Aflatoxin and Ear Rots: Afla-Guard Biological Control Research

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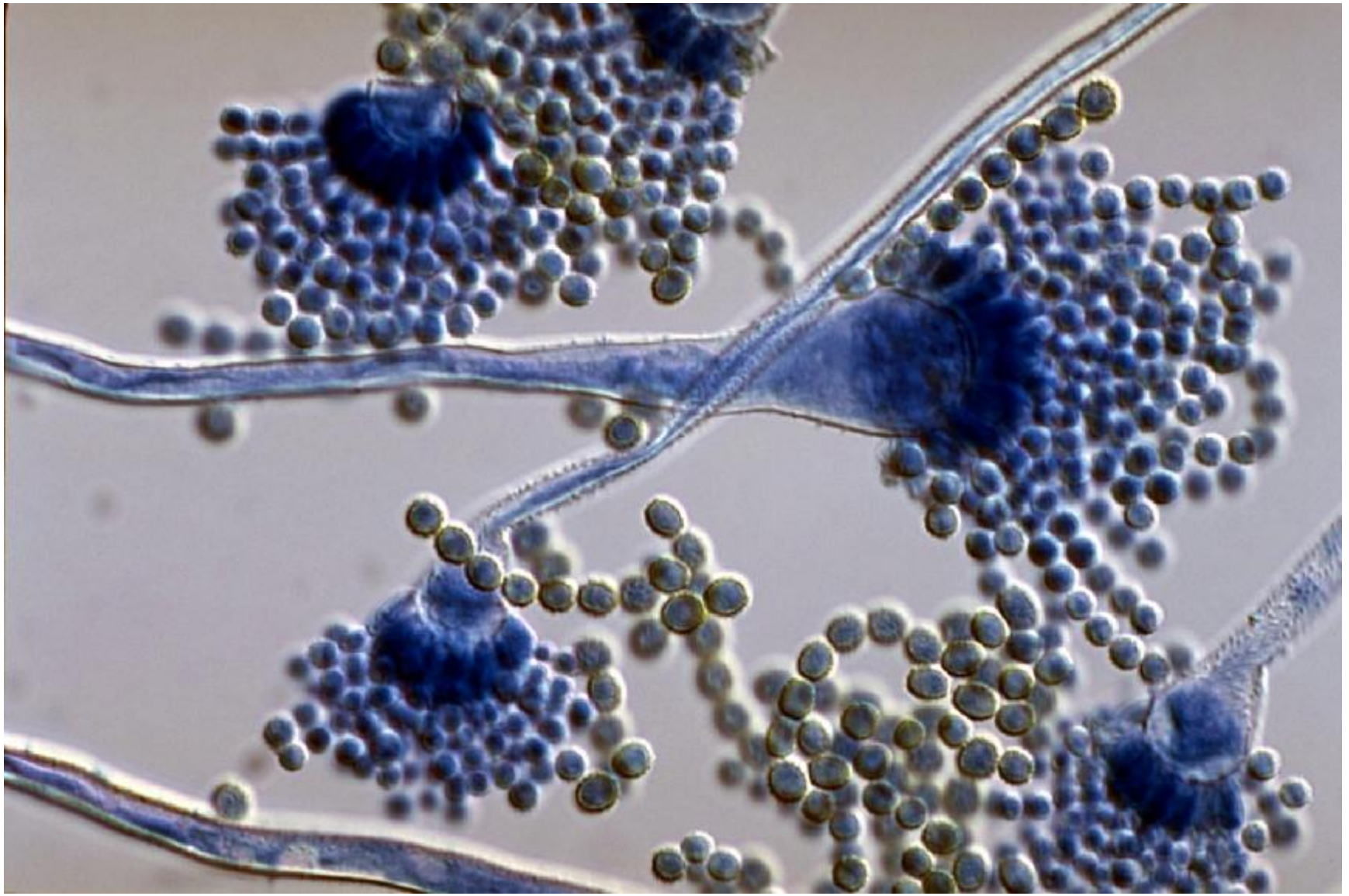
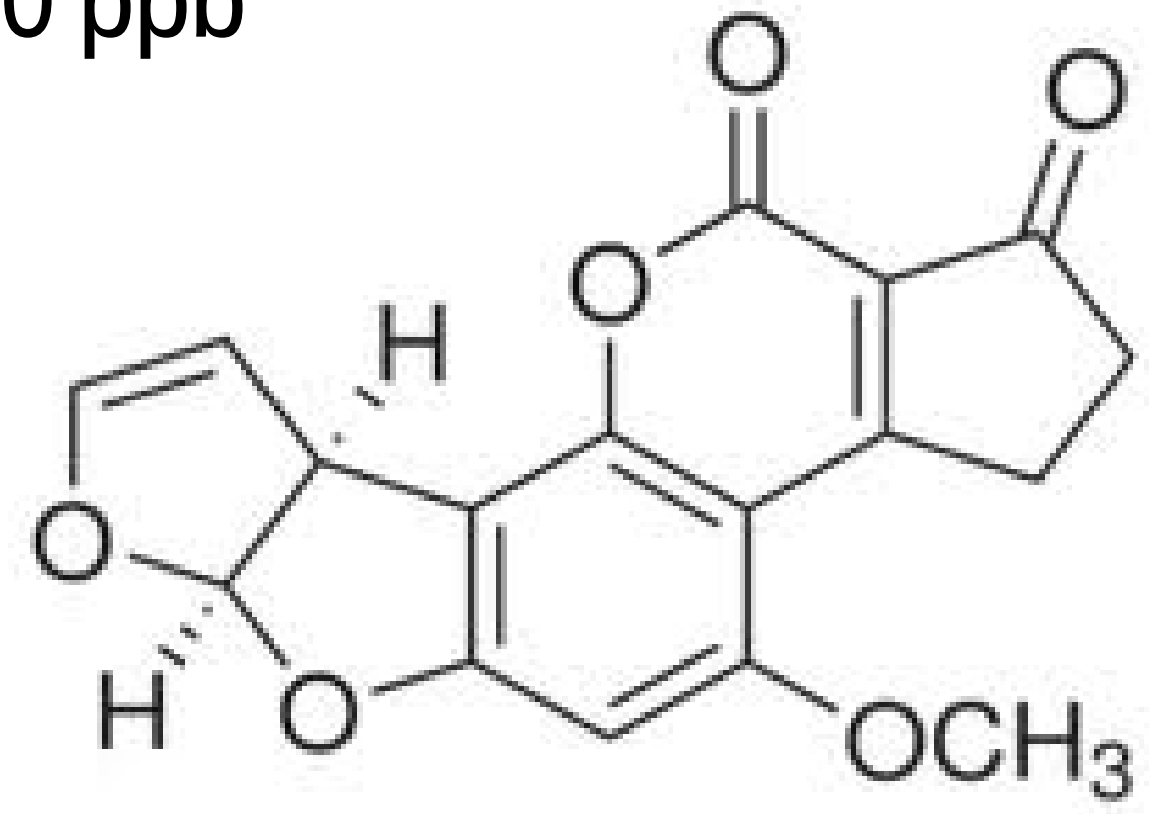
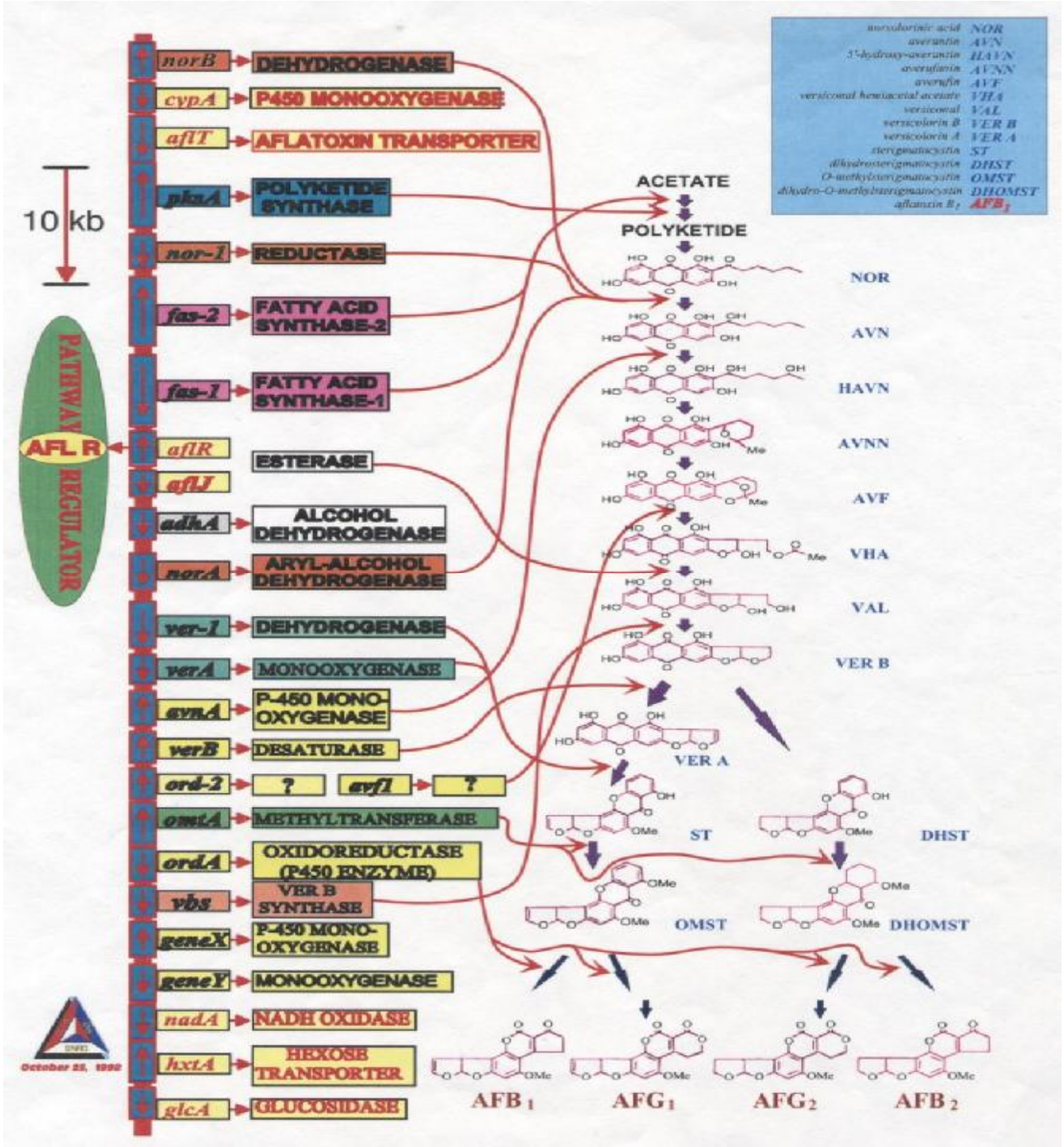


