

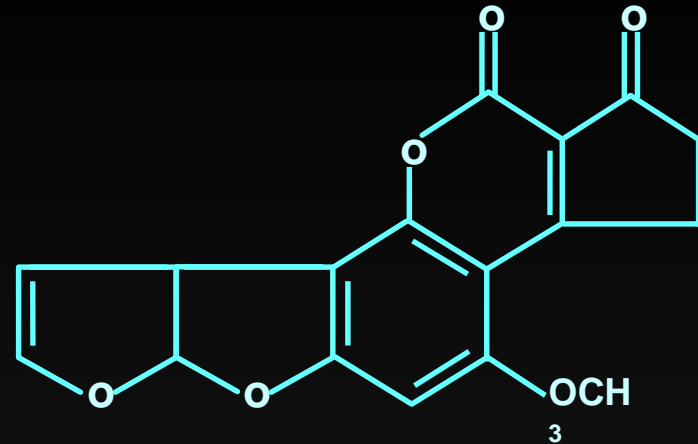


4th ICC Latin American Cereals Conference, Mexico City, March 13, 2018

Aflatoxin Management for The Caribbean Region: Prospects for Biological Control

Peter J. Cotty, Ken Callicott, David Edmunds, Marina Wissotski
Agricultural Research Service, USDA,
School of Plant Sciences, University of Arizona, Tucson

Adriana Murillo Williams
Centro Para Investigaciones en Grano y Semillas
Universitaria Rodrigo Facio, San Jose, Costa Rica



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 Processing, Market Access & Crop Value

Prevent Formation of Dangerous Aflatoxin Levels

Protect Crops From Field...



During Crop Development
Irrigation, Weed Control, Fertilize
Best cultivars, Insect Control



During Storage
Dry, prevent moisture, cool
Prevent damage: insects, rodents



During transport
Rapid, Dry, No Damage



During Processing
Sort, Cull, Discard, Add binders.

...to Mouth



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, Pistachio, Cottonseed, Peanut, Almond & Fig.



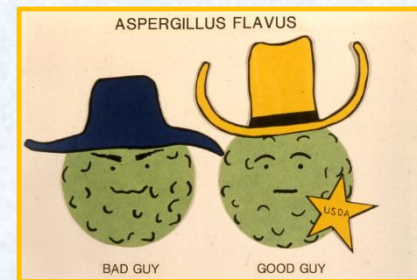
1970s, Arizona

Aflatoxin Biocontrol in Africa

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

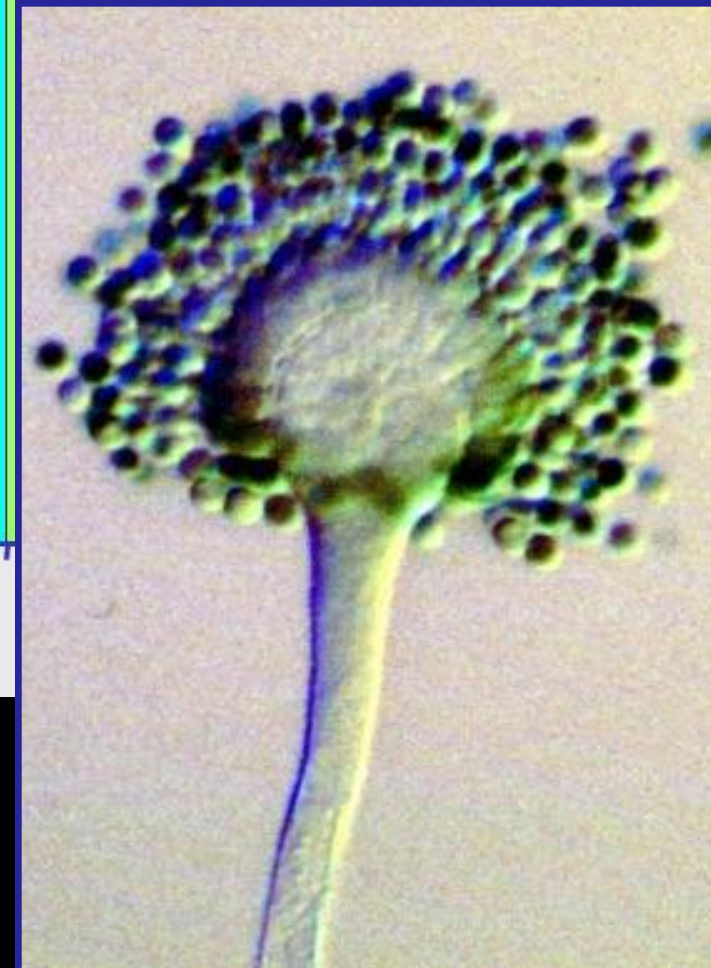
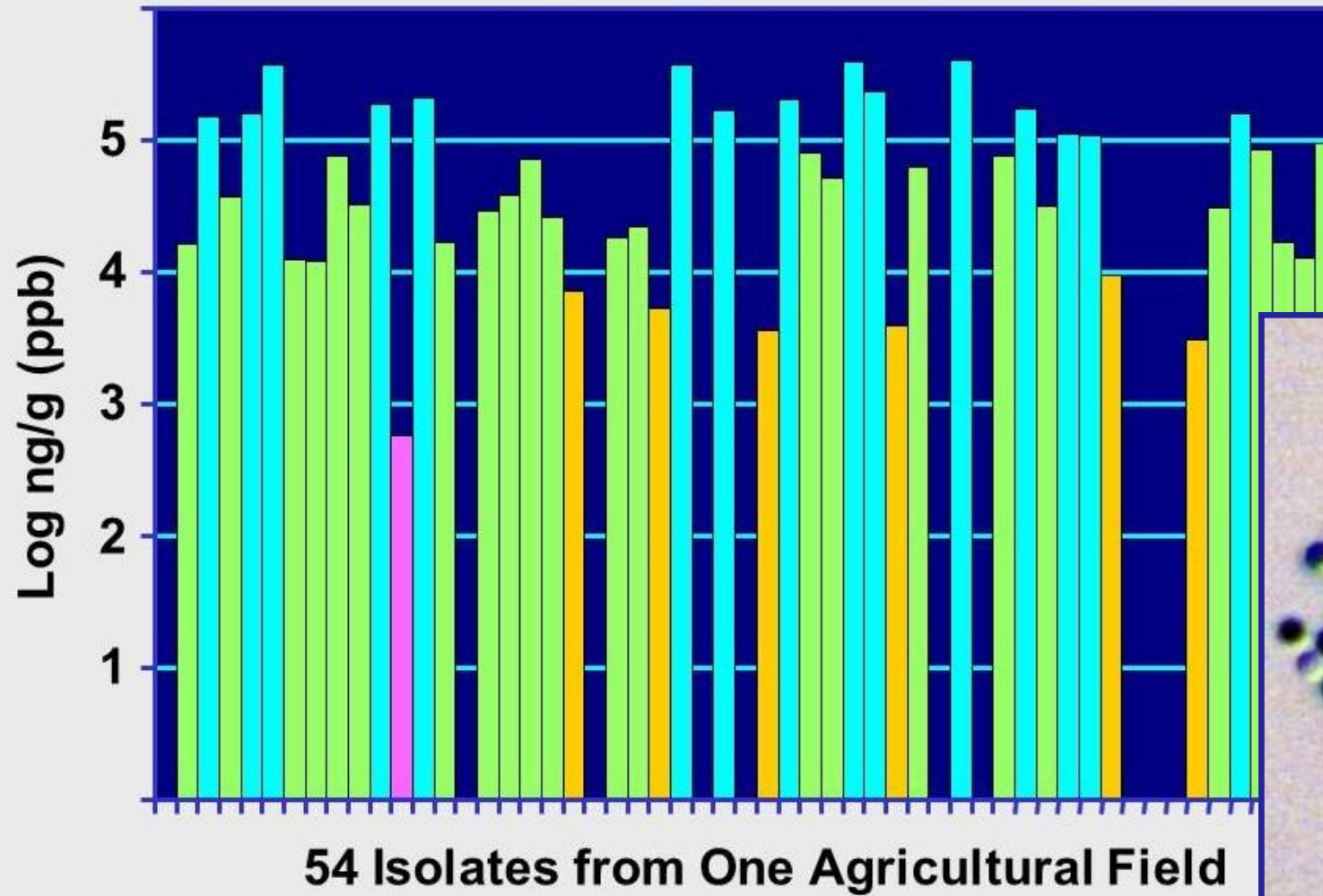


