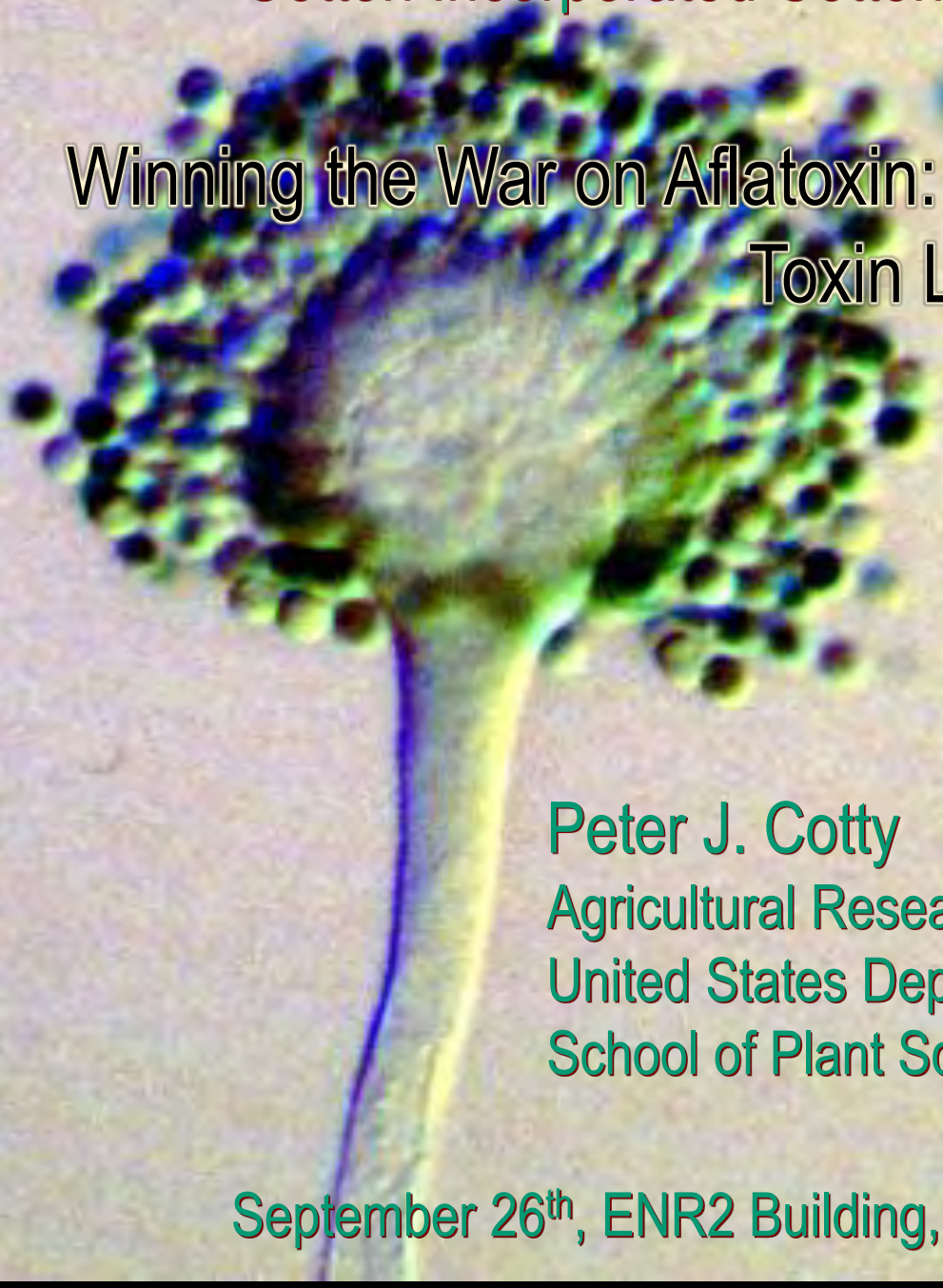
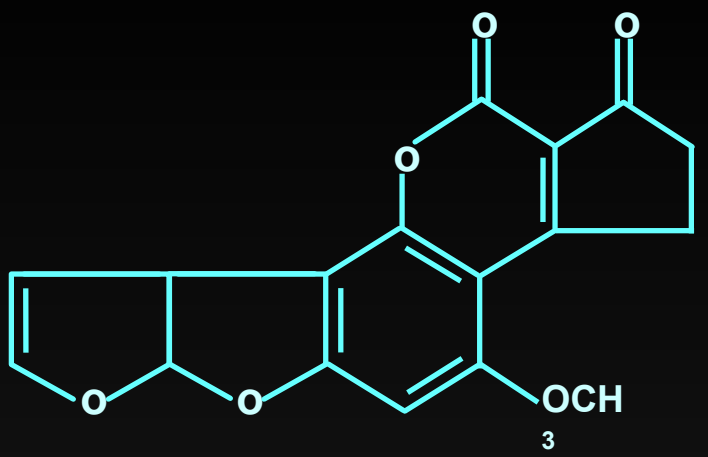
Cotton Incorporated Cotton Breeders Tour (Arizona)



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

Peter J. Cotty
Agricultural Research Service,
United States Department of Agriculture
School of Plant Sciences, University of Arizona

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



Aflatoxin B_1

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

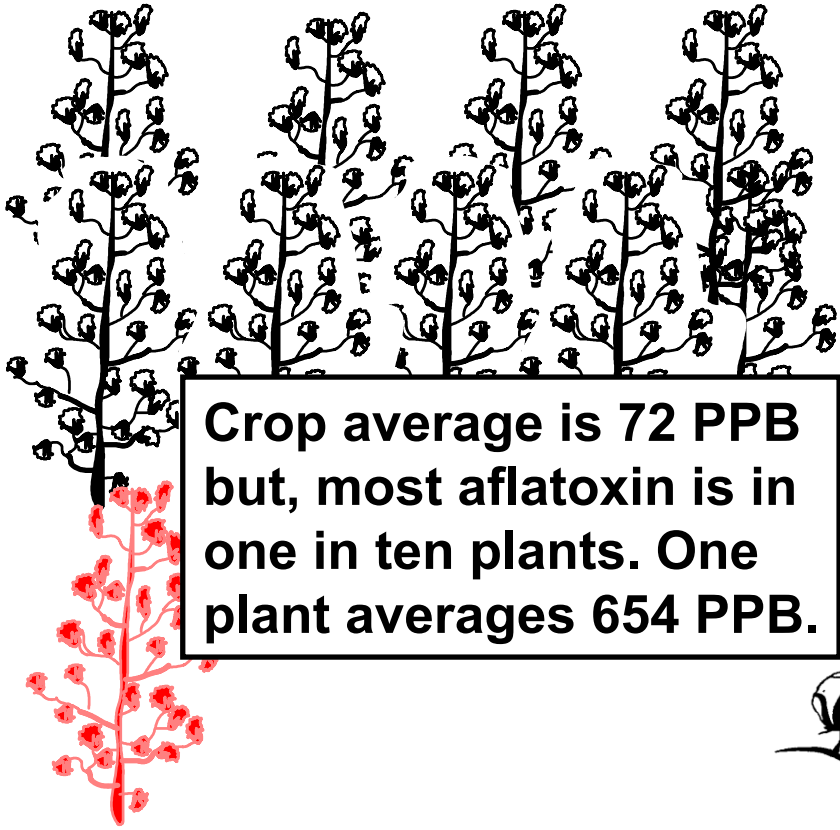
*Contents in Foods & Feed Regulated Worldwide (ppb!)