aflatoxin B1

20 ppb




10 kb
PATHWAY REGULATOR
AFL R
norB
DEHYDROGENASE
cypA
P450 MONOOXYGENASE
aflT
AFLATOXIN TRANSPORTER
pksA
POLYKETIDE SYNTHASE
nor-1
REDUCTASE
fas-2
FATTY ACID SYNTHASE-2
fas-1
FATTY ACID SYNTHASE-1
aflR
aflJ
ESTERASE
adhA
ALCOHOL DEHYDROGENASE
norA
ARYL-ALCOHOL DEHYDROGENASE
ver-1
DEHYDROGENASE
verA
MONOOXYGENASE
avnA
P-450 MONO-OXYGENASE
verB
DESATURASE
ord-2
?
avf1
?
omtA
METHYLTRANSFERASE
ordA
OXIDOREDUCTASE (P450 ENZYME)
vbs
VER B SYNTHASE
geneX
P-450 MONO-OXYGENASE
geneY
MONOOXYGENASE
nadA
NADH OXIDASE
hxtA
HEXOSE TRANSPORTER
glcA
GLUCOSIDASE
ACETATE
POLYKETIDE
NOR
AVN
HAVN
AVNN
AVF
VHA
VAL
VER B
VER A
ST
DHST
OMST
DHOMST
AFB1
AFG1
AFG2
AFB2
versicolorinic acid NOR
averantin AVN
5'-hydroxy-averantin HAVN
averufanin AVNN
averufin AVF
versiconal hemiacetal acetate VHA
versiconal VAL
versicolorin B VER B
versicolorin A VER A
sterigmatocystin ST
dihydrosterigmatocystin DHST
O-methylsterigmatocystin OMST
dihydro-O-methylsterigmatocystin DHOMST
aflatoxin B1 AFB1