1st Biocontrol Talk 1988



1980s, Arizona
First Tests

Aflatoxin Production by Fungal Isolates in Liquid Fermentation

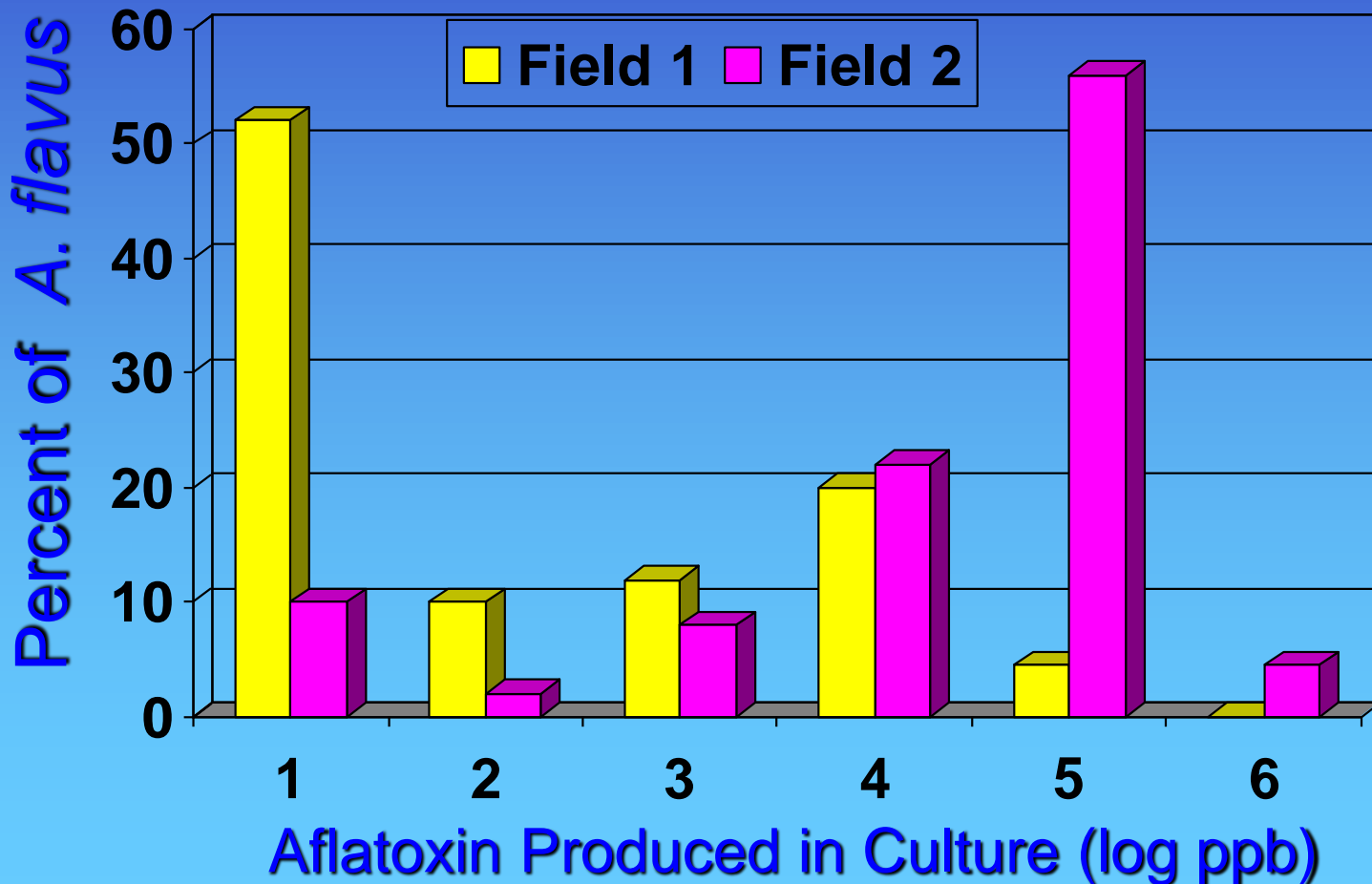


Aspergillus flavus, an important cause of aflatoxin contamination, varies widely in ability to produce aflatoxins – some are “Atoxigenic”.