*Influences Market Access & Crop Value

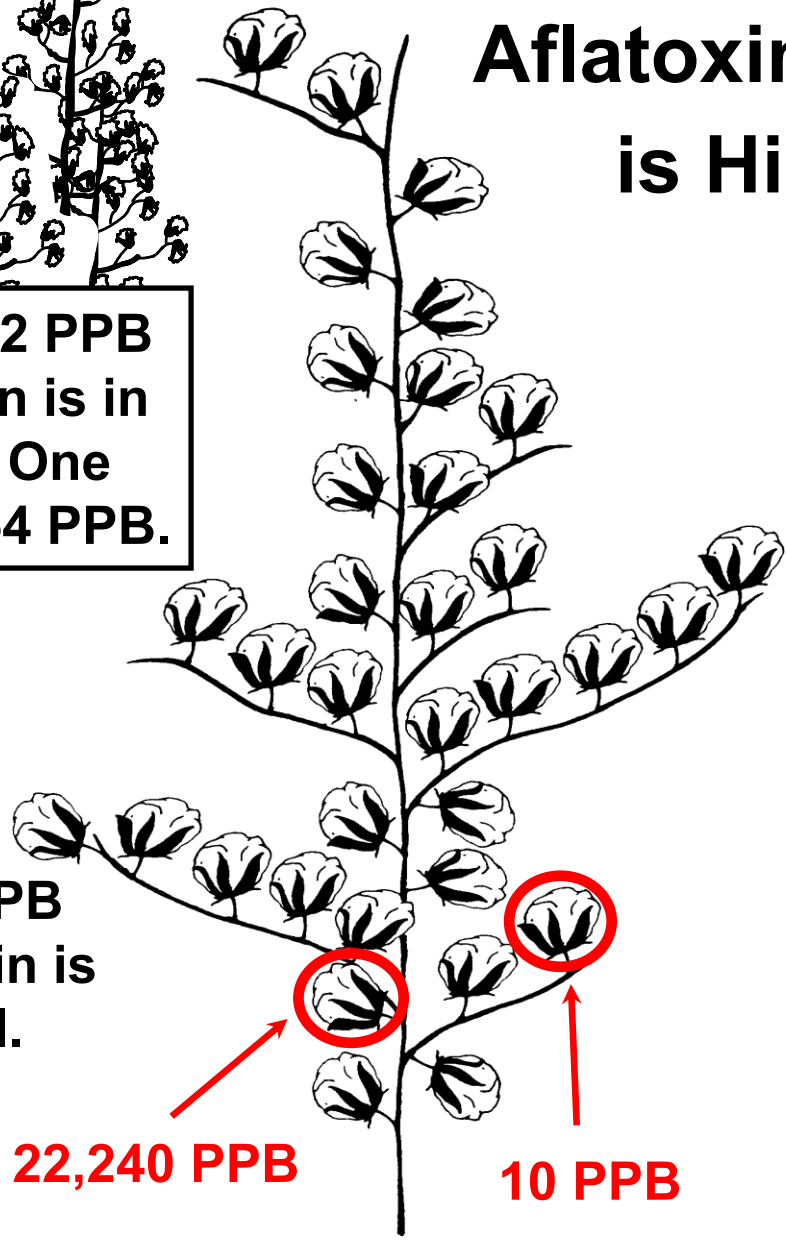
Aflatoxins in Cottonseed

- ✓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

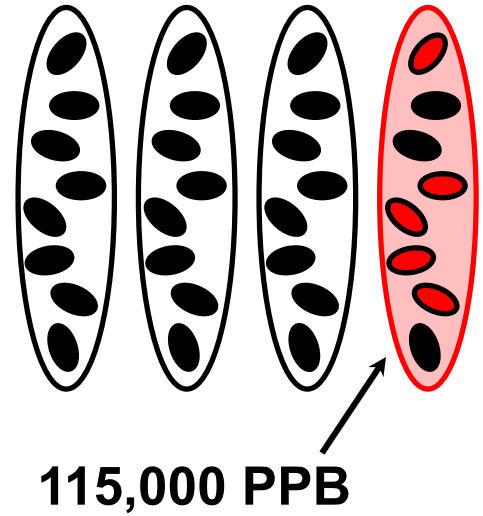


Crop average is 72 PPB but, most aflatoxin is in one in ten plants. One plant averages 654 PPB.



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

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



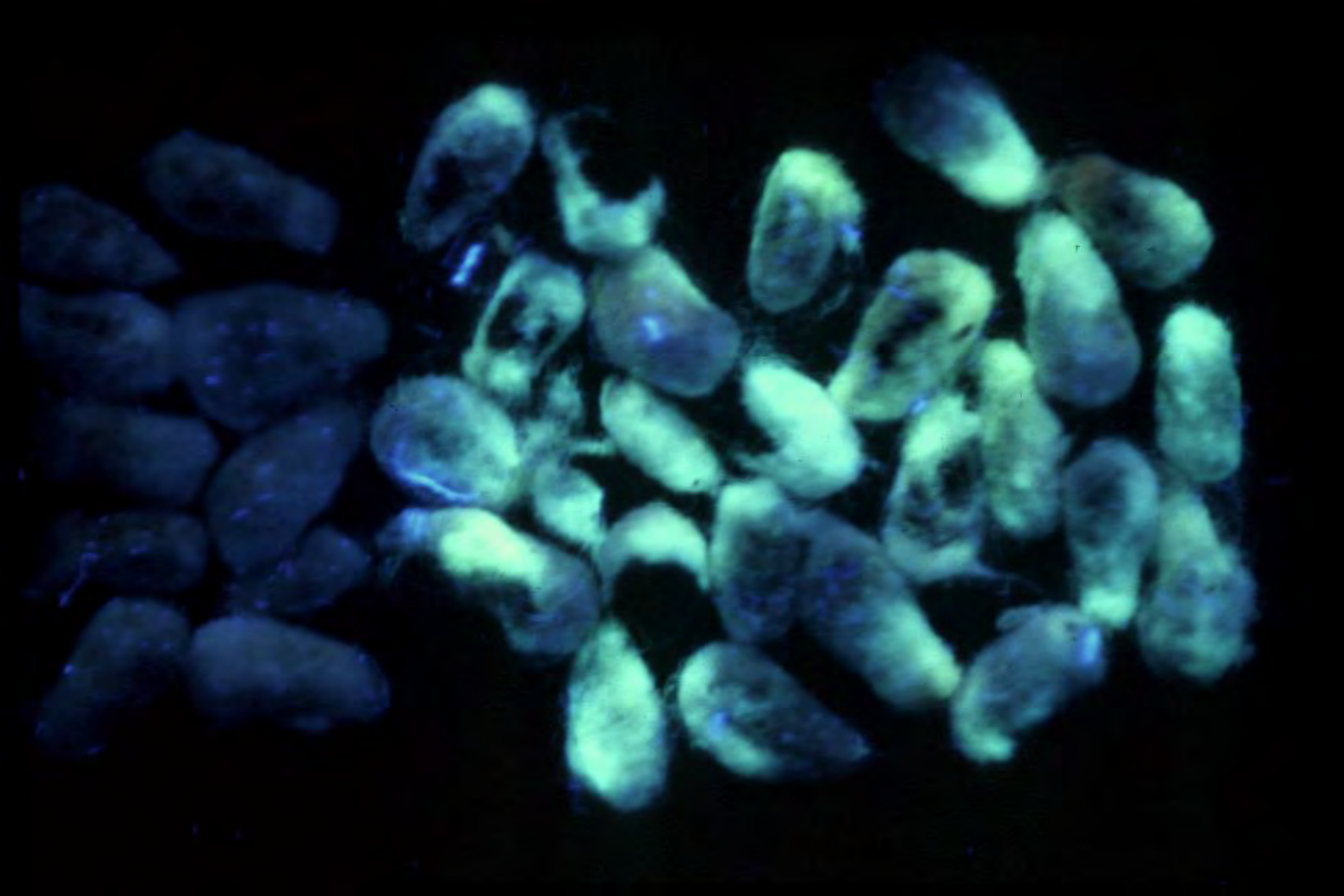
Aflatoxin Contamination Occurs in Two Phases
Climatic Influences Differ Between the Phases

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.

Cotty, P. J., and Lee, L. S. 1989. Tropical Science 29:273-277.

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

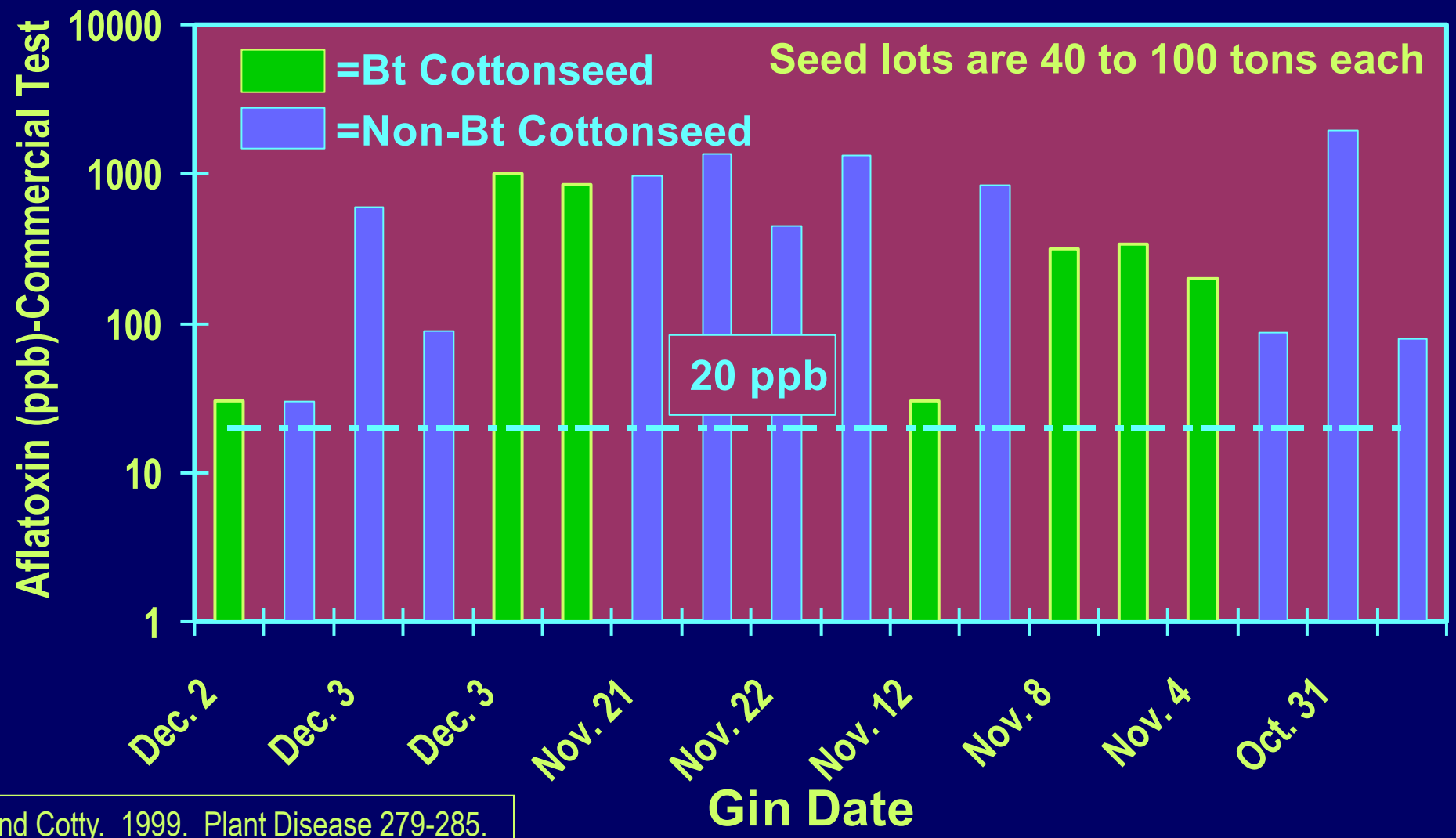
Cultivar	Seed Cotton with BGYF	Larvae dead	Aflatoxin from BGYF	Overall Aflatoxin	Yield
NUCOTN 33B	0.22% b	100%	0 b	2 b	5,485 a
DPL5415	5.80% a	4%	61 a	79 a	3,422 b