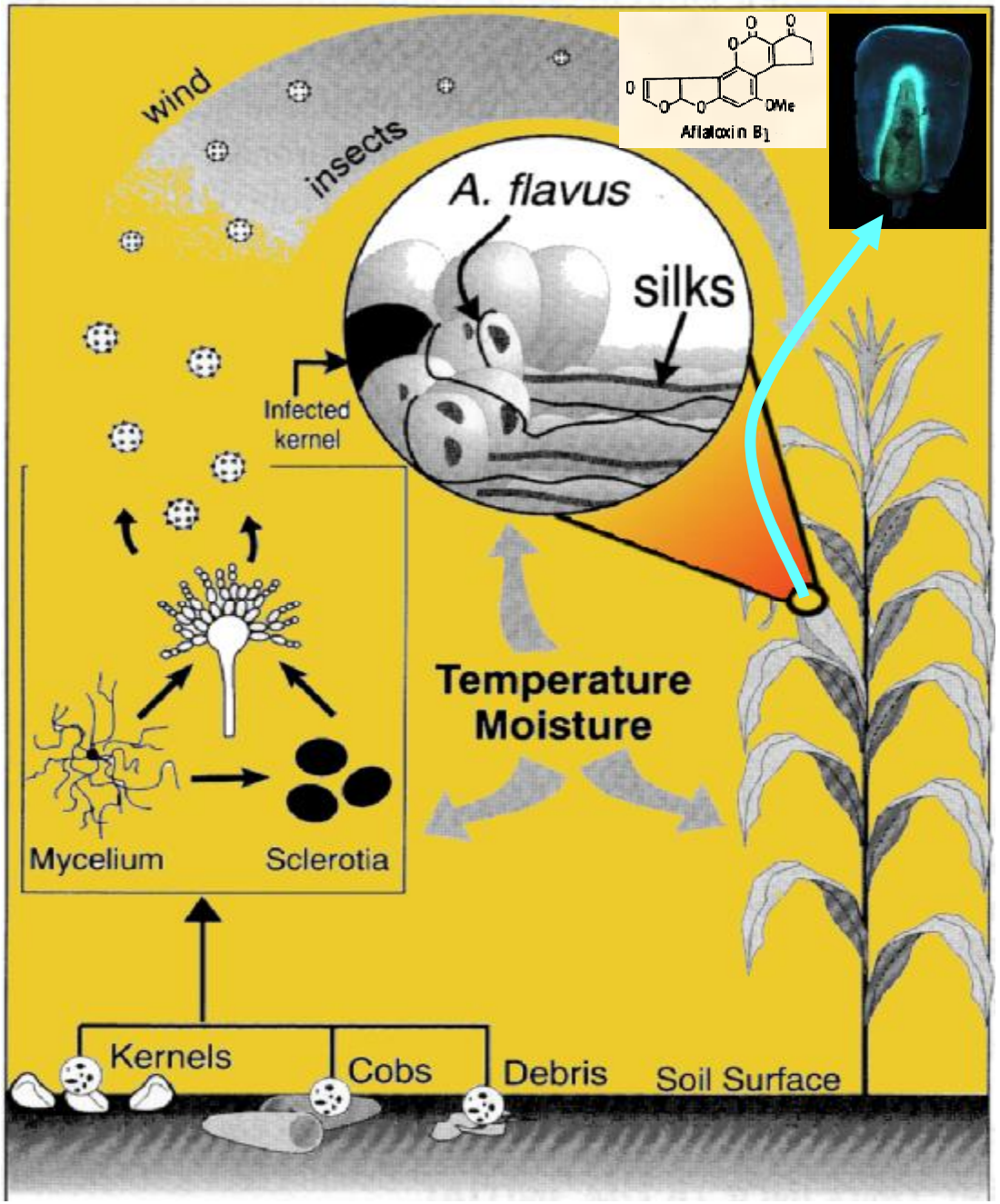


Photo: Gary Payne, N. C. State Univ.

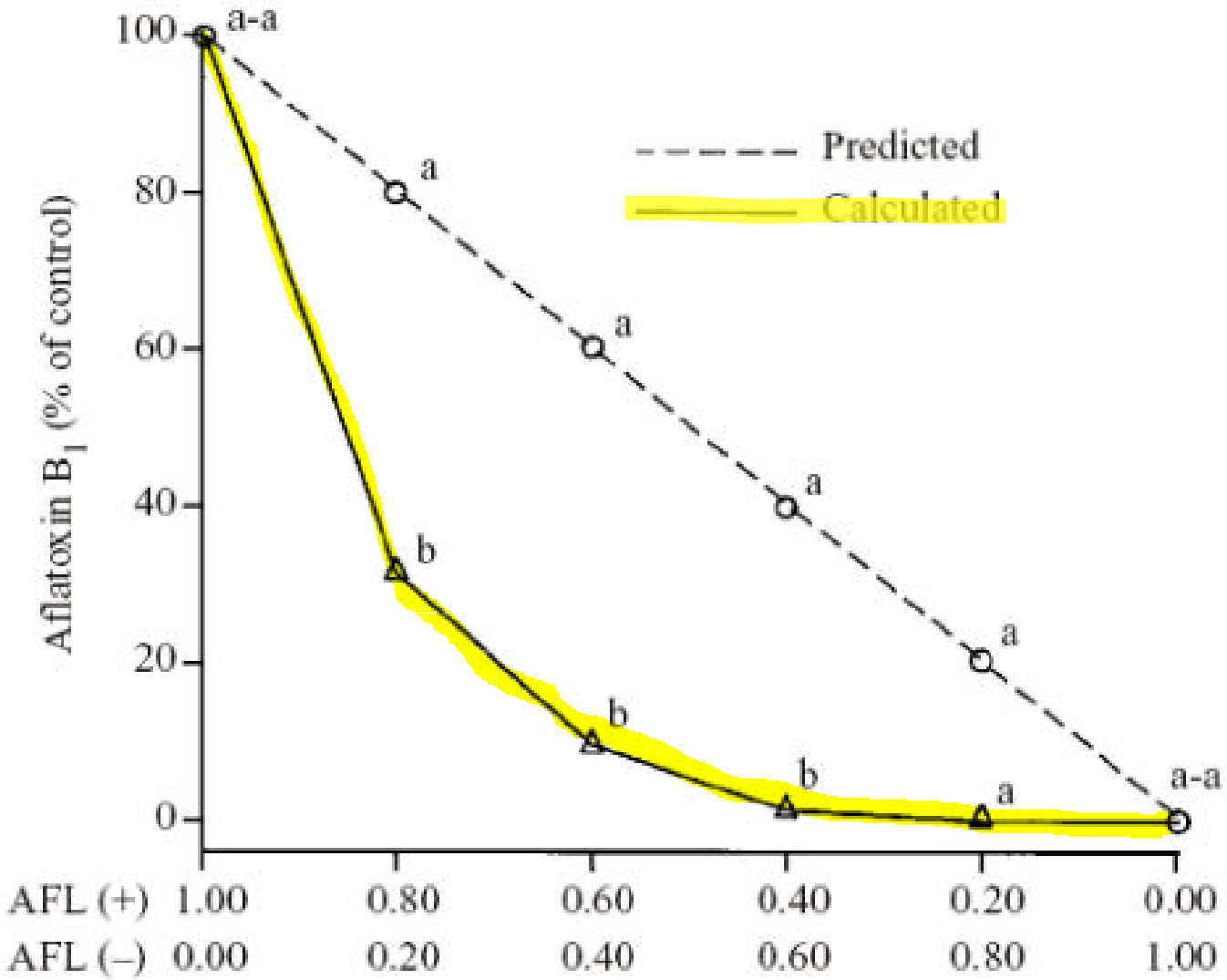
1. Research on the Mechanism of Biocontrol (intra-specific aflatoxin inhibition)

2. Pop. Biology of *A. flavus* in La. Corn Fields

3. Biocontrol Results Using Atoxigenic Strains

Suspended disc method to determine intraspecific aflatoxin inhibition.

Wicklow, *et al*, 2003. Effect of intraspecific competition by *Aspergillus flavus* on aflatoxin formation in suspended disc culture. Mycol. Res. 107:617-623.



Effect of atoxigenic isolates of *Aspergillus flavus* on toxin production using the suspended disc assay. There is a discrepancy between the observed and expected results (Wicklow *et al*, 2003).

