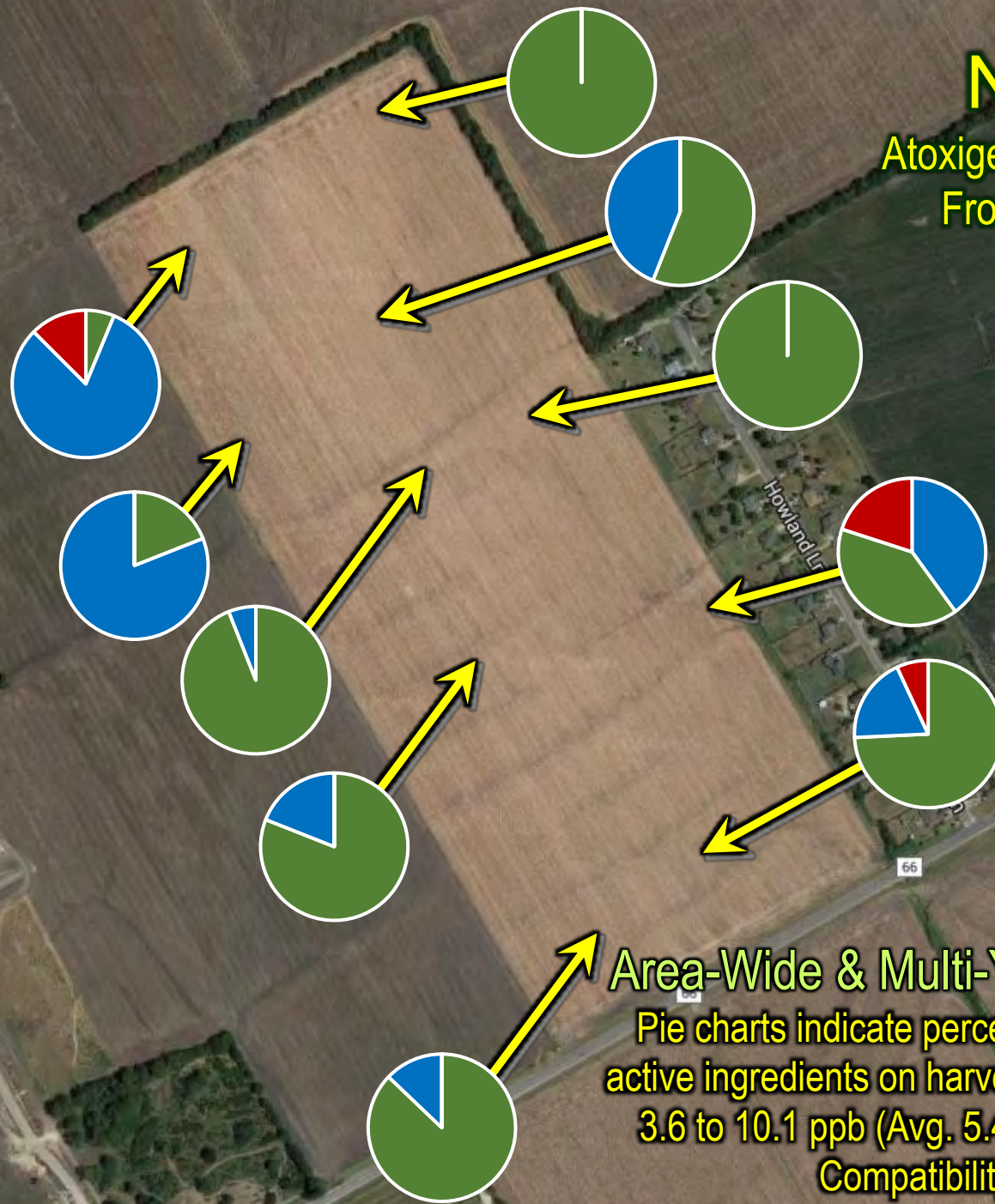
Means in the same column with different letters are significantly different by Tukey's HSD test, $P < 0.001$.