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).

Cotty, P. J., et al., 1997. Proceedings of the Beltwide Cotton Production Conference pp. 108-110.

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



Bock and Cotty. 1999. Plant Disease 279-285.

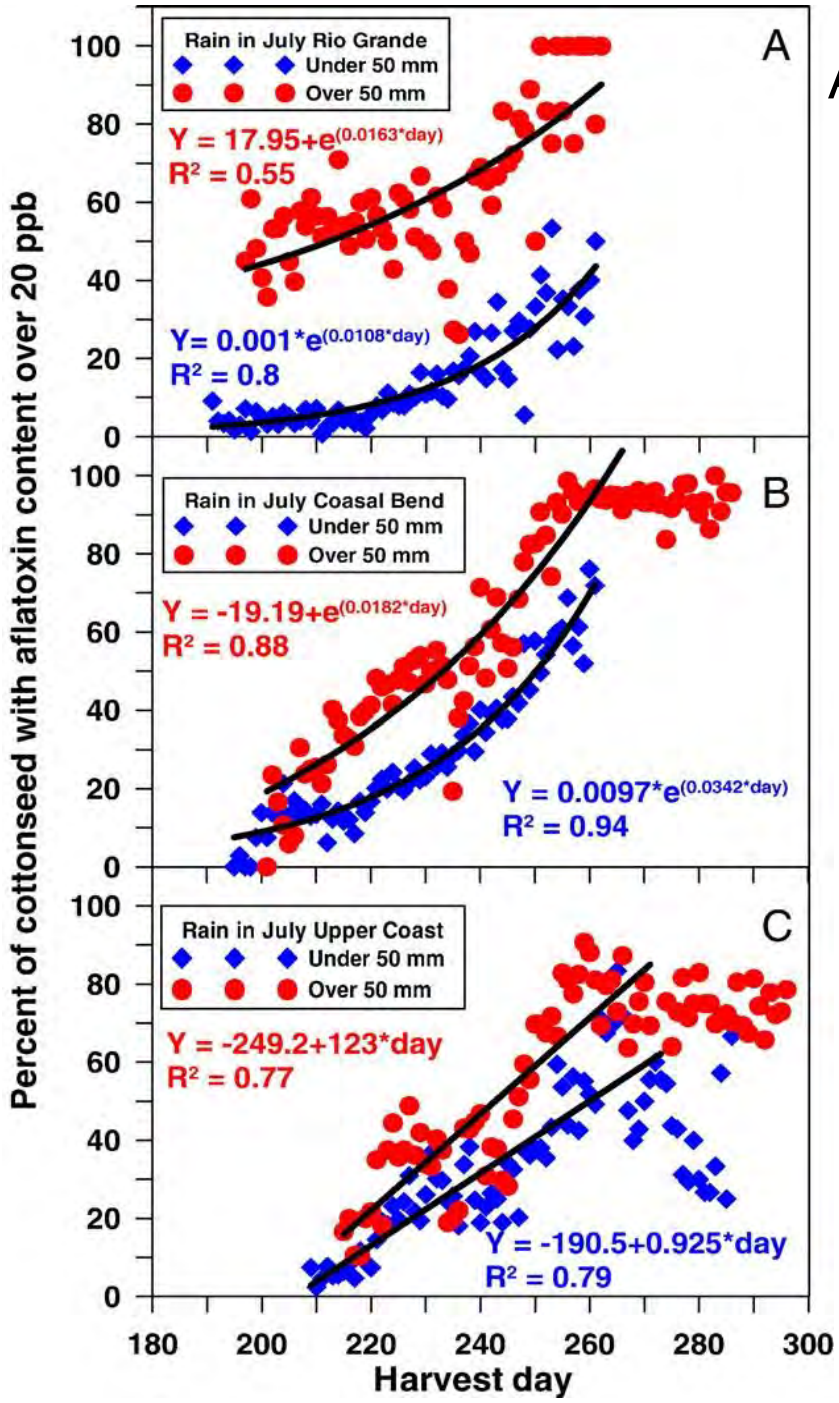
Cottonseed Sampling at the Valley Co-op Oil Mill Harlingen, TX

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



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.

Phytopathology 2003. 93:1190-1200; Int. J. Food Micro. 2007. 119:109-115.

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

Aflatoxin Biocontrol in the US

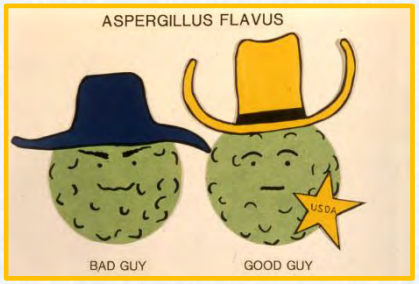
- First Patent Application: 1989
- 1st Conference with U.S. Environmental Protection Agency: 1992
- **Used on commercial crops in US since 1996.**
- Three Products with Unrestricted Registrations (more coming).
- **Over 1 Million Acres Treated Annually**
- Registered Target Crops: Maize Grain & Silage,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