Effect of atoxigenic isolates on toxin production by 53 (Jha, A., *et al*, 2005)

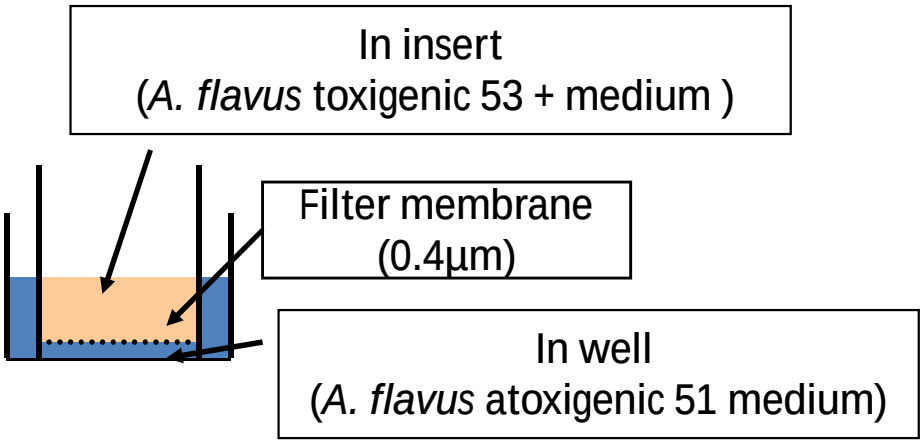
Isolate #	50:50 (ppb AFB1)	80:20 (ppb AFB1)
1	1396	112
3	17	8
4	7	50
13	8	32
14	0	73
15	125	242
16	15	218
17	5	244
18	0	1
19	1	152
20	615	502
21	11	22
22	0	304
23	1	115
25	18	397
26	43	484
27	2	136
28	20	80
29	42	199
30	104	321

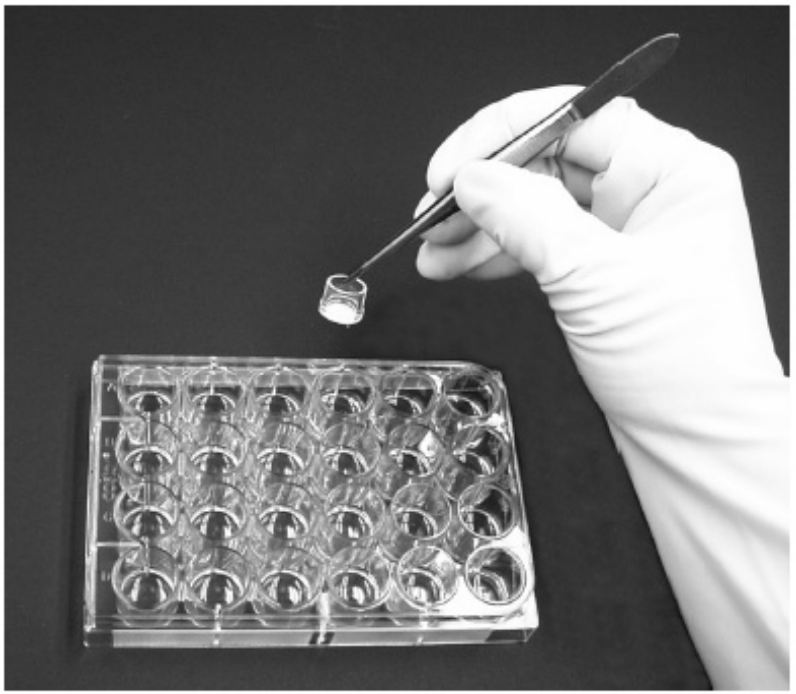
Isolate #	50:50 (ppb AFB1)	80:20 (ppb AFB1)
31	21	298
32	20	0
33	34	0
34	105	21
35	136	24
36	387	60
37	29	34
38	108	10
39	395	1
40	78	5
41	0	1
42	0	0
43	0	0
45	0	0
46	0	0
47	8	0
48	0	0
49	0.3	0
50	0	0
51	0	0
52	0	0

Distribution of completely and highly inhibitory isolates across vegetative compatibility groups.

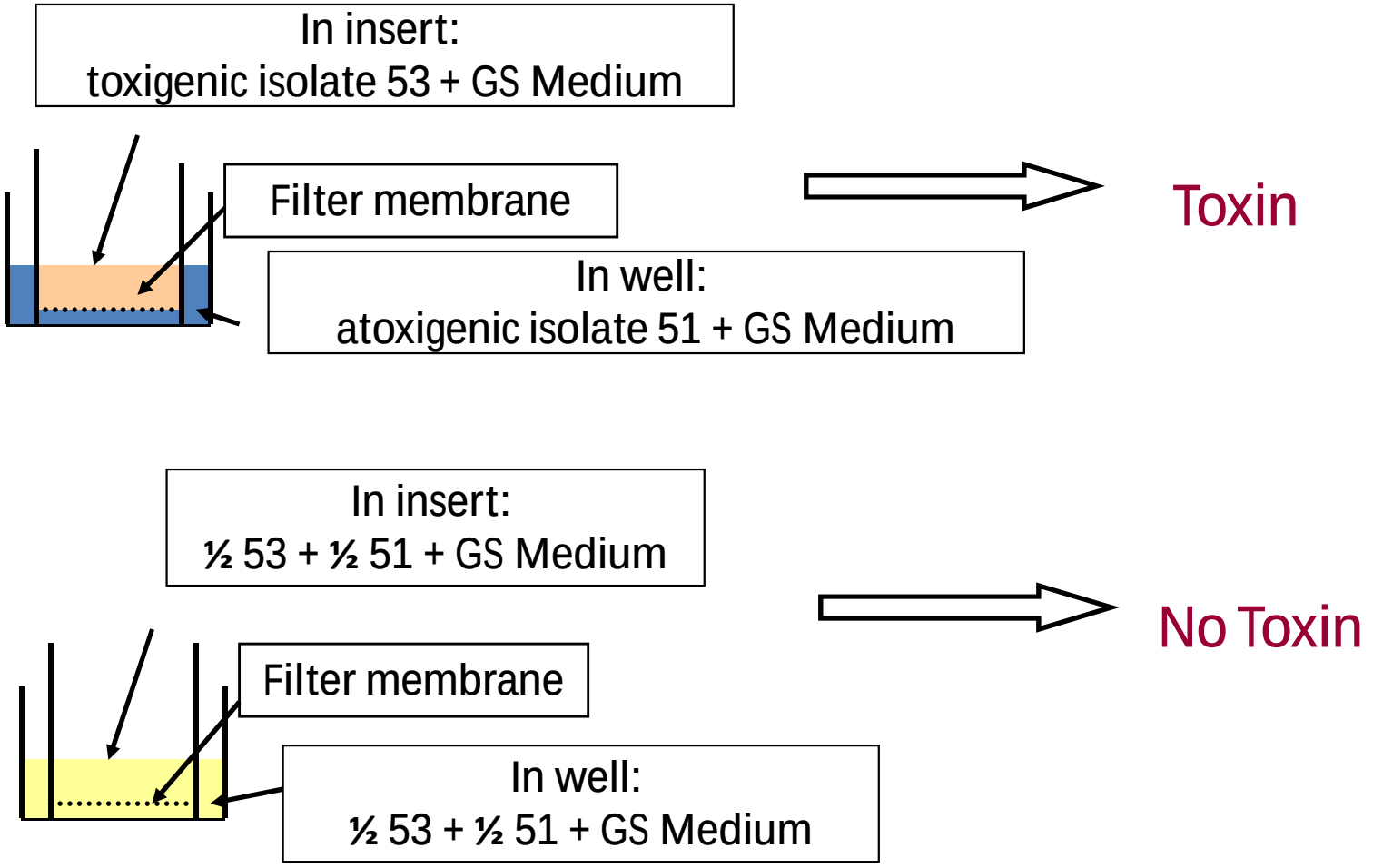
A	B(**53**)	C	Other
45	**50**	**42**	**48**
18	**51**	**43**	**52**
		46	
		41	
		47	
		49	

Filter insert- plate well system





Janisiewicz *et. al.,* 2000. Characterizing the mechanism of biological control of postharvest disease on fruits with a simple method to study competition for nutrients. Phytopathology 90:1196-1200.