Fungi Vary Across Areas in Aflatoxin-Producing Ability

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

Aflatoxin Production by *A. flavus* from Two Fields



Aflatoxin-Producing Potential

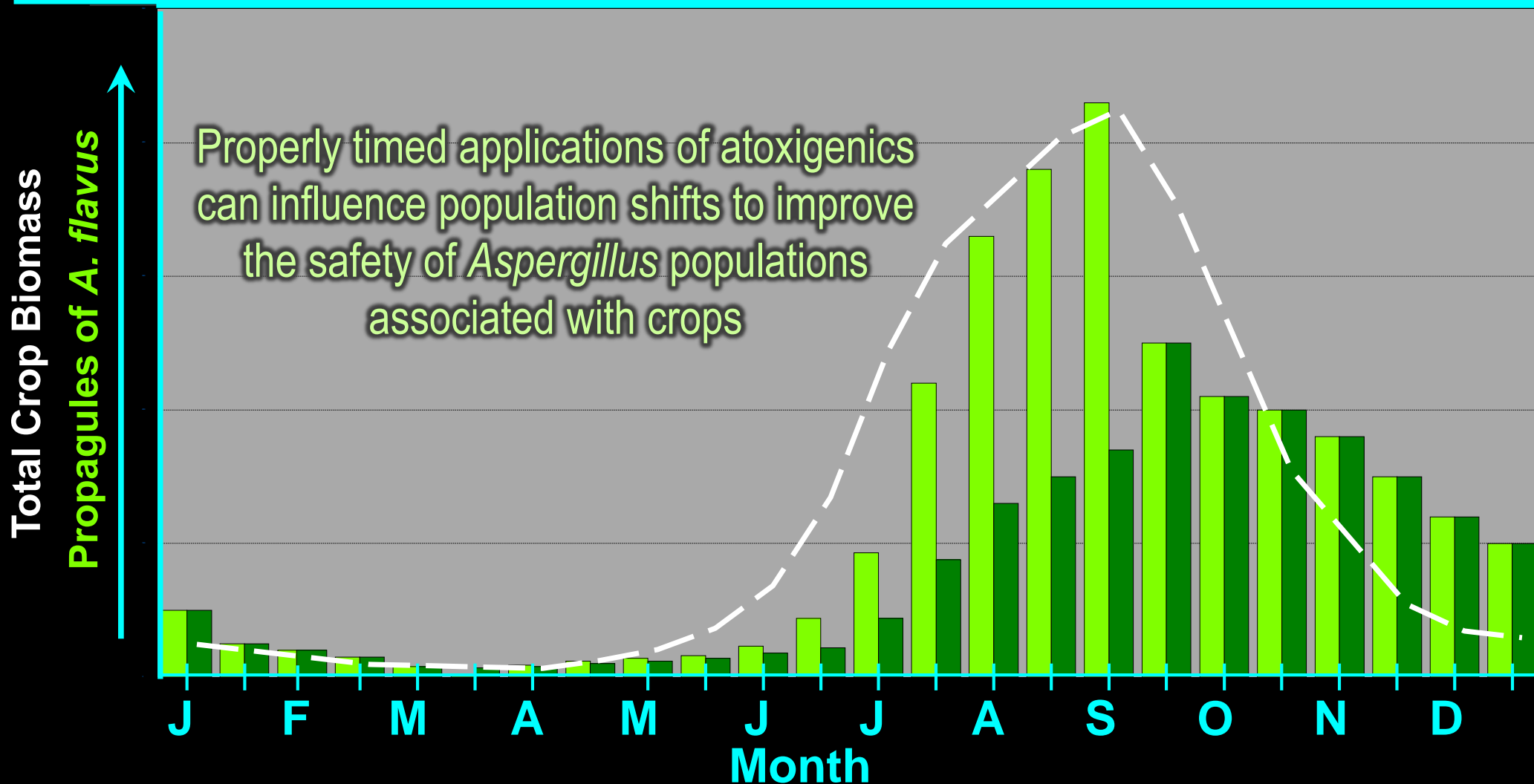
Field 1 = Low,
3,400 ppb

Field 2 = High,
54,000 ppb

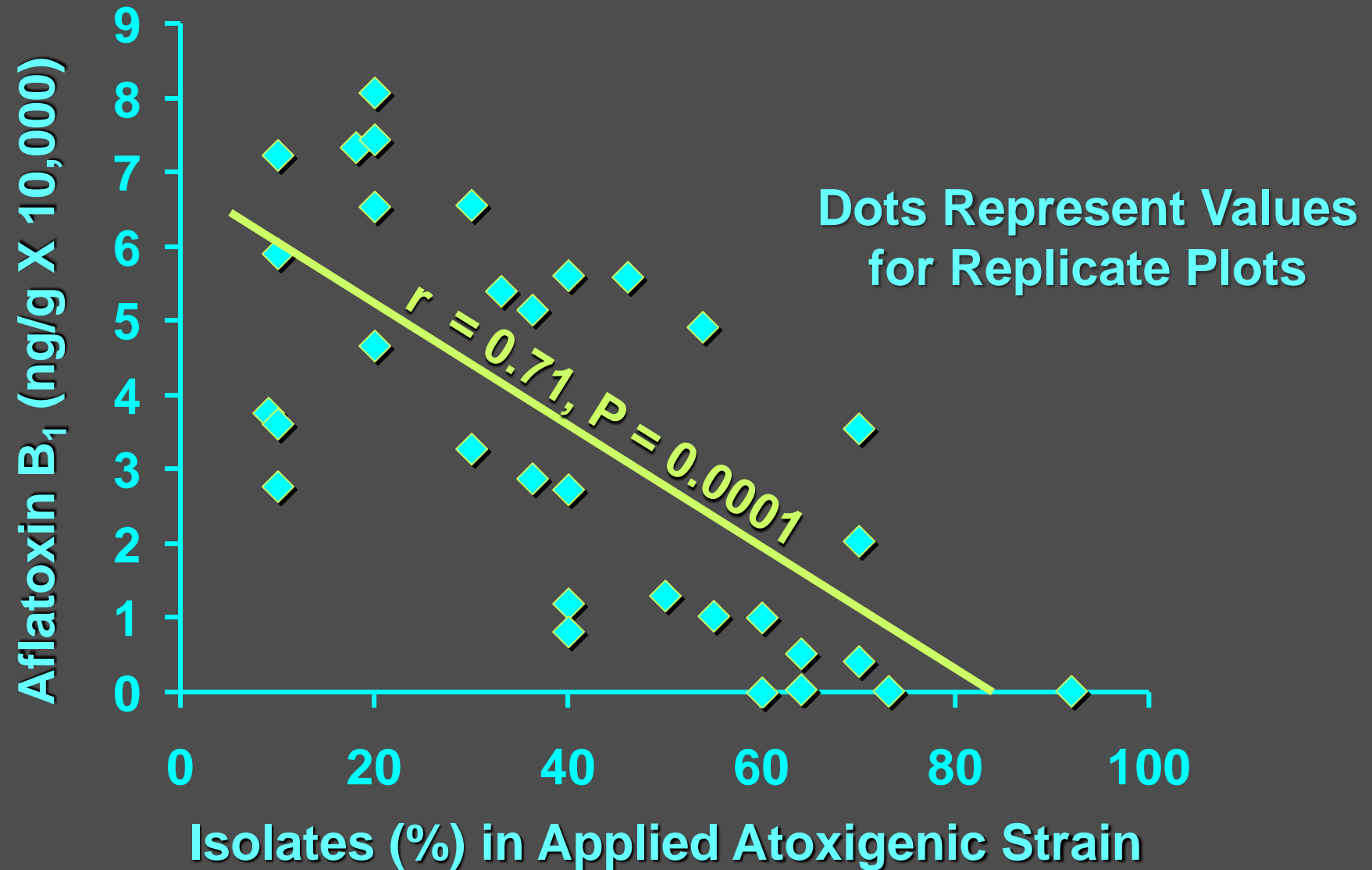
Aspergillus Populations Rapidly Increase with Crop Biomass

Founder Effects Drive Shifts in Population Structure.

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



Aflatoxin in Crop versus Atoxigenic Incidence



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

Registrations for use on maize, silage, pistachios, almonds, peanuts, and figs.

As Applied



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, $P < 0.001$.



AF-X1TM
2016

GRANULARE 12,5 Kg e



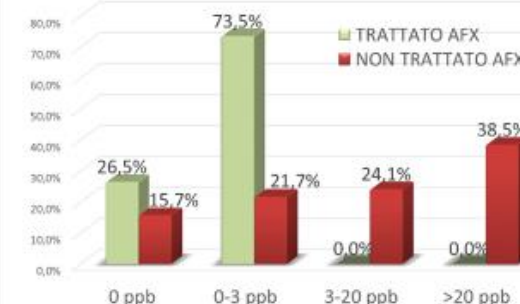
In collaborazione con:

CONSORZI AGRARI D'ITALIA
SOCIETÀ CONSORTILE PER AZIONI



UNIVERSITÀ
CATTOLICA
del Sacro Cuore

Monitoraggio granelle 2016: ambienti a alto rischio



2016 - Modena

TRATTATO AF-X1

PRODOTTO < 3 ppb

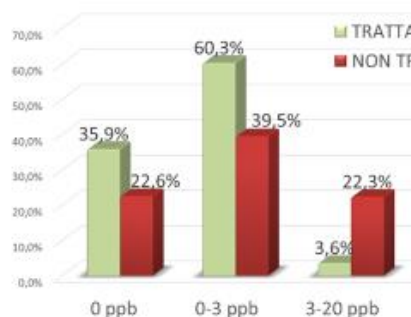
NON TRATTATO

- 62,6 % DEL PRODOTTO > 3 ppb
- 38,5 % DEL PRODOTTO > 20 ppb



UNIVERSITÀ
CATTOLICA
del Sacro Cuore

2016



TRATTATO AF-X1

- 96,2 % DEL PRODOTTO < 3 ppb
- 99,8 % DEL PRODOTTO < 20 PPB

NON TRATTATO

- 37,9 % DEL PRODOTTO > 3 ppb
- 15,6 % DEL PRODOTTO > 20 ppb



NON TRATTATO

- 42,8 % DEL PRODOTTO > 5 ppb
- 23,8 % DEL PRODOTTO > 20 ppb

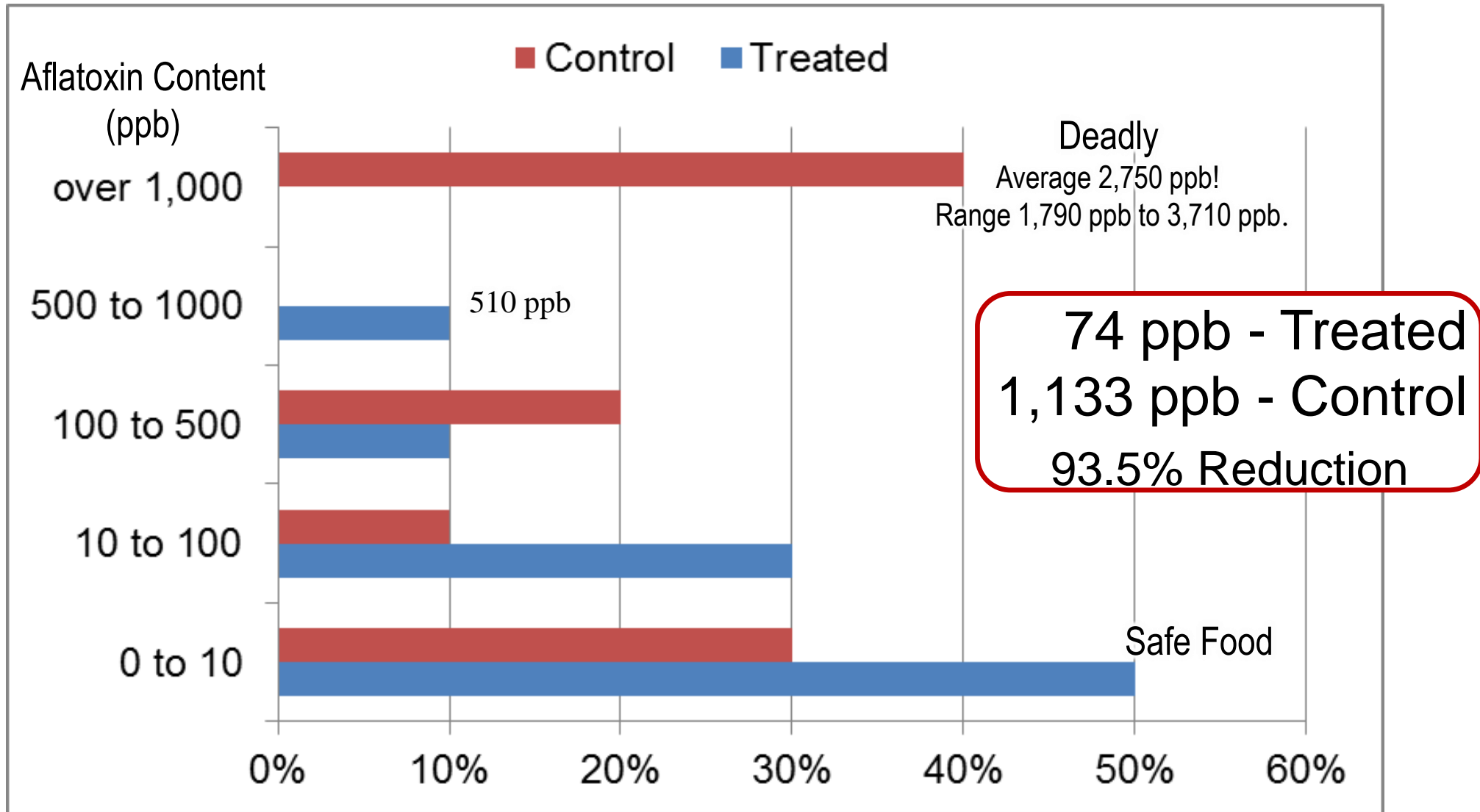
Dr. Paola Battilani
Università Cattolica del Sacro Cuore
Piacenza, Italy