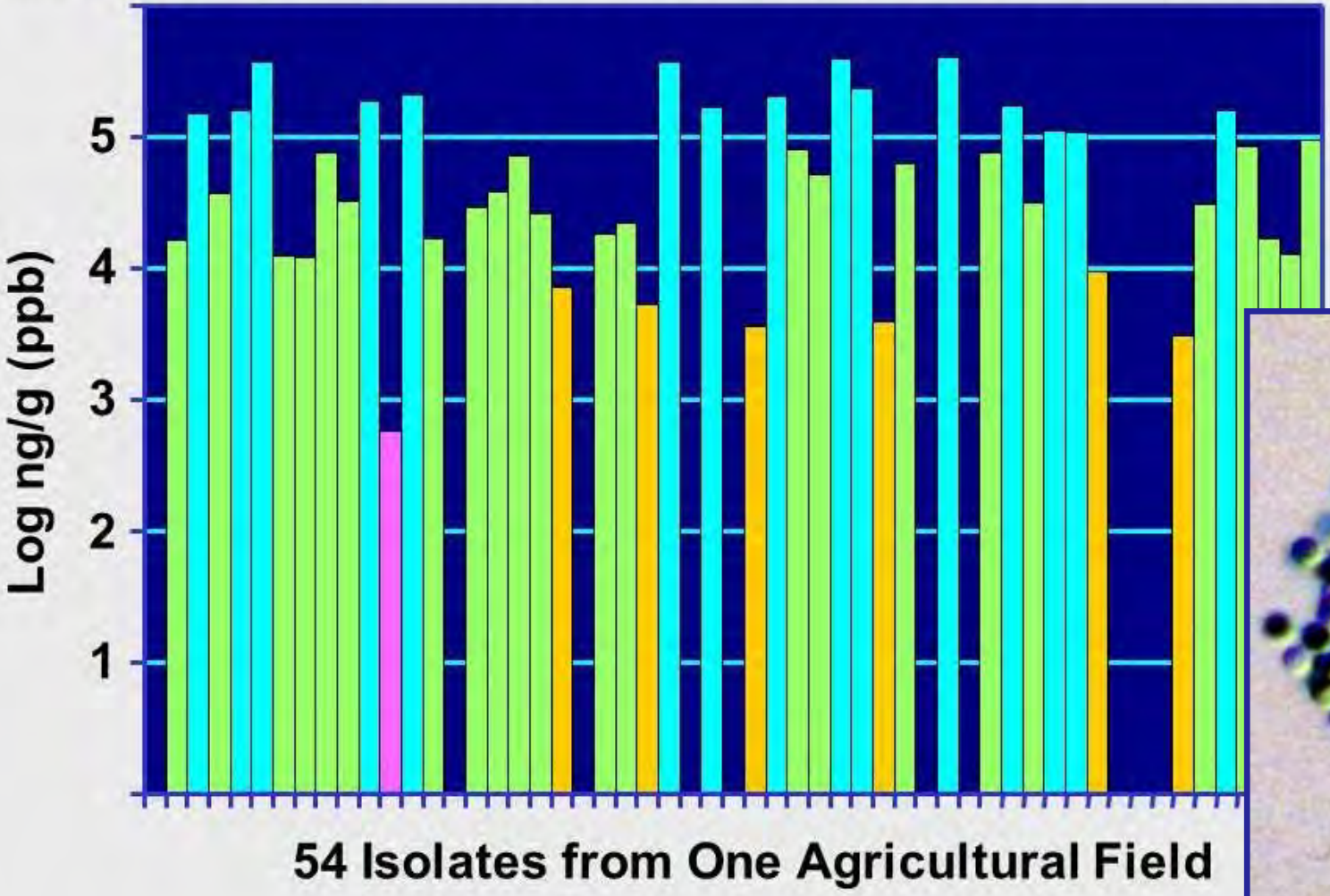
Aflatoxin Biocontrol in Europe

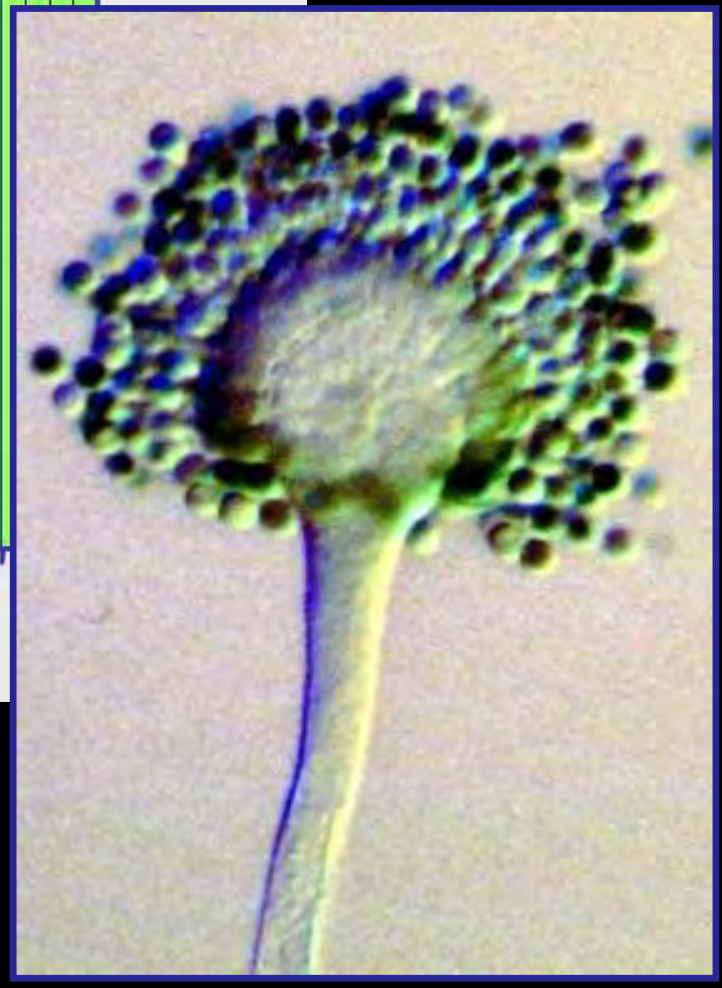
- Target Crop: Maize.
- 35,000 acres treated in 2016 – very effective.
- Maize required to be below 3 ppb for cheese industry.



1st Biocontrol Talk 1988

Aflatoxin Production by Fungal Isolates in Liquid Fermentation





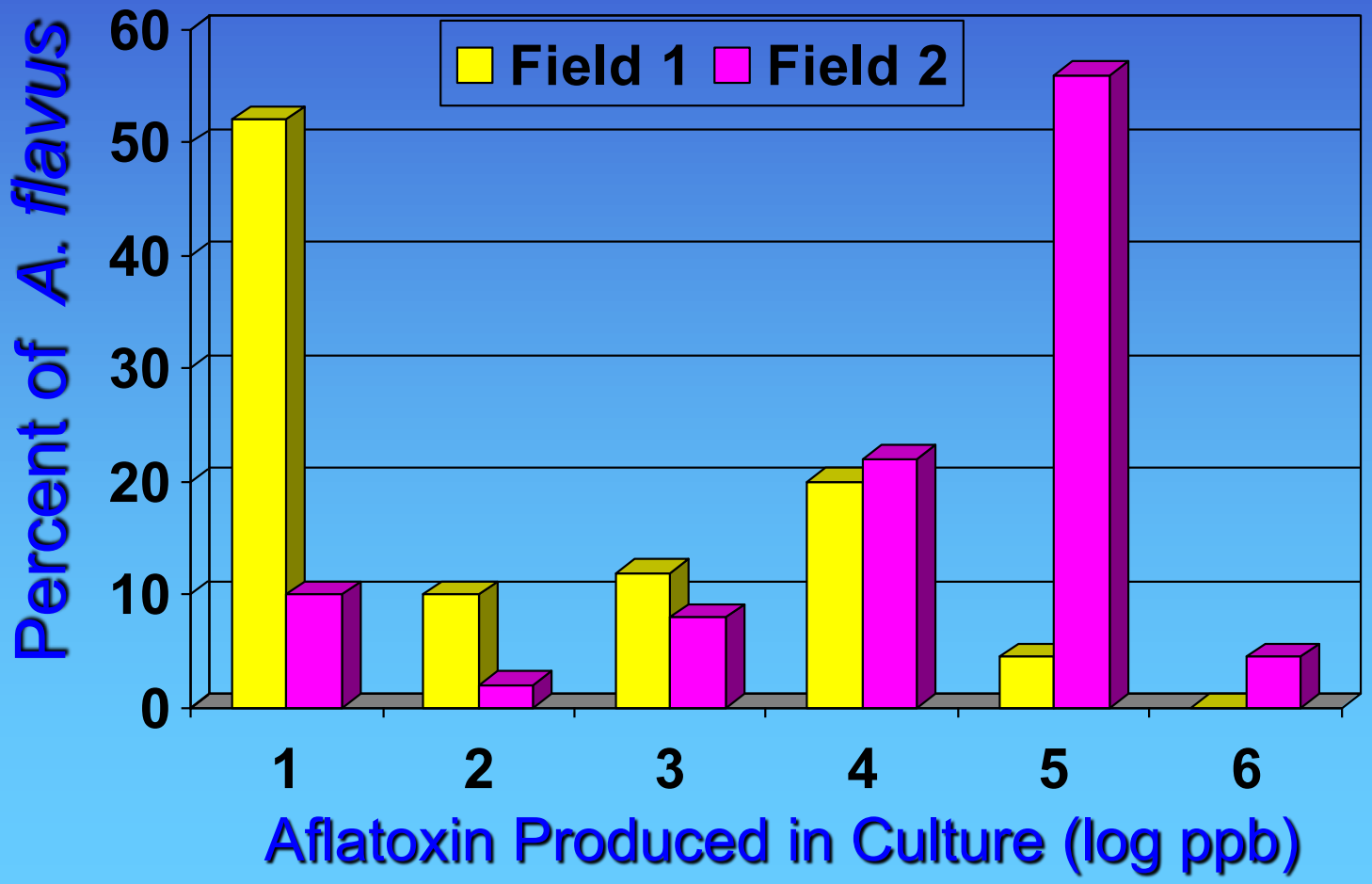
Aspergillus flavus primary causal agent– Phenotypically Variable!