In insert:
toxigenic isolate 53 + GS Medium
Filter membrane
In well:
atoxigenic isolate 51 + GS Medium
Toxin
In insert:
½ 53 + ½ 51 + GS Medium
Filter membrane
In well:
½ 53 + ½ 51 + GS Medium
No Toxin

Conclusions:

* Touching or physical interaction is required for the intraspecific inhibition of toxin production.

* Nutrient competition nor soluble signals can explain toxin inhibition mechanism by atoxigenic isolates.

Hypothesis:
A role for touching or physical interaction can be confirmed by letting the fungus pass the membrane.
This should result in toxin inhibition.

- The diameter of *A. flavus* conidia and hyphae is between 3.5-7.0 µm

The effect of the pore size on toxin inhibition in separated culture system

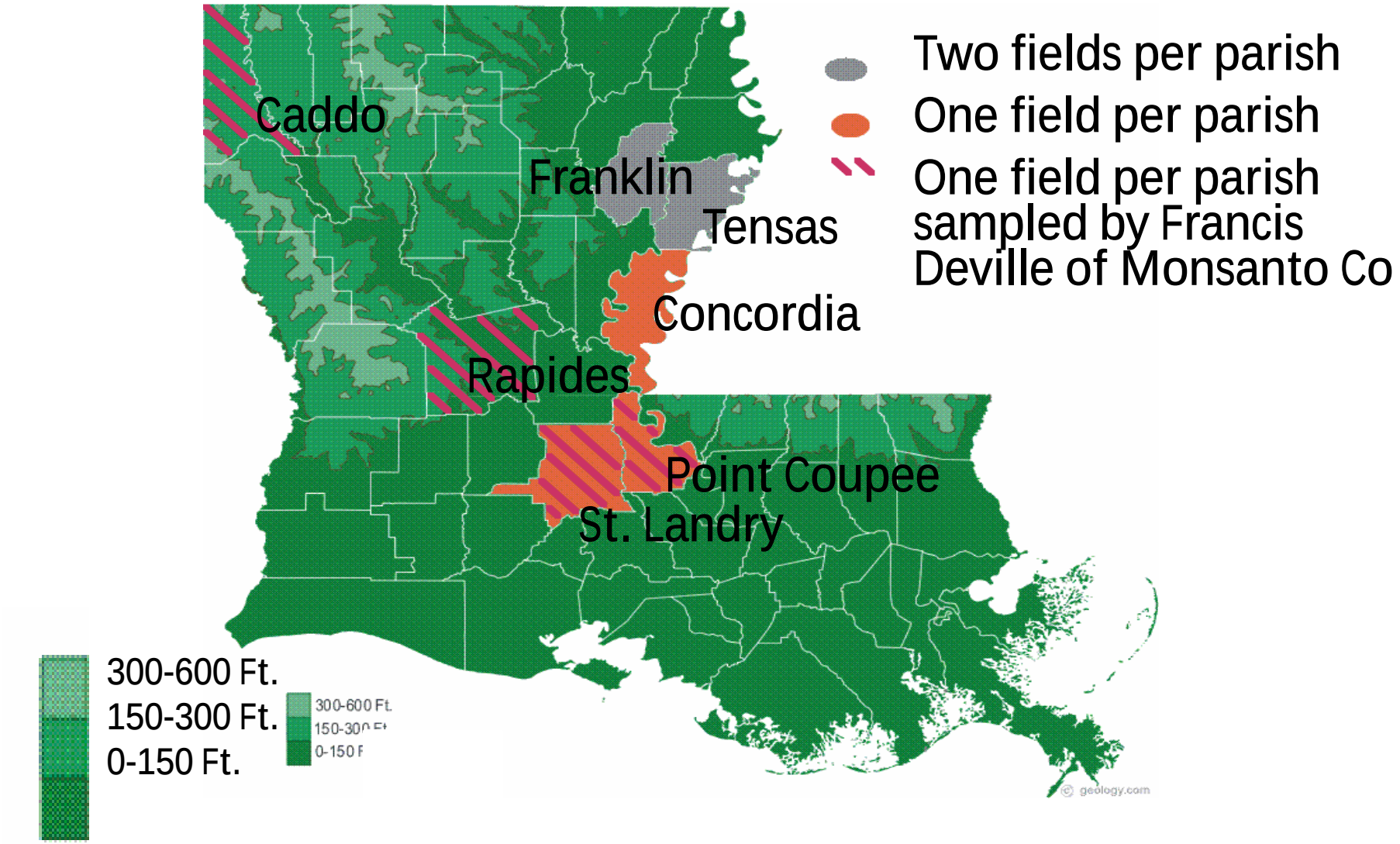
treatment	pore size	membrane material	plate material	mean amount of B1 (ppb)± SD
Check (53/0.01%TX)	0.4 µm	Hydrophilic PTFE	Polystyrene	605.93±45.78 **a**
	74 µm	Polyester Mesh	Polystyrene	399.40±111.79 **bc**
	200 µm	Polyester Mesh	Polystyrene	528.02±80.38 **ab**
Separate (53/51)	0.4µm	Hydrophilic PTFE	Polystyrene	507.54±87.49 **ab**
	0.4µm	Polycarbonate	Polystyrene	586.10±44.52 **a**
	3µm	Polycarbonate	Polystyrene	511.34±115.7 **ab**
	12µm	Polycarbonate	Polystyrene	317.59±102.09 **c**
	74µm	Polyester Mesh	Polystyrene	1.82±1.65 **d**
	200µm	Polyester Mesh	Polystyrene	0.02±0.02 **d**
Together (53+51/53+51)	0.4µm	Hydrophilic PTFE	Polystyrene	10.65±2.18 **d**
	74µm	Polyester Mesh	Polystyrene	0.08±0.1 **d**
	200µm	Polyester Mesh	Polystyrene	0.01±0.01 **d**

Conclusions:

- Inhibitory ability is VCG independent.
- Competition for nutrients nor soluble inhibitor signal molecules can explain intraspecific toxin inhibition in *Aspergillus flavus.*
- Touch or physical interaction is needed in toxin inhibition which initiates an unknown signaling pathway to prevent toxin synthesis.
- There is specificity in intraspecific toxin inhibition.
- Window of sensitivity in 53 to 51 inhibition is open during the first 24 hrs.
- Inhibitory competence of 51 is always on.