Prior Student: Dr. Antonio Mauro
Now with IITA Tanzania



Mutomo, Kitui County, Kenya: 2012 Tests

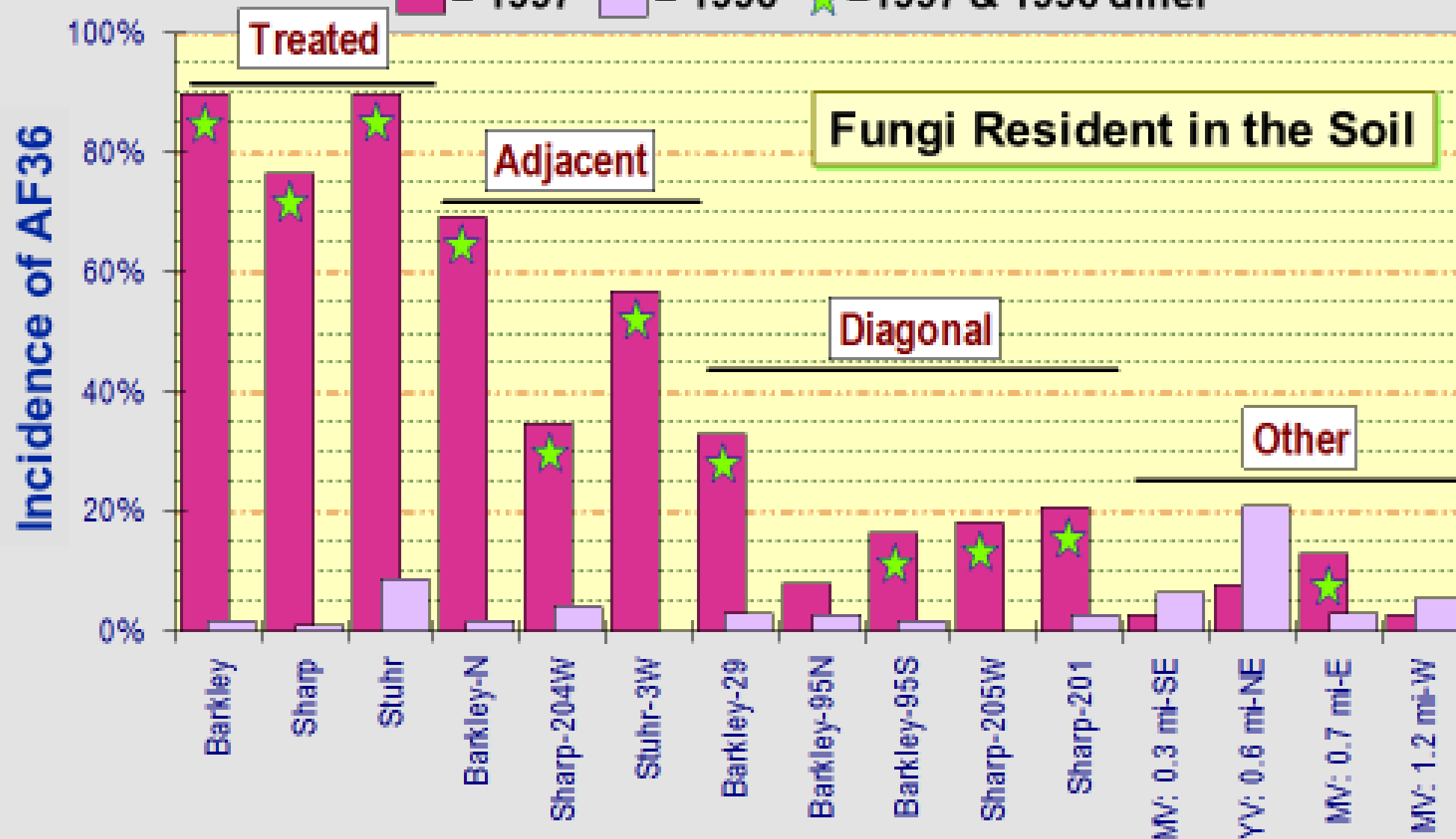
Short Rain Season Farmer Field Trials



Selection of atoxigenics for Kenya: Probst, et al. 2011. Plant Disease 95:212-218.

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

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

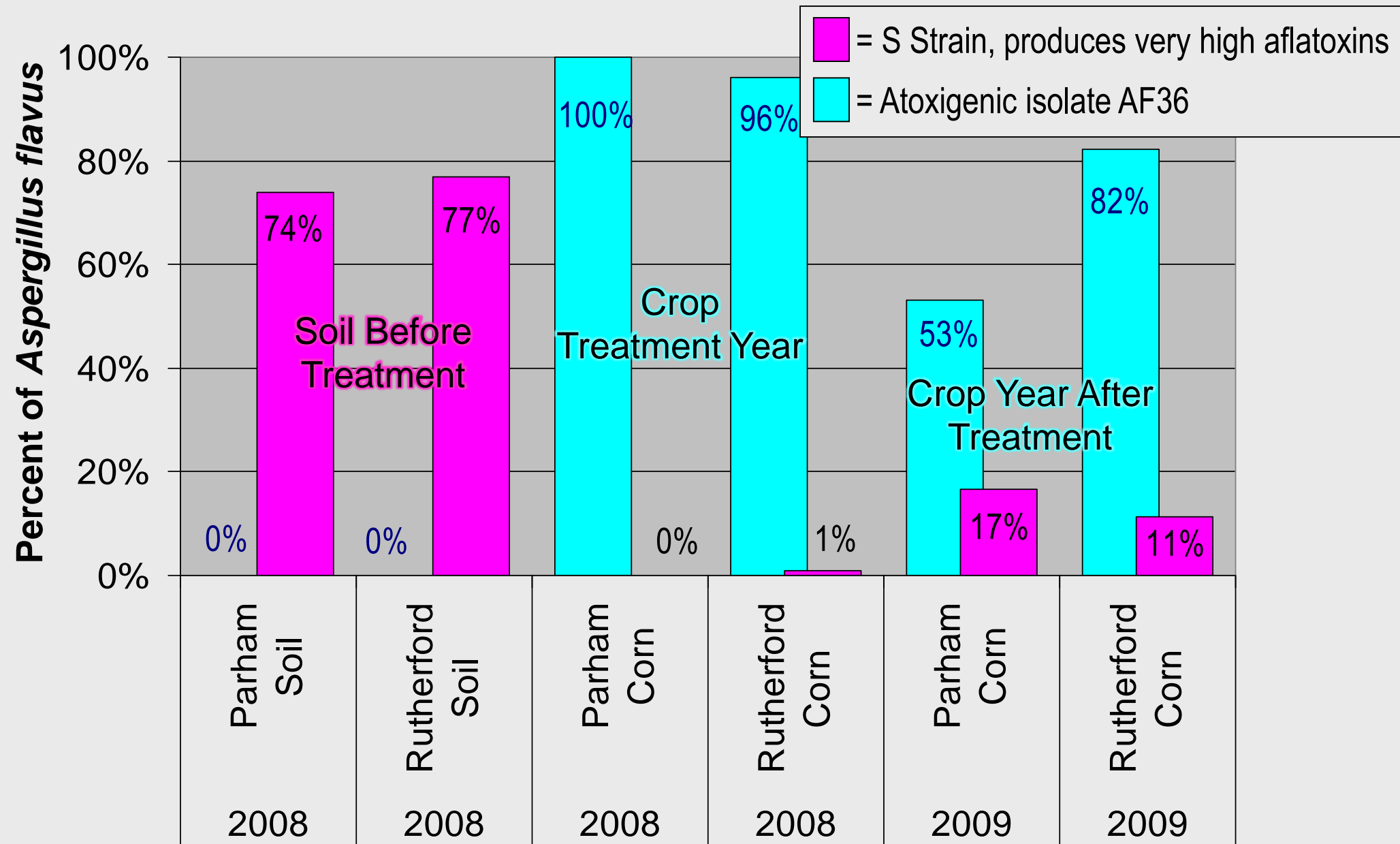


Commercial Maize: North Central Texas 2008

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



Fungi move between fields and across areas.