There are Many Atoxigenic Strains

Fungi Vary Across Areas in Aflatoxin-Producing Ability

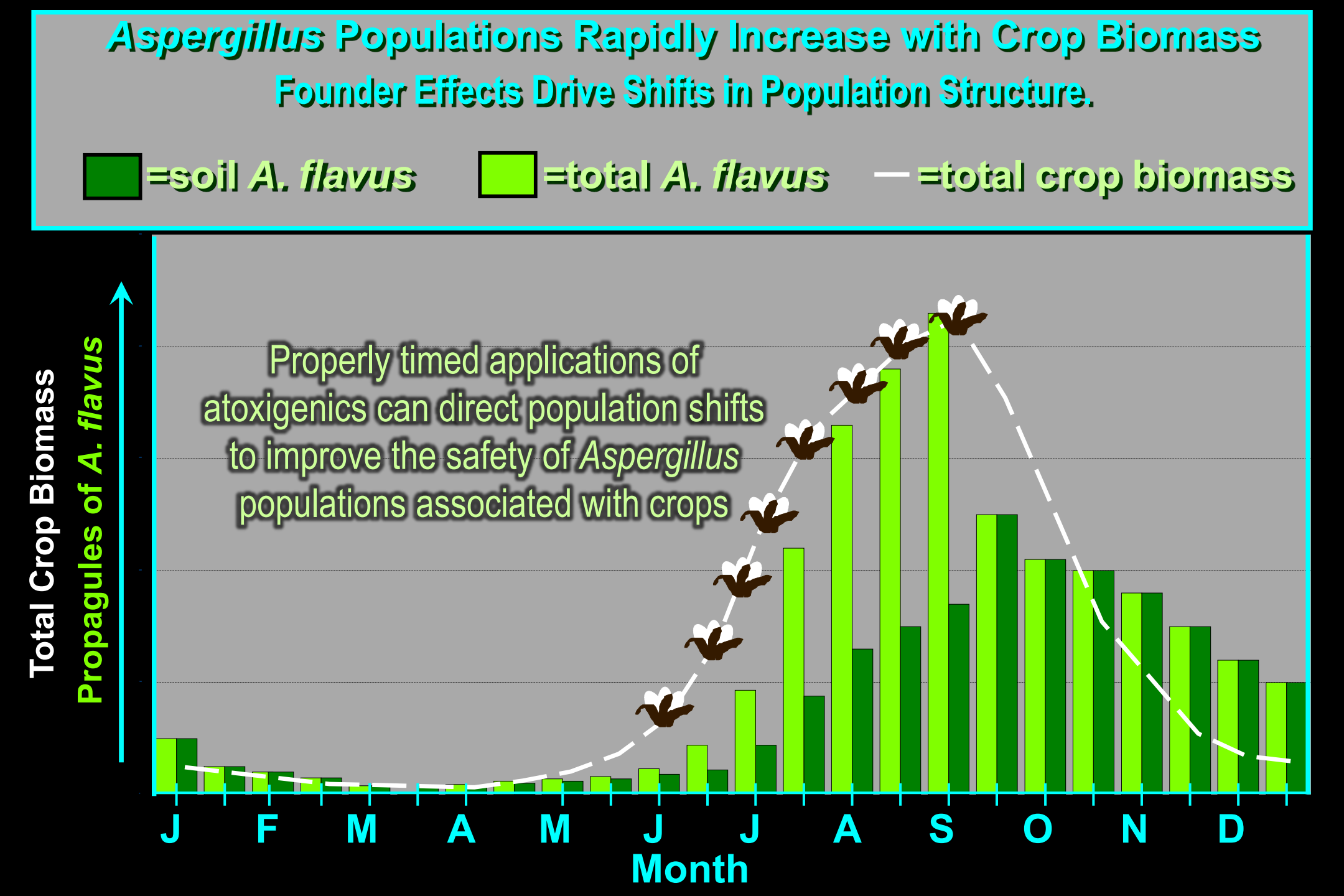
Farmers apply atoxigenic strains to reduce the average aflatoxin-producing potential of fungi on farms & thus the vulnerability of crops to aflatoxin contamination

Aflatoxin Production by *A. flavus* from Two Fields

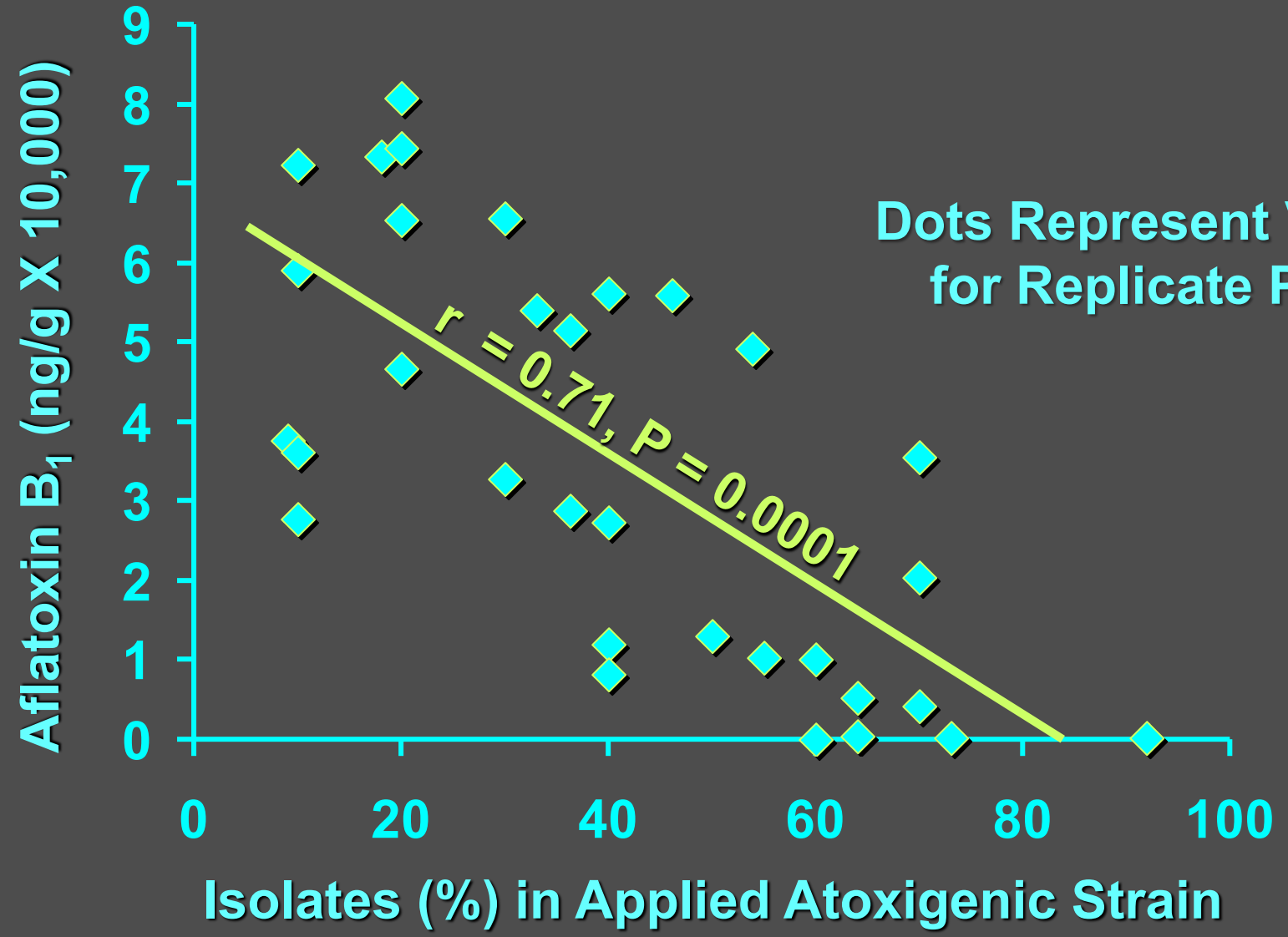


Aflatoxin-Producing Potential
Field 1 = Low, 3,400 ppb
Field 2 = High, 54,000 ppb