Huang, Jha, Sweany, DeRobertis, Damann. (2011) Intraspecific aflatoxin inhibition in *Aspergillus flavus* is thigmoregulated, independent of vegetative compatibility group and is strain dependent. PLoS ONE 6(8):e23470 doi:10.1371/journal.pone.0023470

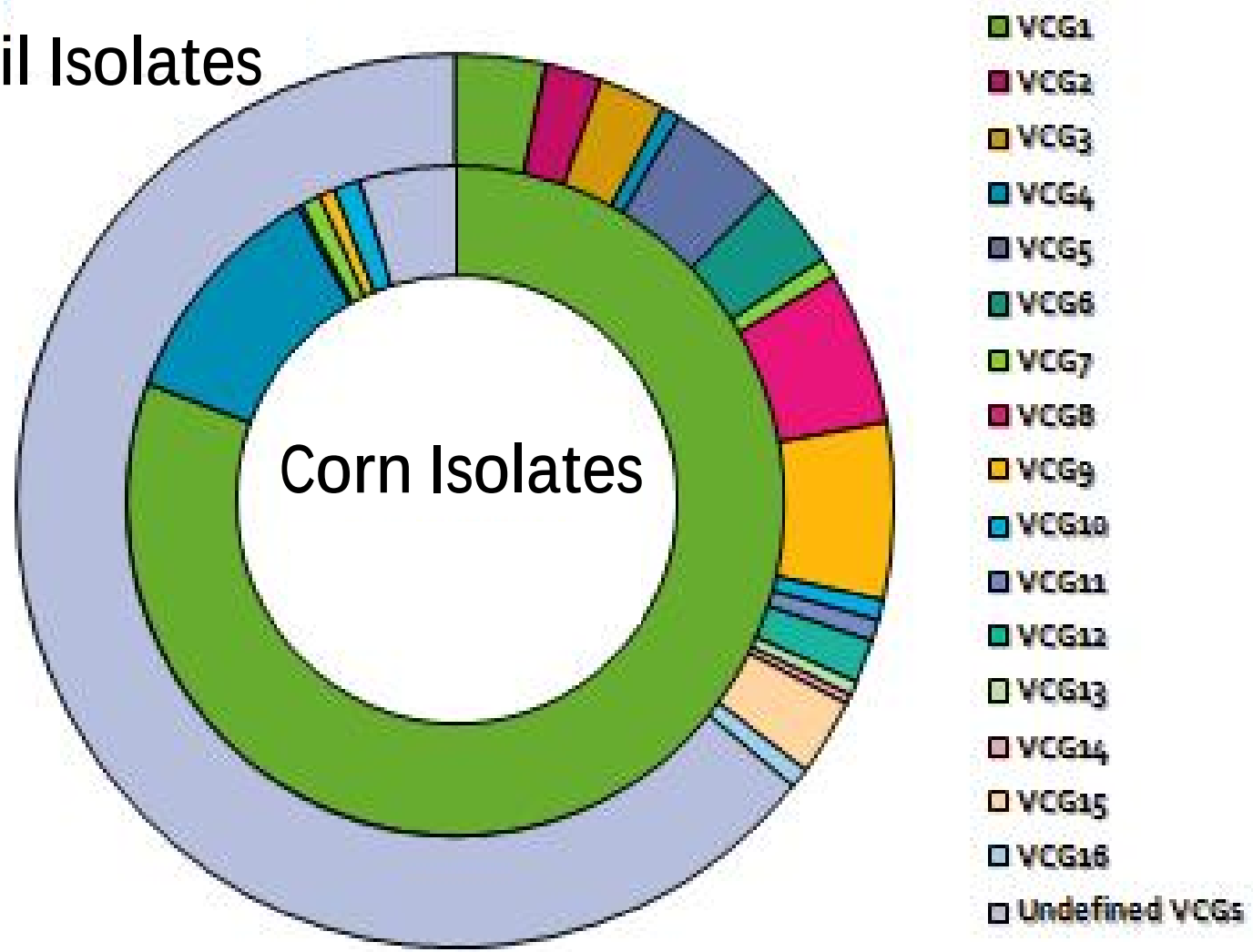
POPULATION BIOLOGY OF *A. flavus*
11 Field Locations:10 ear & 5 soil/ site



The fungus is soil-borne and a saprophyte.

Is there a subset of strains of the fungus in the soil which go to corn (parasitic) and another subset which does not (saprophytic only) appear in corn?

Different mean proportion of corn and soil isolates in 16 VCGs



Sweany, Damann, Kaller. 2011. Comparison of soil and corn kernel *Aspergillus flavus* populations: evidence for niche specialization. Phytopathology 101:952-959.

Aflatoxin B1 production (ppb) on rice by the 2007 *A. flavus* isolates by VCG and niche: ( ) #of isolates; #=locations/11

	(Soil isolates)			(Kernel isolates)			Mating Type
Vcg1	3.3+/-0.7	(4)	2	4.5+/- 2.8	(483)	11	2
Vcg4	22,565+/-3,789	(5)	2	28,868+/- 9,016	(56)	11	2
Vcg5	0	(1)	1	19,137+/-6,927	(10)	2	1
Vcg7	3.2+/-1.2	(10)	1	8.8+/-7.4	(2)	2	1
Vcg9	1,589+/-404	(2)	2	6,261+/-8,159	(14)	2	1
Vcg10	0	(10)	1	2.6+/-0.2	(2)	1	2

			Mating Type	
Vcg2	25,589+/-6,821	(29)	2	2
Vcg3	15,116+/-6,319	(6)	4	1
Vcg6	2.8 +/-1.8	(5)	2	2
Vcg8	13,602+/-12,479	(14)	4	1
Vcg11	10,372+/-1,133	(2)	2	2
Vcg12	25,961+/-21,644	(4)	3	2
Vcg13	9.5+/-6.0	(3)	1	1
Vcg14	2.5+/-0.1	(2)	2	2
Vcg15	21,320+/-6,856	(3)	2	2
Vcg16	10,273+/-63	(2)	1	2

Not all strains in the soil end up infecting corn.

The presence of VCG 1 in ~80% and VCG 4 in ~10% of the kernel isolates from all 11 locations and the low toxin (<5ppb) suggests that VCG 1 is a natural biocontrol strain which protects against VCG 4 (>28,000ppb) and other high toxin producing strains.

Looking at non-toxigenic VCG 1 isolates as potential biocontrol strains.

We have had many years since the 1998 corn aflatoxin contamination when the environmental conditions conducive to contamination were as severe or more severe than 1998 yet the contamination has not occurred.

Why?

Possible that the high toxin infective strains are not indigenous to the fields where the corn is infected. That is they fly in from elsewhere?

ALIENS

To have a bad year we need:

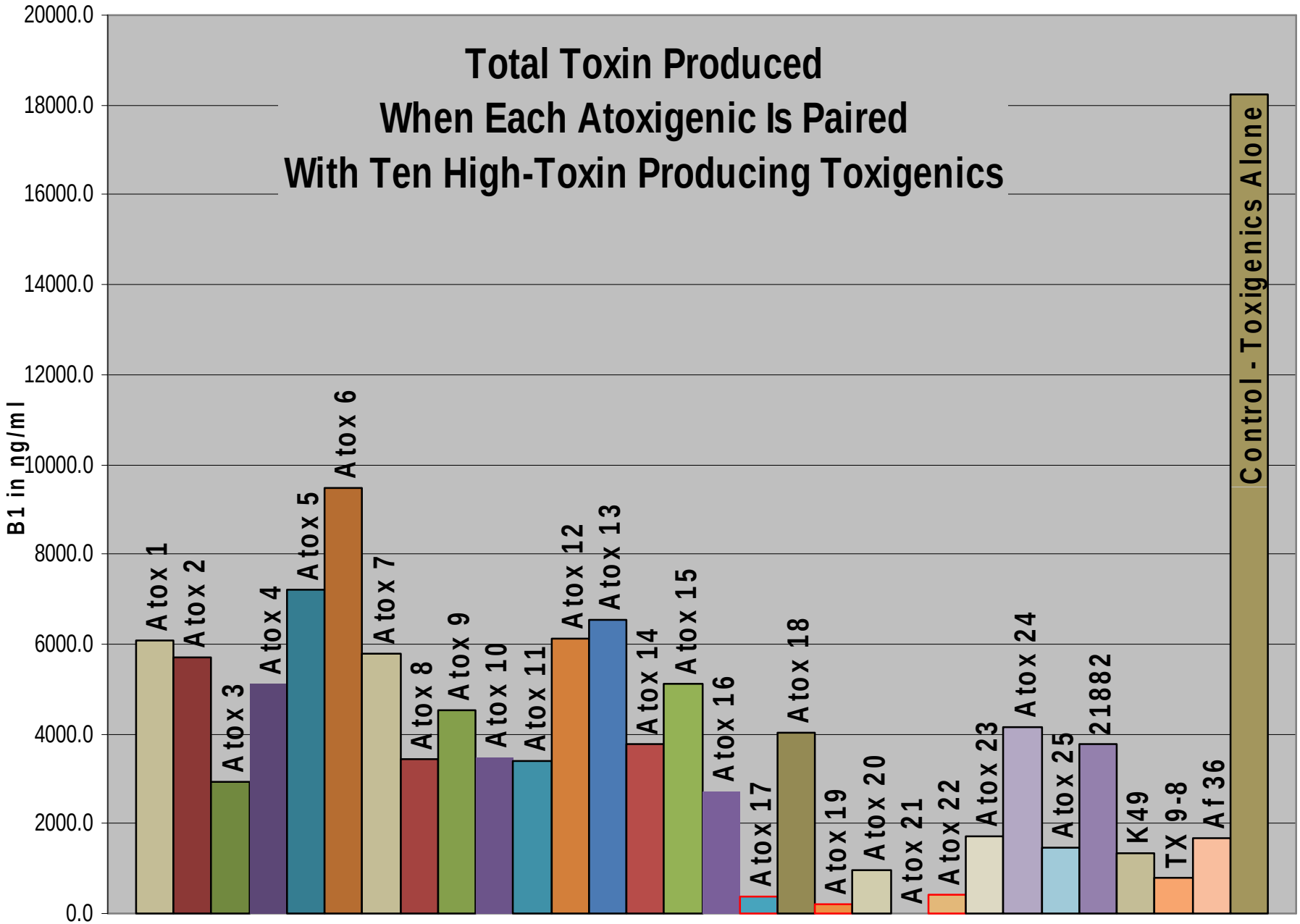
1) conducive stressful environment (hot & dry),
2) highly infective high toxin producing strains,
3) and no indigenous natural biocontrol strains.

What do those bad strains look like?
What are their characteristics?

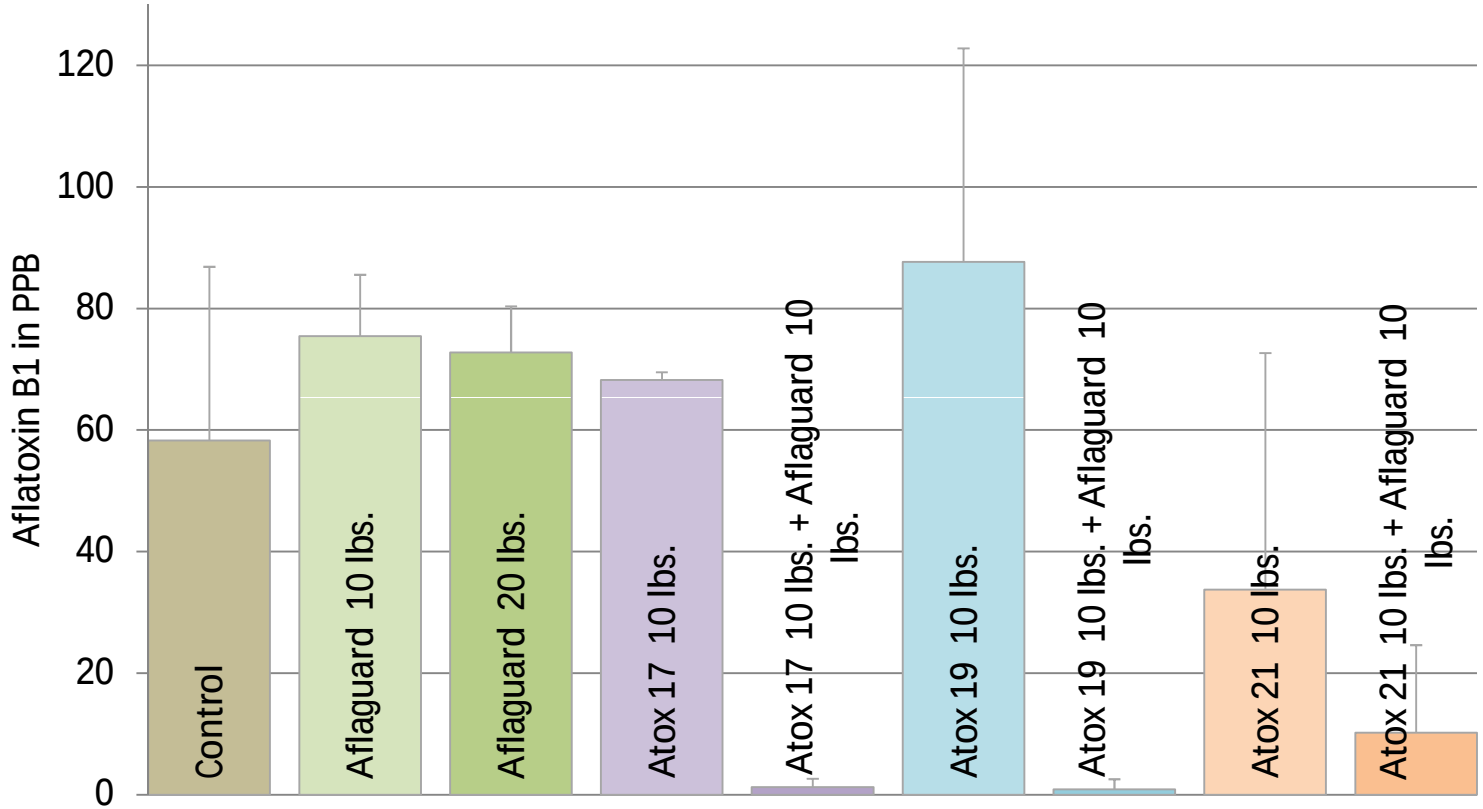
How do we determine their characteristics?

What about Biocontrol Results!