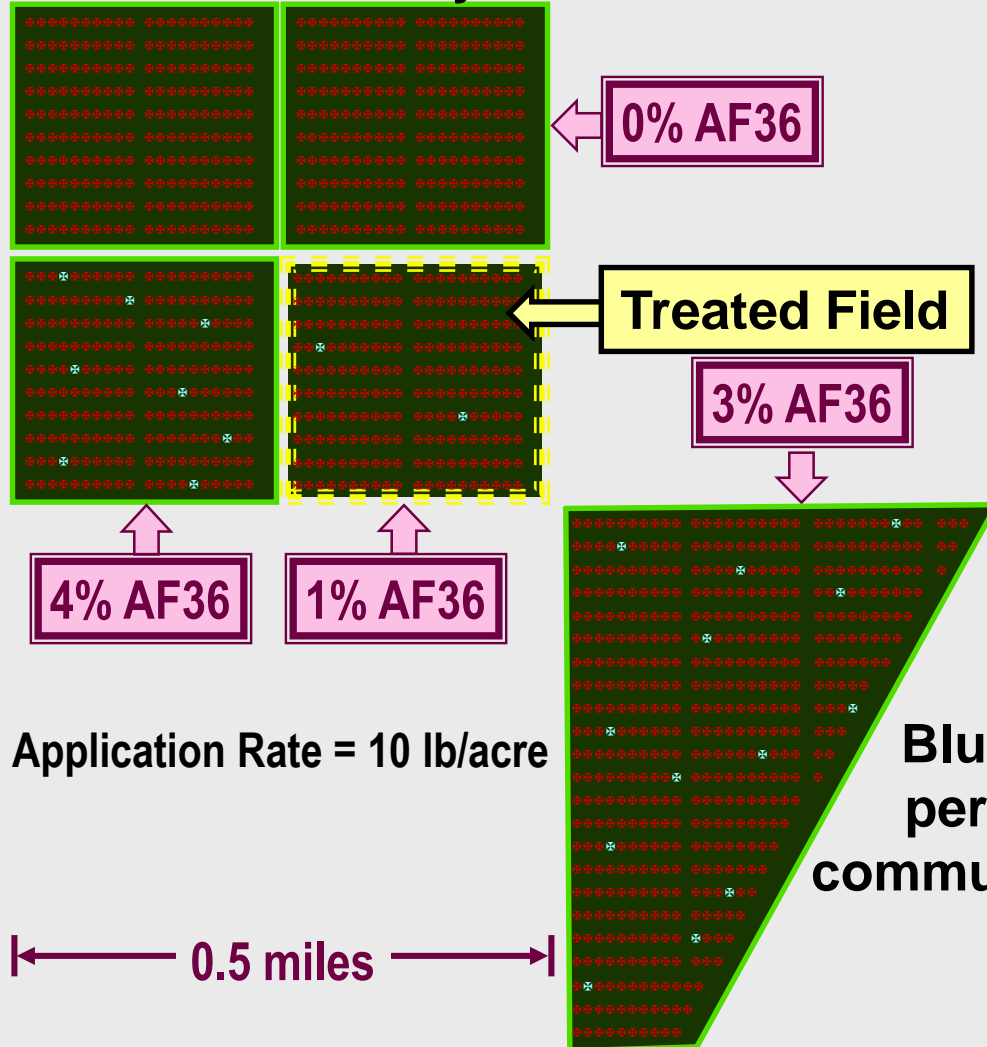
Take a bigger view: Not just one field, one farmer, one crop.

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 yellow and 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 impression is one of a complex, interconnected agricultural system.

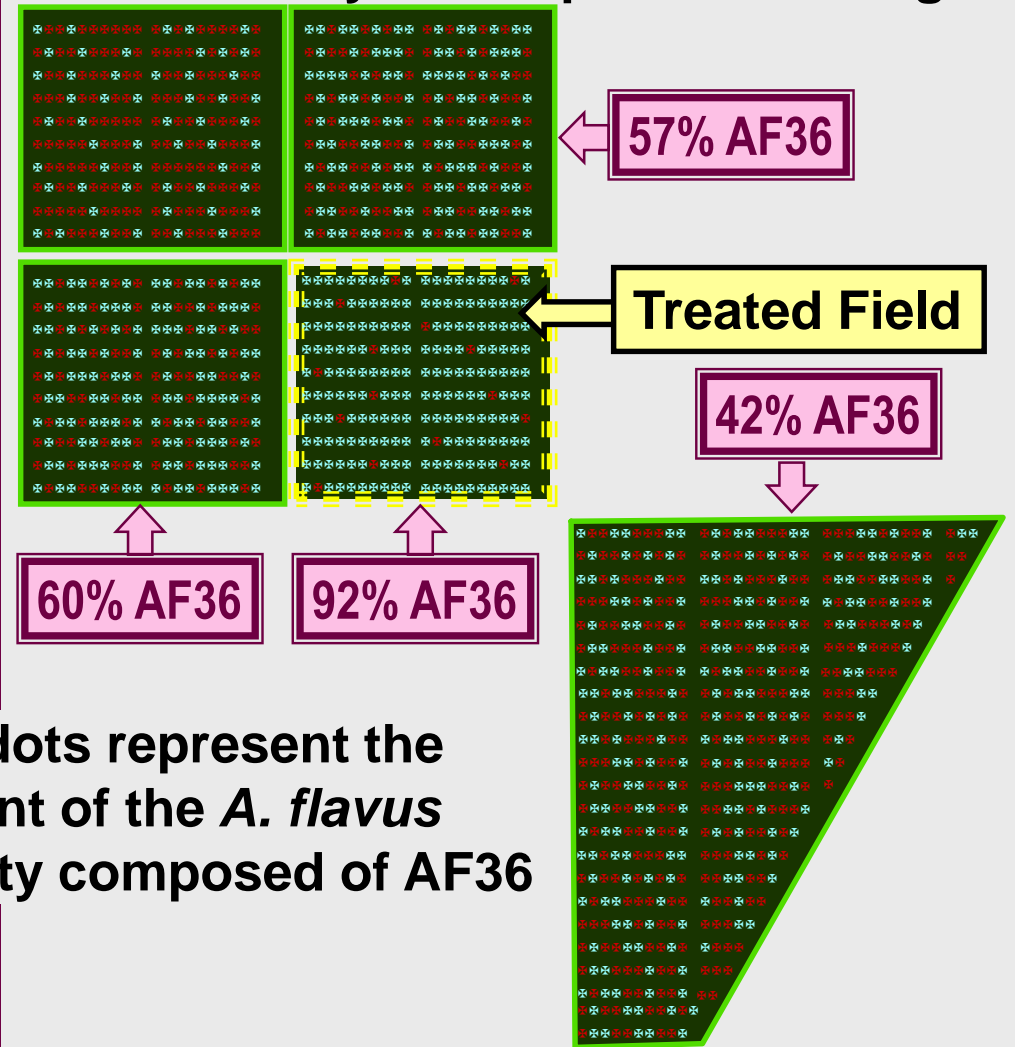
We can protect whole agricultural systems from aflatoxins including all affected industries and improve the environment.