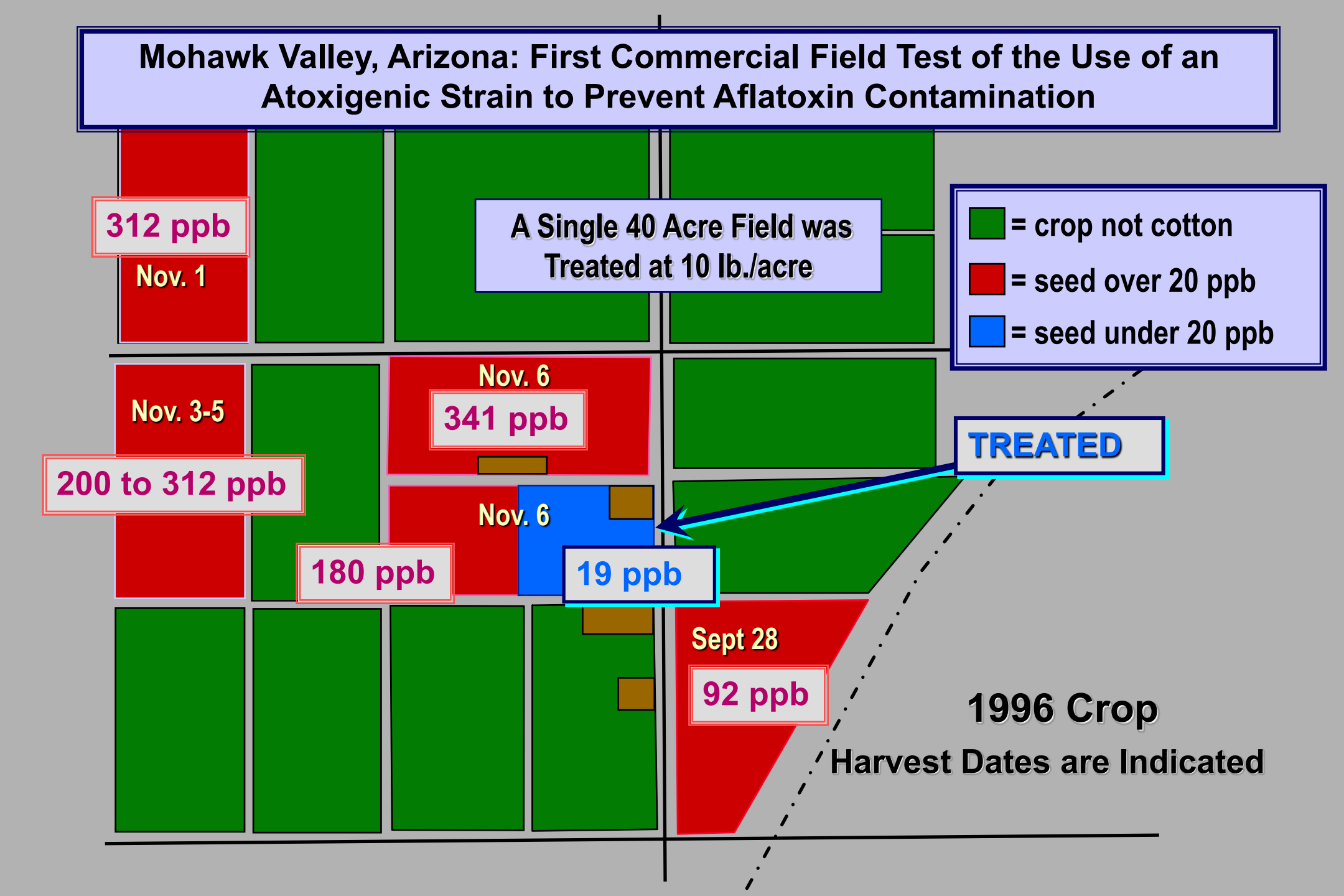
Mycological Research 101:698-704; 1997.


Aspergillus Populations Rapidly Increase with Crop Biomass
Founder Effects Drive Shifts in Population Structure.
=soil *A. flavus*
=total *A. flavus*
=total crop biomass
Total Crop Biomass
Propagules of *A. flavus*
Properly timed applications of atoxigenics can direct population shifts to improve the safety of *Aspergillus* populations associated with crops
J
F
M
A
M
J
J
A
S
O
N
D
Month

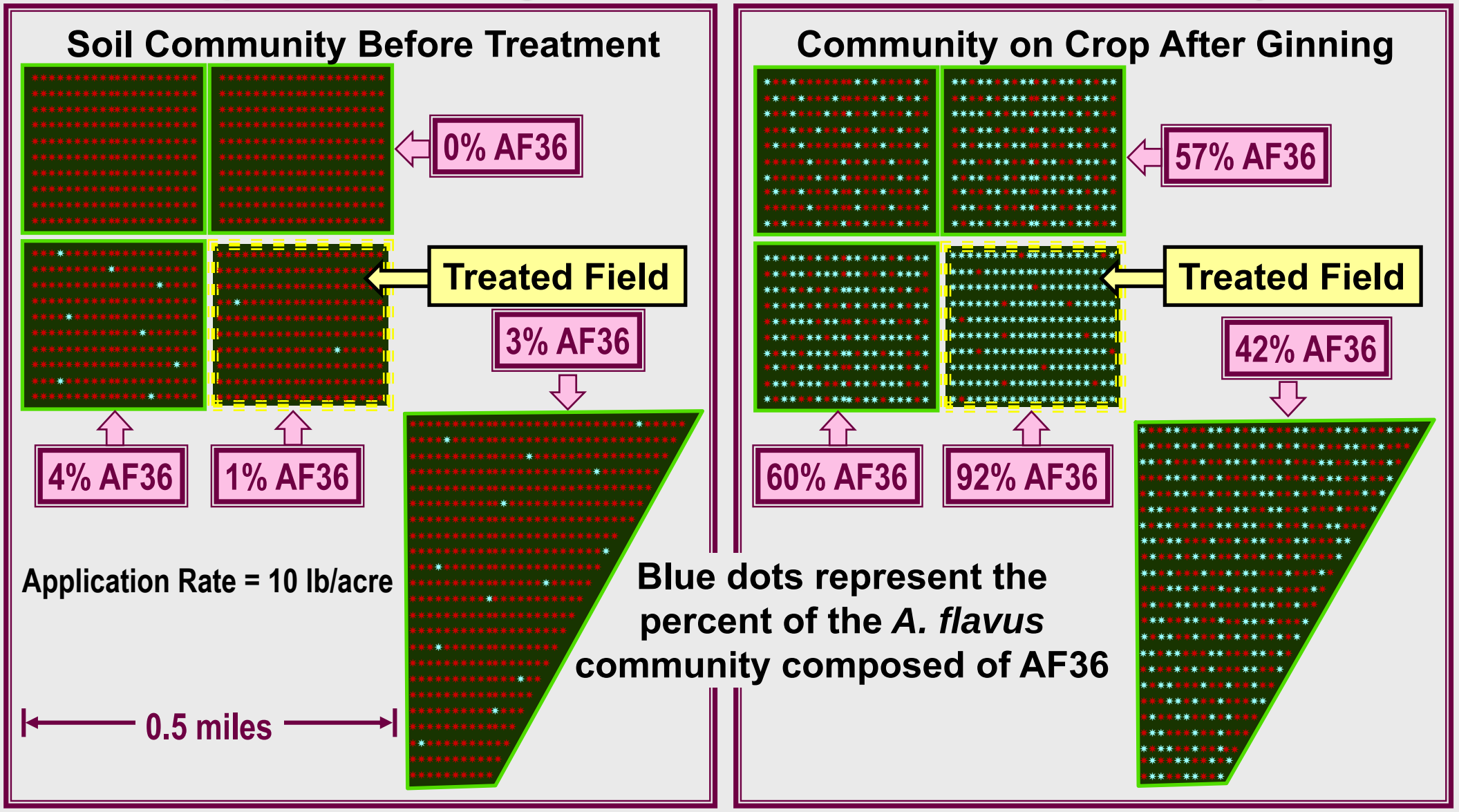
Aflatoxin in Crop versus Atoxigenic Incidence



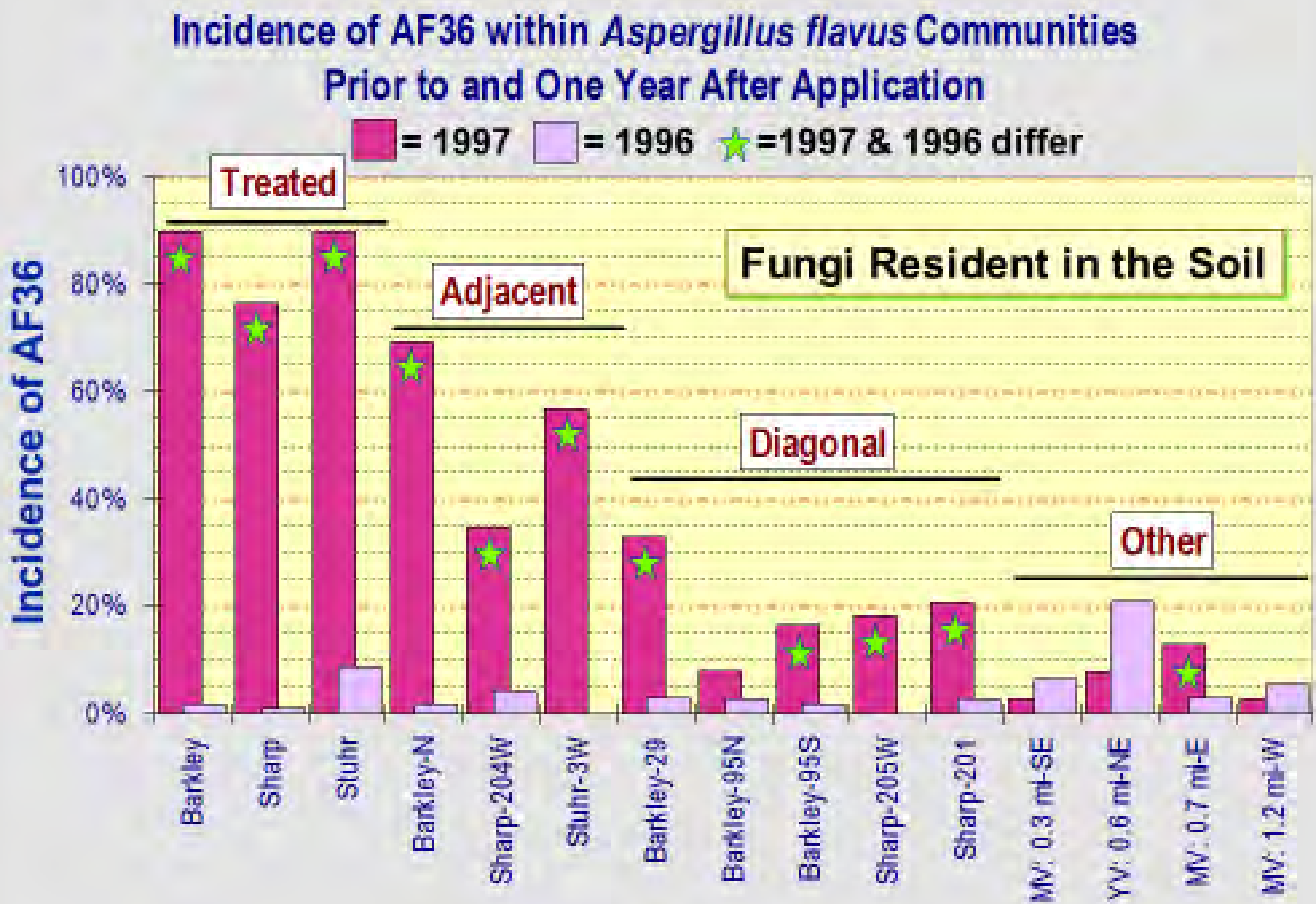
Cotty, 1994. Phytopathology:1270-1277.