Grayson County, Texas: Carry Over to the Second Year Crop



Not Treated in 2015

Atoxigenic Biocontrols Agents on Harvested Corn
From Commercial Field Northcentral Texas



Area-Wide & Multi-Year Effects in Commercial Agriculture

Pie charts indicate percent *A. flavus* composed of the two EPA registered active ingredients on harvested maize from the indicated spots. Aflatoxin was 3.6 to 10.1 ppb (Avg. 5.4 ppb). Values in each pie result from Vegetative Compatibility Analyses on 16 isolates (160 total).

Fungi move between fields and across areas.

Take a bigger view: Not just one field, one farmer, one crop.

An aerial photograph showing a vast, patchwork landscape of agricultural fields. The fields are in various stages of growth or harvest, displaying a range of colors from vibrant green to golden-brown. A winding river or stream flows through the landscape, creating a natural boundary between different sections of the land. Small clusters of buildings, likely farmhouses or small villages, are scattered throughout the fields. The overall view emphasizes the interconnectedness of the agricultural system.

We can protect whole agricultural systems from aflatoxins including all affected industries and improve the environment.

Biocontrol is Area-wide Management

Area-wide Aspects can be Optimized

Biocontrol fungi move from treated fields across areas

Atoxigenics persist in soils, on crop debris, on non-crop plants



Multiple Crops Benefit From the Same Biocontrol Fungus

Area-wide Programs provide efficacy across cropping systems and additional health benefits. **Area-wide Programs** are less expensive (per hectare), provide for long-term commitment to aflatoxin elimination, protect all crops and remediate the area's reputation.





Aspergillus flavus AF36 Prevail

For displacing aflatoxin-producing fungi

Arizona Cotton Research and Protection Council

"for growers by growers"

COTTON: FOR USE ONLY IN THE STATES OF ARIZONA, TEXAS AND CALIFORNIA (Imperial, Riverside and San Bernardino counties only)

CORN: FOR USE ONLY IN THE STATES OF ARIZONA AND TEXAS

PISTACHIO: FOR USE ONLY IN THE STATES OF CALIFORNIA, ARIZONA, TEXAS AND NEW MEXICO

Aspergillus flavus AF36 is a strain of *Aspergillus flavus* that occurs naturally. When applied to cotton just prior to first bloom, to corn from the 7 leaf stage (V7) until silking, or to pistachio from late May through early July, *Aspergillus flavus* AF36 competes with strains of *Aspergillus flavus* that produce large amounts of aflatoxin and, in doing so, limits the amount of these high aflatoxin producers that become associated with the crop.

Active ingredient: <i>Aspergillus flavus</i> strain AF36*	0.0008%
Other inert ingredients	99.9992%
Total:	100.0000%

* Contains a minimum of 3,000 CFU/gram in the End-Use Product

KEEP OUT OF REACH OF CHILDREN
CAUTION

First Aid

IF INHALED:	Move person to fresh air. If person is not breathing, call 911 or an ambulance, then give artificial respiration, preferably mouth-to-mouth if possible. Call a poison control center or doctor for further treatment advice.
IF ON SKIN OR CLOTHING:	Take off contaminated clothing. Rinse skin immediately with plenty of water for 15-20 minutes. Call a poison control center or doctor for treatment advice.
IF IN EYES:	Hold eye open and rinse slowly and gently with water for 15-20 minutes. Remove contact lenses, if present, after the first 5 minutes, then continue rinsing eye. Call a poison control center or doctor for treatment advice.
Have the product container or label with you when calling a poison control center or doctor, or going for treatment.	

SEE PRECAUTIONARY STATEMENTS BELOW AND ON OTHER PANEL

EPA Registration No.: 71693-2
EPA Establishment No.: 71693-AZ-001

Arizona Cotton Research and Protection Council
3721 E. Wier Avenue
Phoenix, Arizona 85040

NET WEIGHT: 50 lb bags, 1000-3000 lb bulk bags or boxes

PRODUCT INFORMATION

Aspergillus flavus AF36 is for application to cotton, corn and pistachio to displace aflatoxin-producing strains of *Aspergillus flavus*.

Aspergillus flavus AF36 is a living fungus growing on grain, which serves as both a carrier and a nutrient source. After application and once the colonized grain is exposed to sufficient moisture (this may occur at irrigation), *Aspergillus flavus* AF36 will grow out and the grain will be covered with green spores. This growing fungus will first appear as white fuzz and later as green fuzz. The green spores will eventually be spread to the crop by wind and insects in the same manner that aflatoxin-producing fungi are spread.