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

Soil Community Before Treatment



Community on Crop After Ginning

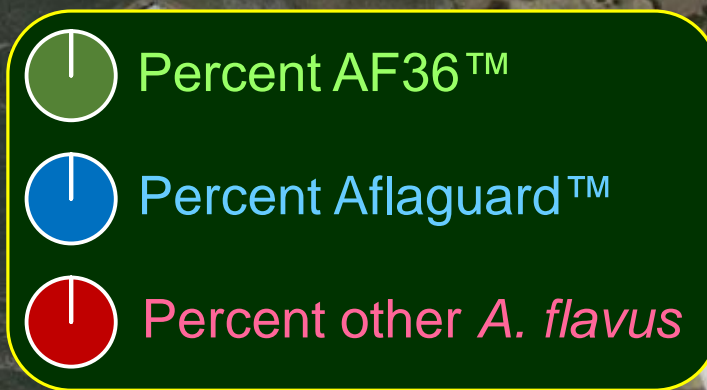
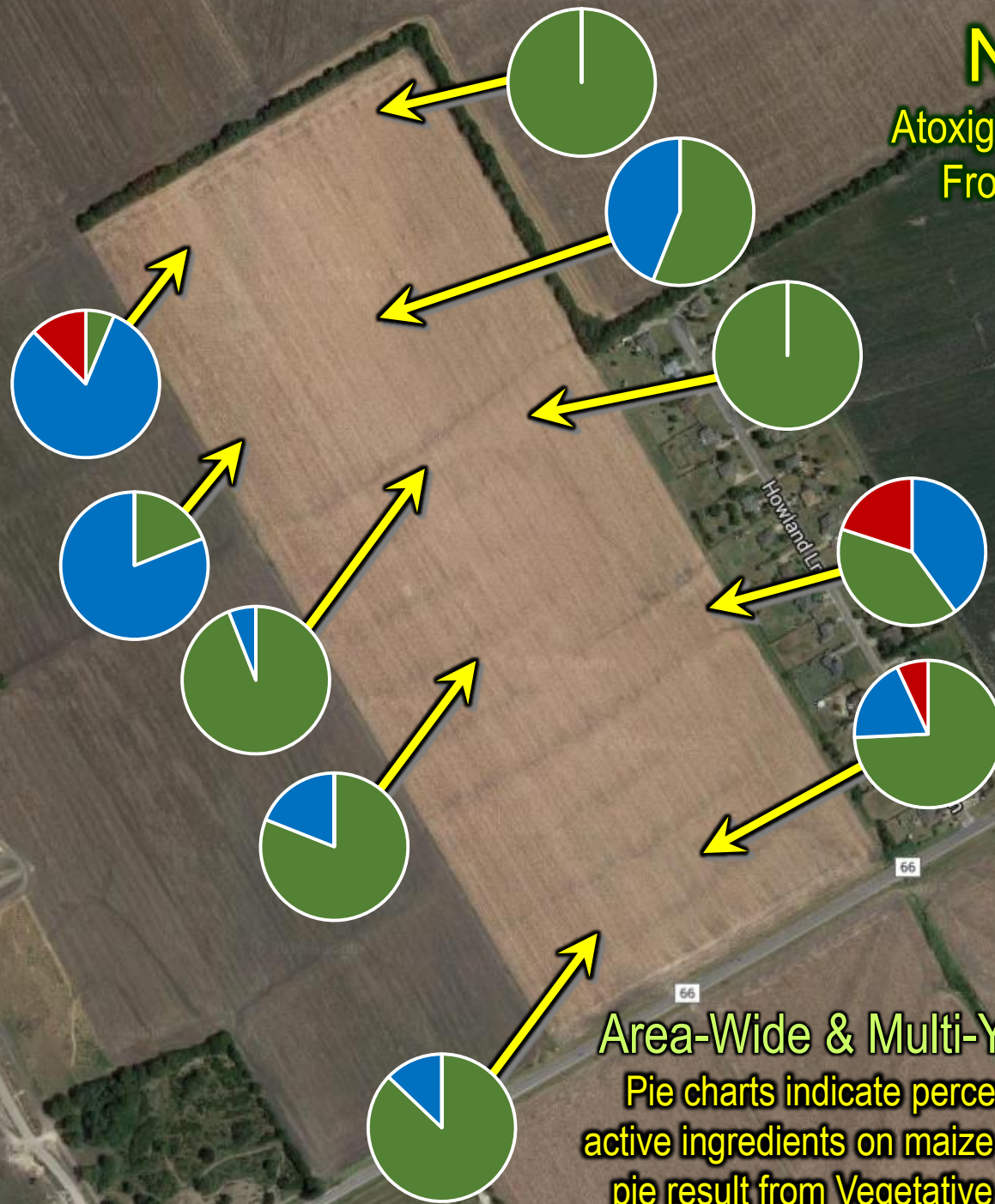


Blue dots represent the percent of the *A. flavus* community composed of AF36

Data from 564 vegetative compatibility analyses.

Not Treated in 2015

Atoxigenic Biocontrol 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 maize harvested from the indicated spots. Values in each pie result from Vegetative Compatibility Analyses on 16 isolates (160 total).

Atoxigenic strain technology is being adapted to the Caribbean. Selection of atoxigenics began in Haiti and Costa Rica in 2016. In 2018 Guatemala, Nicaragua, and Columbia will be added.

Haiti:
1236 isolates
43 crop samples

Costa Rica:
1405 isolates
109 crop samples

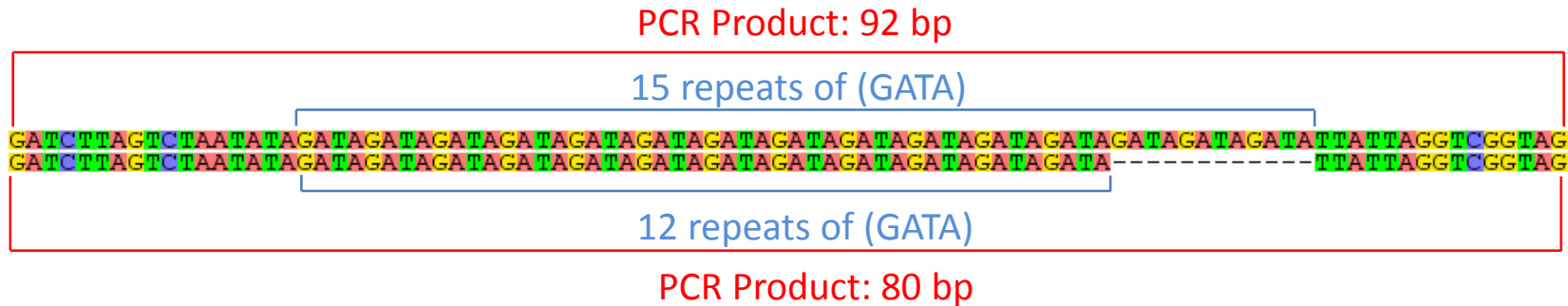


Adriana Murillo Williams

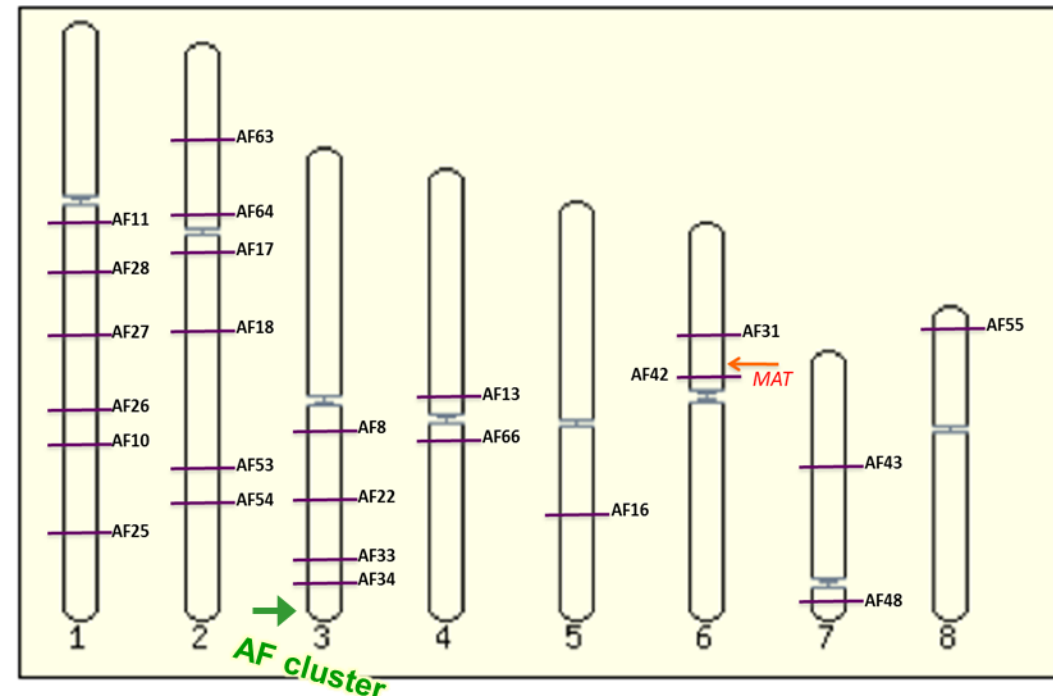
There Are Many Atoxigenic *A. flavus* that may be used as Active Ingredients in Biocontrol Products