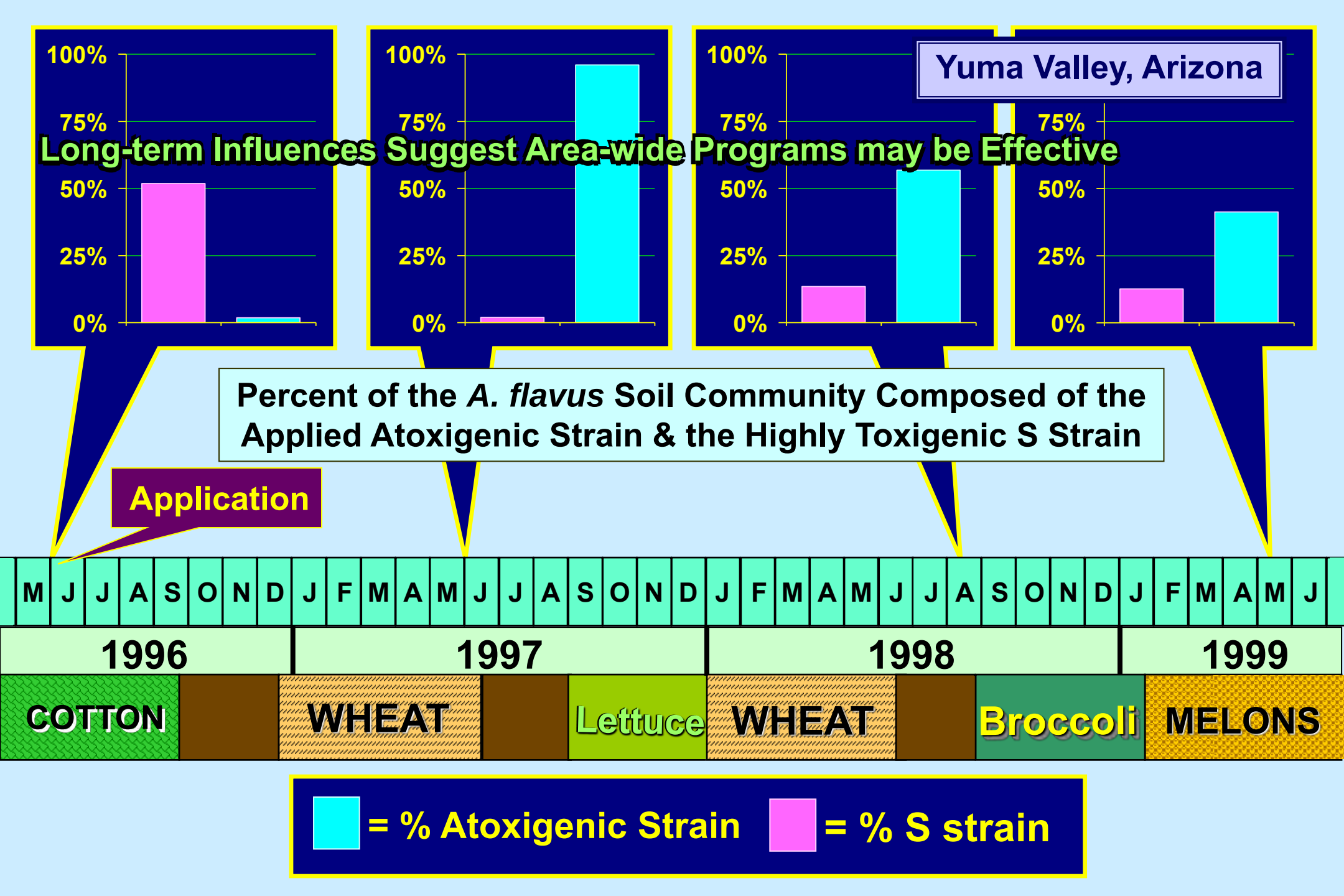

Mohawk Valley, Arizona: First Commercial Field Test of the Use of an Atoxigenic Strain to Prevent Aflatoxin Contamination
312 ppb
Nov. 1
A Single 40 Acre Field was Treated at 10 lb./acre
= crop not cotton
= seed over 20 ppb
= seed under 20 ppb
Nov. 6
341 ppb
Nov. 3-5
200 to 312 ppb
TREATED
Nov. 6
180 ppb
19 ppb
Sept 28
92 ppb
1996 Crop
Harvest Dates are Indicated

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
Incidence of AF36
100%
80%
60%
40%
20%
0%
Treated
Adjacent
Diagonal
Other
Fungi Resident in the Soil
Barkley
Sharp
Sluhr
Barkley-N
Sharp-204W
Sluhr-3W
Barkley-29
Barkley-95N
Barkley-95S
Sharp-205W
Sharp-201
MV: 0.3 mi-SE
YV: 0.6 mi-NE
MV: 0.7 mi-E
MV: 1.2 mi-W