USE PRECAUTIONS

Do not apply as a tank mixture with fertilizers, insecticides, or fungicides. Apply *Aspergillus flavus* AF36 only when the potential for drift to adjacent, sensitive areas (e.g., residential areas, bodies of water, known habitat for threatened or endangered species, nontarget crops) is minimal. *Aspergillus flavus* AF36 may be applied to irrigated cotton, corn and pistachio fields.

APPLICATION INSTRUCTIONS

Ground Application: COTTON

1. Apply *Aspergillus flavus* AF36 to the surface of the soil under the plant canopy with a tractor-mounted, granular applicator. DO NOT COVER THE AF36 COLONIZED GRAIN WITH SOIL.
2. Adjust the applicator to optimize delivery of *Aspergillus flavus* AF36 under the canopy and to minimize delivery of *Aspergillus flavus* AF36 to furrows.
3. *Aspergillus flavus* AF36 has been shown to be effective when applied from late May through June, prior to first bloom. Make a single application during the last cultivation before bloom.
4. Furrow irrigating the crop with at least 2 inches of water within 3 days after application of *Aspergillus flavus* AF36 will provide best results.
5. Use 10 lbs of *Aspergillus flavus* AF36 per acre of cotton (per 13,000 linear feet based on 40-inch rows).

Ground Application: CORN

1. Apply *Aspergillus flavus* AF36 directly to the soil or under the plant canopy after last cultivation with a tractor-mounted, granular applicator. Make a single application from the V7 growth stage until emergence of the silks from the husk. Cultivation must be completed before application. DO NOT COVER THE AF36 COLONIZED GRAIN WITH SOIL.
2. Adjust the applicator to optimize delivery of *Aspergillus flavus* AF36 under the canopy and to minimize delivery of *Aspergillus flavus* AF36 to areas that do not get wet.
3. Rain or irrigation within 3 days of application of *Aspergillus flavus* AF36 will improve results.
4. Use 10 lbs of *Aspergillus flavus* AF36 per acre of corn (per 13,000 linear feet based on 40-inch rows).

Ground Application: PISTACHIO

1. Apply *Aspergillus flavus* AF36 to the surface of the soil under the plant canopy with a granular applicator. DO NOT COVER THE AF36 COLONIZED GRAIN WITH SOIL.
2. Adjust the applicator to optimize delivery of *Aspergillus flavus* AF36 under the canopy and to minimize delivery of *Aspergillus flavus* AF36 to areas that do not get wet.
3. *Aspergillus flavus* AF36 has been shown to be effective when applied from late May through early July. A single



Arizona Cotton Research
and Protection Council

Atoxigenic Strains are Biopesticides Under Development for over Two Decades

Initial Registration was by the Public Sector; Initial Production by a Farm Organization

Time Line for Development of First Atoxigenic in U.S.

- 1989: First Patent Application.
- 1993: Preregistration meeting with EPA on atoxigenic strain potential.
- 1995: IR-4 Biopesticide Program Joined Registration Effort.
- 1996: First Experimental Use Permit (EUP) for Cotton (1120 acres).
- 1998: ACRPC initiates first Atoxigenic Manufacturing Facility.
- 1999 to 2002: EUP repeatedly expanded to finally 22,000 acres/yr.
- 2003: Unrestricted registration allows unlimited cotton treatments.
- 2007: EUP to Treat 3,000 acres of pistachios.
- 2008: EUP to treat 6,000 acres of corn.
- 2011: Unrestricted registration for Corn.
- 2012: Unrestricted registration for Pistachio.
- 2016: Unrestricted registration for Preval.
- 2017: Unrestricted registration for almond & fig.



Biocontrol Products: AF36, Prevail™, Aflaguard™, AflaSafe™, AF-X1™, FourSure™

The atoxigenic fungi are applied on non-viable grain (sorghum, wheat, and barley are used).

As Applied

After Fungal Growth

As Applied





Agricultural Research Service, USDA
School of Plant Sciences, University of Arizona



USAID
FROM THE AMERICAN PEOPLE

BILL & MELINDA
GATES foundation



AFRICAN AGRICULTURAL TECHNOLOGY FOUNDATION
FONDATION AFRICAINE POUR LES TECHNOLOGIES AGRICOLES