Individual Atoxigenics can be identified with Simple Sequence Repeats (SSRs)

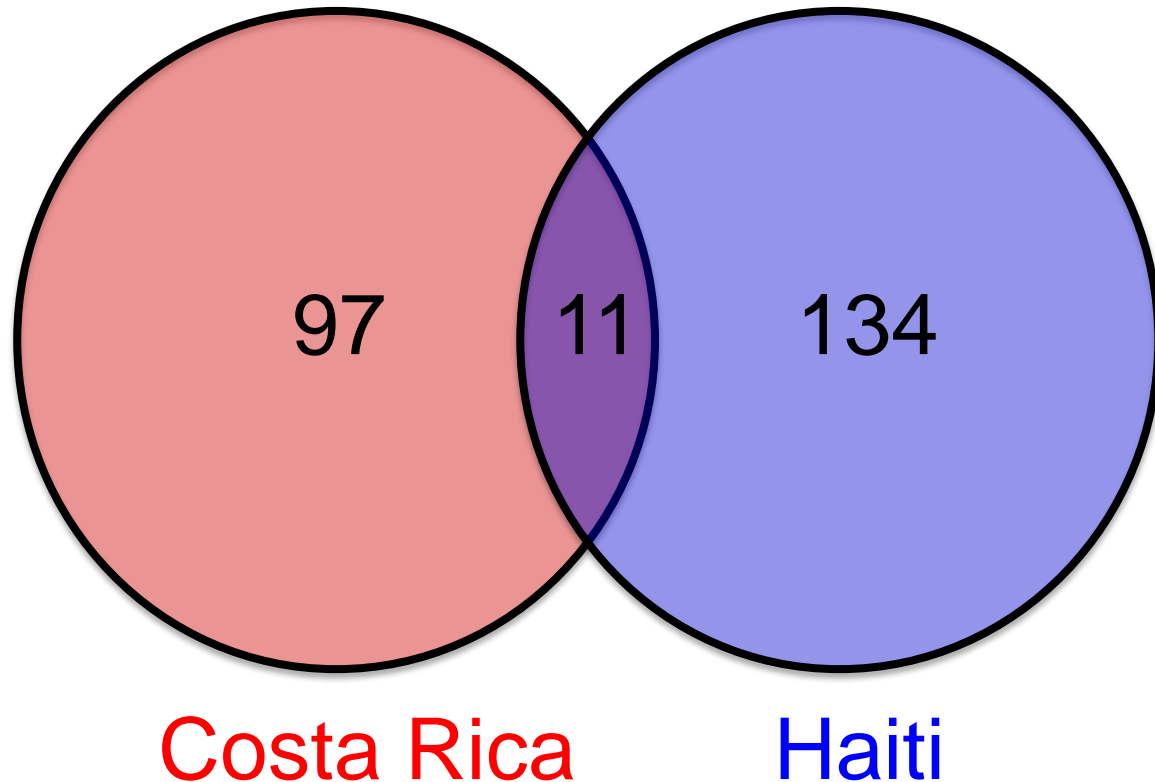
- Also called Microsatellites
 - 2-6 nucleotides repeated a variable number of times (e.g. (GATA)_n)



- Highly variable
 - Faster divergence than sequence data
 - Different rates at different loci
 - Good at discriminating closely related isolates



242 Haplotypes Detected Multiple Times 2641 Isolates Characterized at 17 SSR Loci

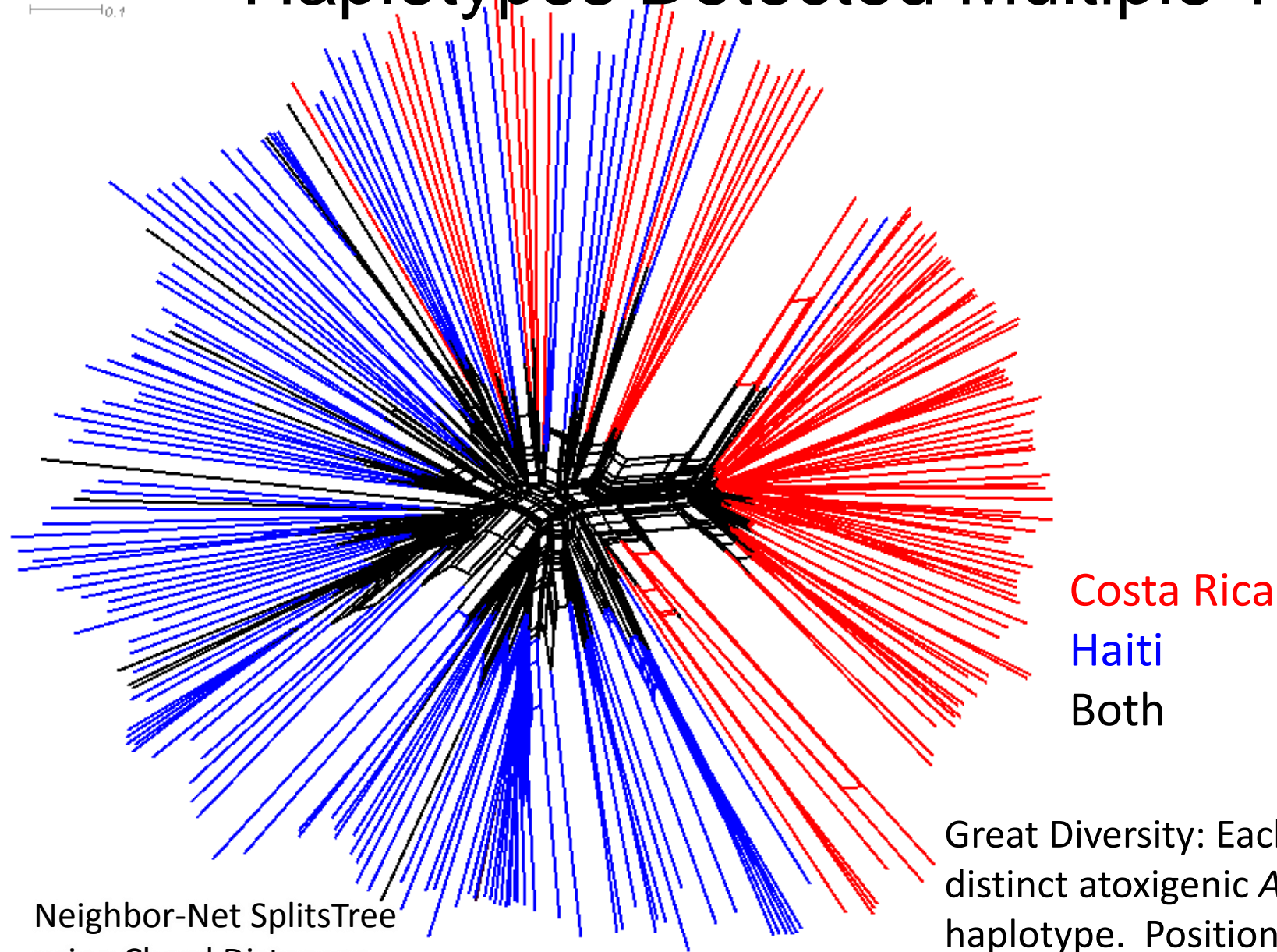


199 single sample haplotypes
In Costa Rica

689 single sample haplotypes
In Haiti

Haplotypes Detected Multiple Times

0.1



Neighbor-Net SplitsTree
using Chord Distances

Great Diversity: Each line indicates a distinct atoxigenic *A. flavus* based on SSR haplotype. Positions on the tree indicate relationships among the fungi.