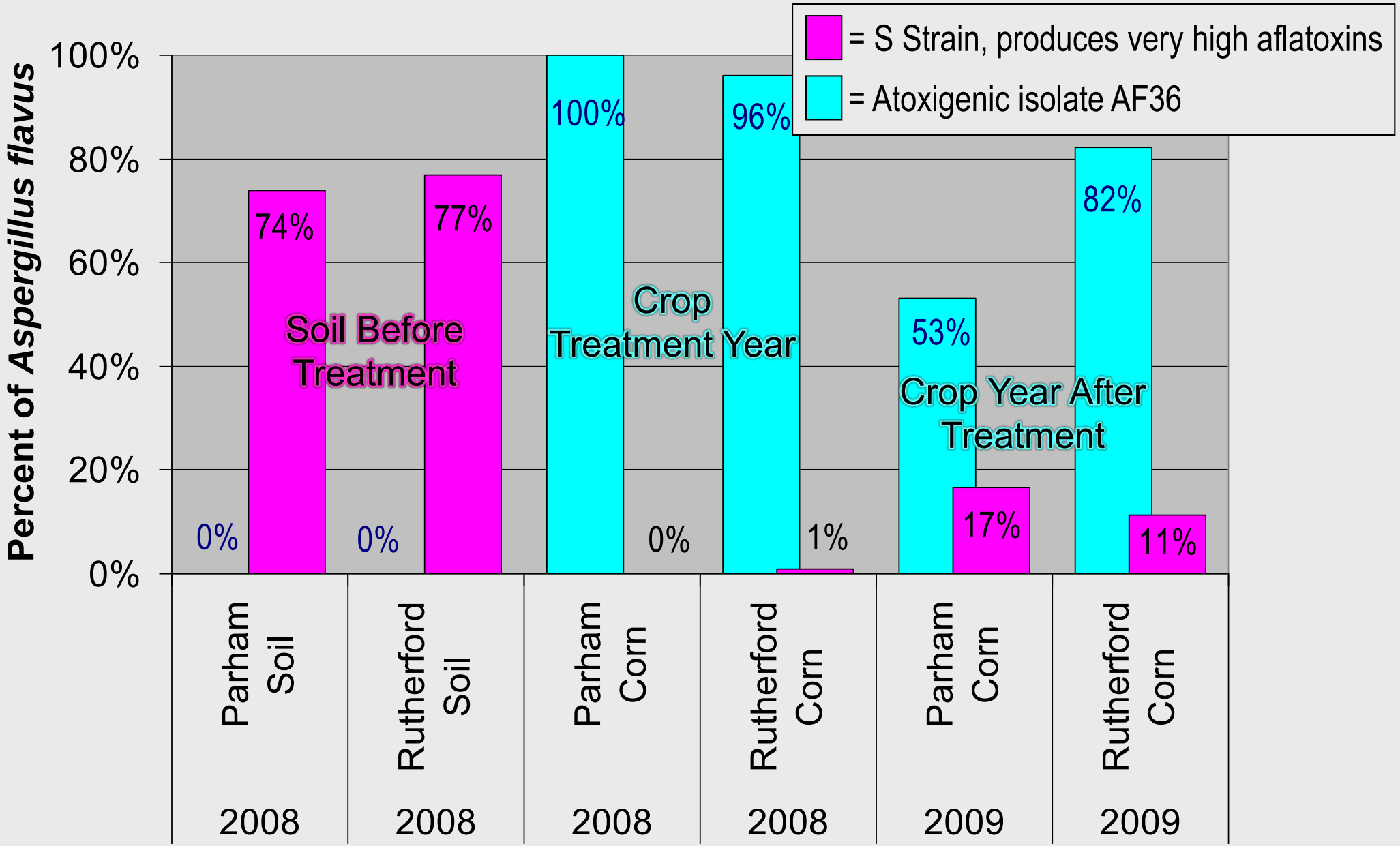

Yuma Valley, Arizona
Long-term Influences Suggest Area-wide Programs may be Effective
100%
75%
50%
25%
0%
Percent of the A. flavus Soil Community Composed of the Applied Atoxigenic Strain & the Highly Toxigenic S Strain
Application
M J J A S O N D J F M A M J J A S O N D J F M A M J J A S O N D J F M A M J J A S O N D J F M A M J
1996
1997
1998
1999
COTTON
WHEAT
Lettuce
WHEAT
Broccoli
MELONS
= % Atoxigenic Strain
= % S strain

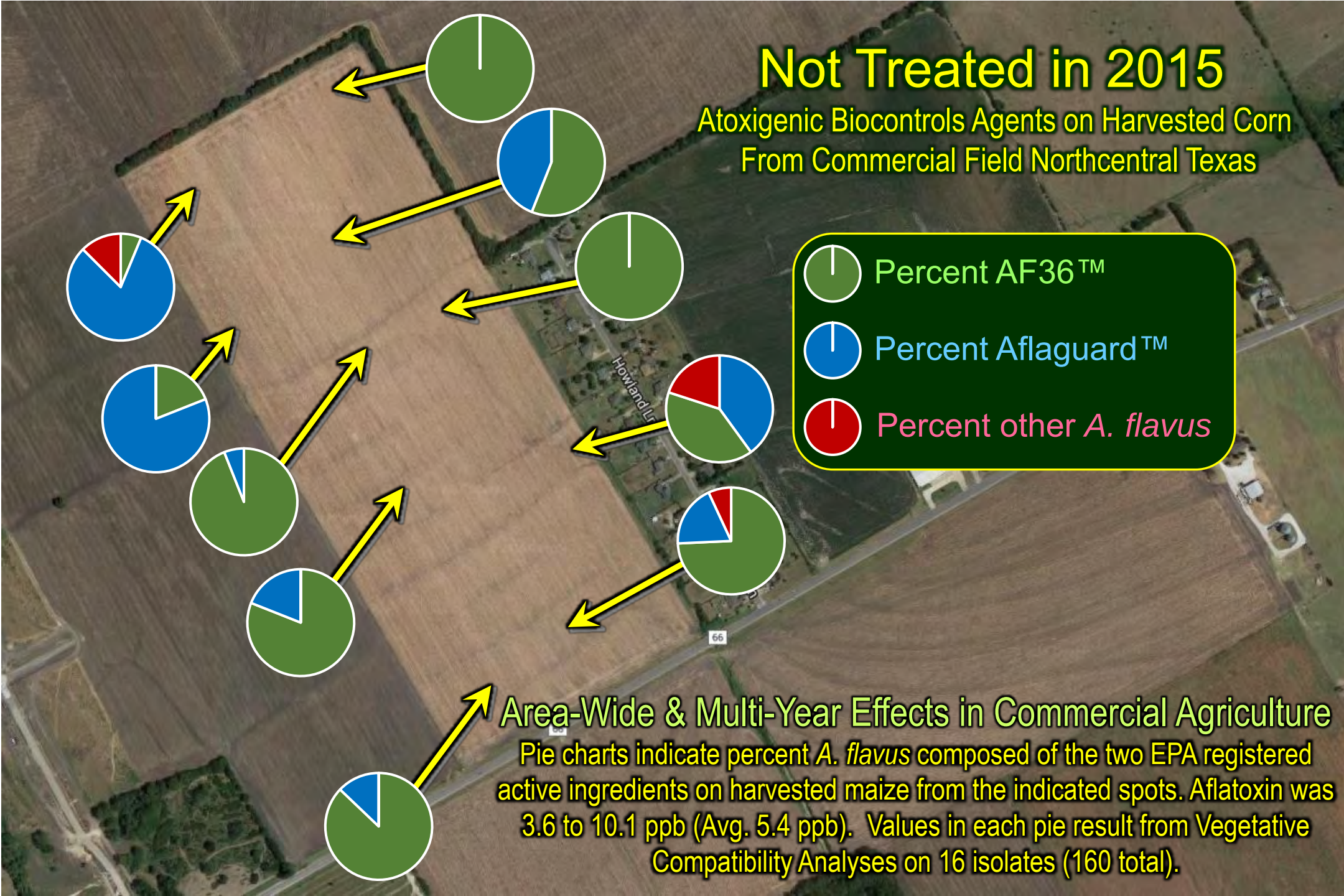
Commercial Maize: North Central Texas 2008

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
Percent AF36™
Percent Aflaguard™
Percent other A. flavus
Howland Ln
66
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.

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: *Aspergillus flavus* 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 without corn.
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 Prevail.

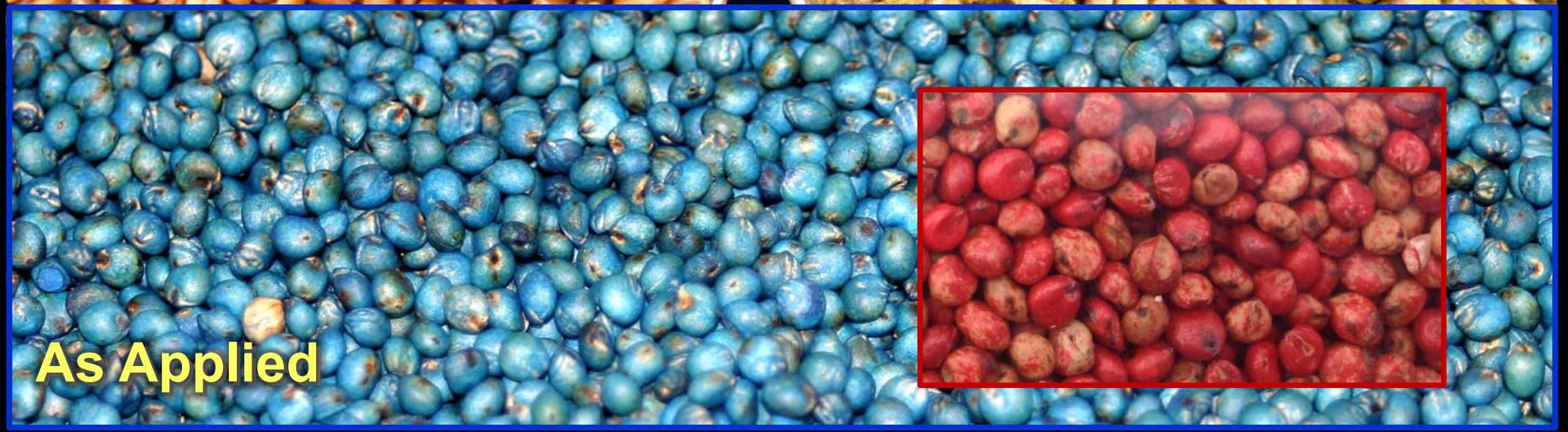
2017: Unrestricted registration for almond & fig.

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



The IR-4 Project

ACRPC

cotton
COTTON INCORPORATED

IITA

FOREIGN AGRICULTURAL SERVICE
USDA

USDA
Agricultural Research Service, USDA
School of Plant Sciences, University of Arizona
THE UNIVERSITY OF ARIZONA

TEXAS CORN PRODUCERS

USAID
FROM THE AMERICAN PEOPLE

BILL & MELINDA GATES foundation

The Cotton Foundation

AgResults
INNOVATION IN RESEARCH AND DELIVERY

NATIONAL CORN GROWERS ASSOCIATION

National Cotton Council OF AMERICA

PACA Partnership for Aflatoxin Control in Africa

NCPA
National Cottonseed Products Association
Established 1897

STCGA South Texas Cotton & Grain Association
Serving South Texas Agriculture Since 1963

AATF AFRICAN AGRICULTURAL TECHNOLOGY FOUNDATION
FONDATION AFRICAINE POUR LES TECHNOLOGIES AGRICOLES