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



Work in Africa led by:
Dr. Ranajit Bandyopadhyay
Int. Institute Tropical Agriculture
Ibadan, Nigeria

Work in Italy led by:
Dr. Paola Battilani
U. Cattolica del Sacro Cuore
Piacenza, Italy



Work on Tree Crops led by:
Dr. Themis Michailides
University of California, Davis
Parlier, California



Crops are infected by complex communities of diverse fungi

We can influence aflatoxin-producing ability of fungal communities resident in production areas by properly timed application of atoxigenic strains

There are many atoxigenic strains

Select strains best adapted to rotations, ecosystems, & climates

Atoxigenics are Already Present on the Crop

Just increasing the frequency of endemic strains & natural interference with contamination

Treatments have Long-Term Influences & Cumulative Benefits

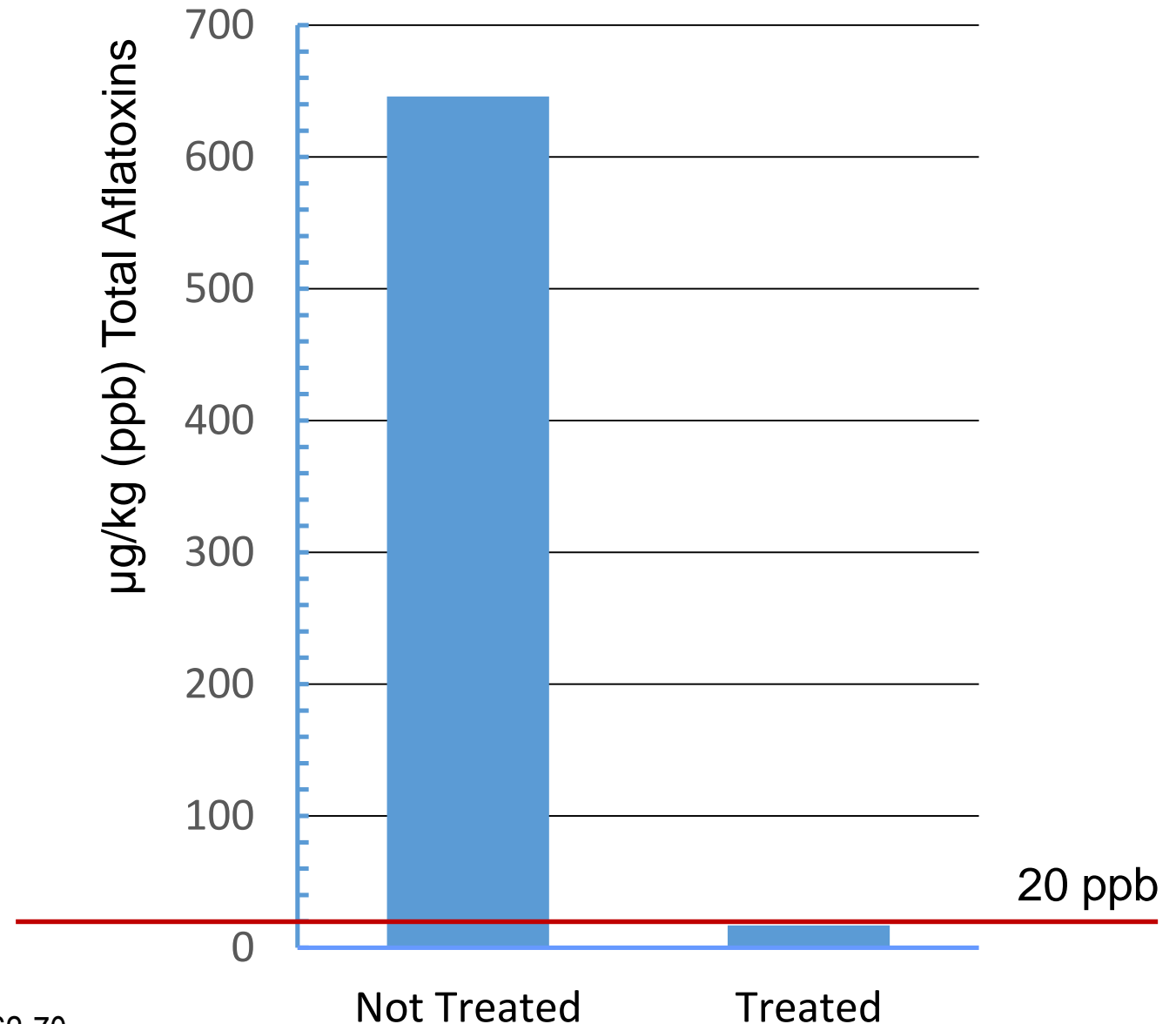
All Crops in an Area May Benefit From Applications

Atoxigenic Strains can be Applied Without Increasing Infection

and without increasing the overall quantity of *A. flavus* on the crop & throughout the environment

Atoxigenics stay with the crop from treatment,
.....through harvest, storage, and consumption.

Nigeria: Treated and control crops were harvested and stored under poor conditions for four months.



Biocontrol is Area-wide Management

Area-wide & Multi-year Aspects should be Optimized

Biocontrol fungi move from treated fields across areas

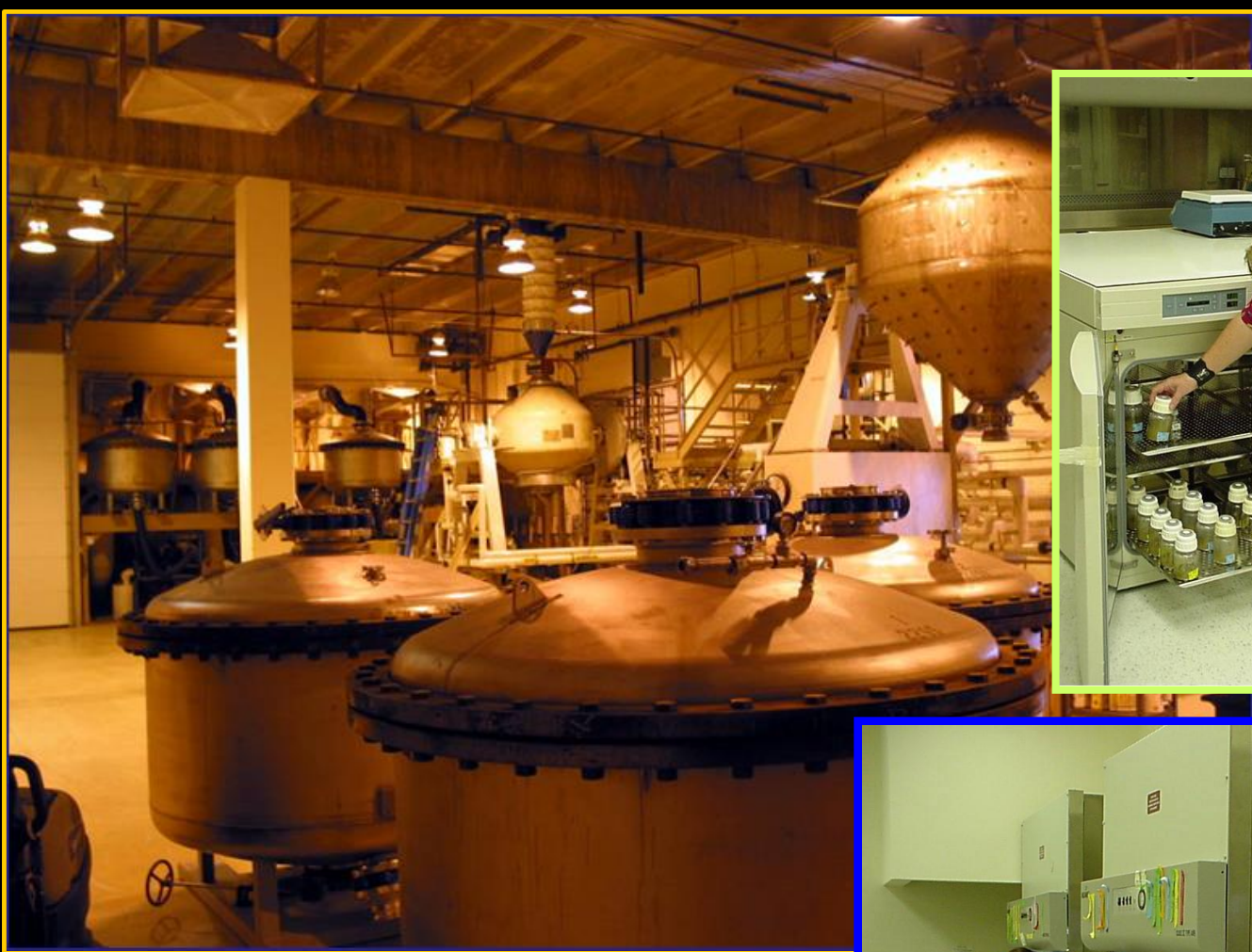
Atoxigenics persist in soils, on crop debris, on non-crop plants



Multiple Crops Benefit From the Same Biocontrol Product

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.





The partnership between commodity groups and USDA developed the first atoxigenic strain biocontrol product, registered it with EPA, and assembled the first manufacturing active from 1995 till present.