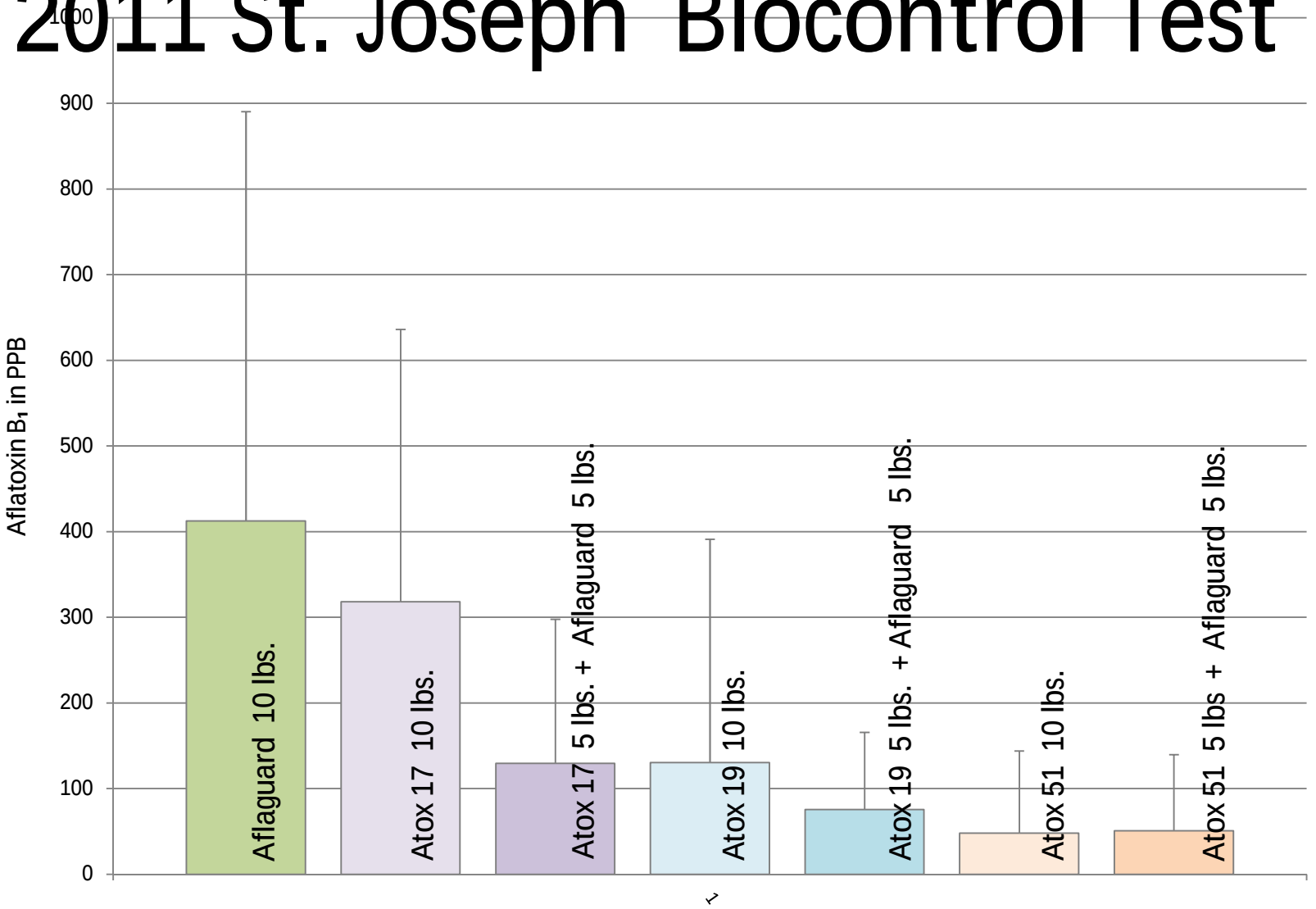
Aspergillus flavus on sterile wheat seed used as inoculum of bio-control and toxic isolates

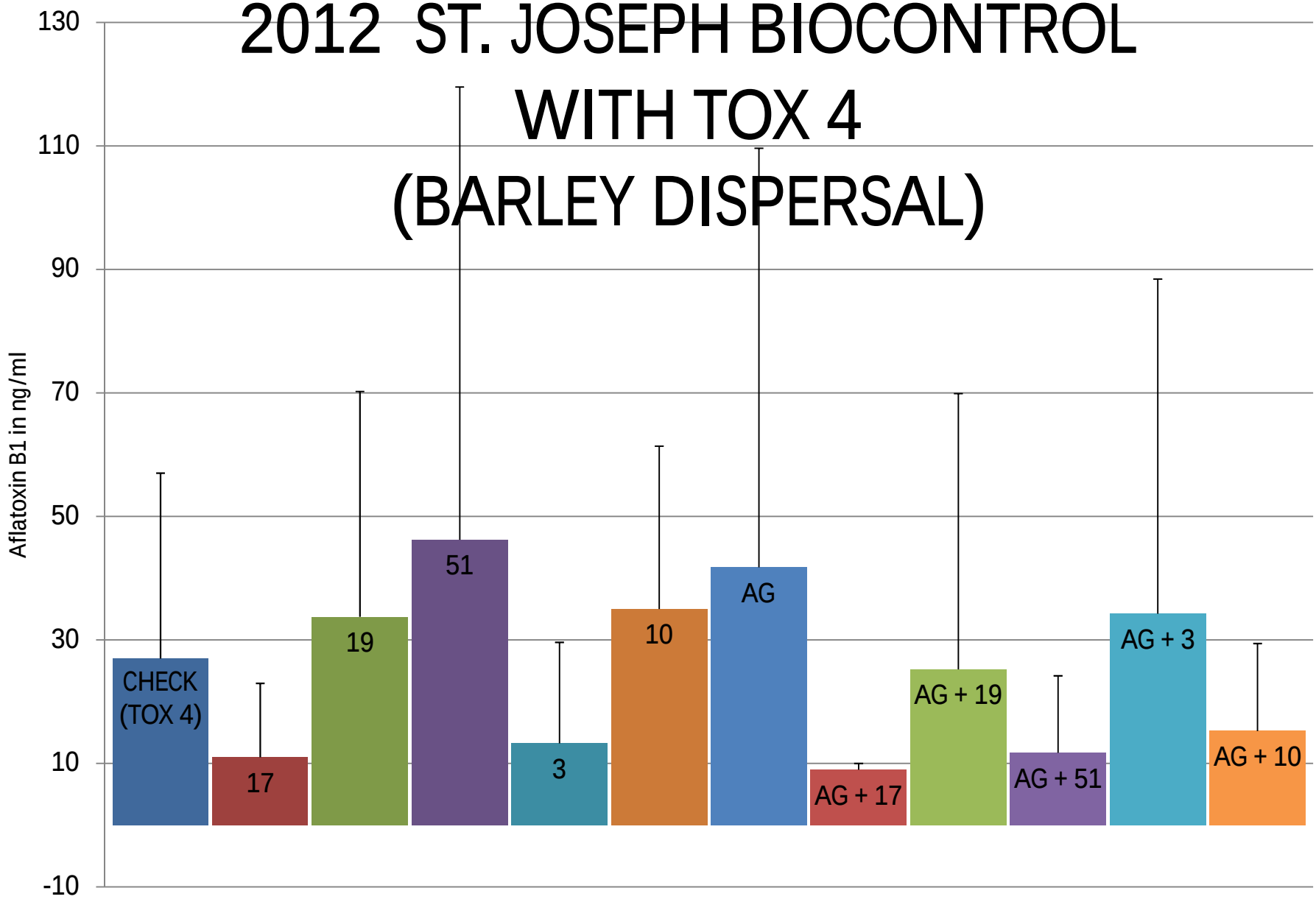

Total Toxin Produced
When Each Atoxigenic Is Paired
With Ten High-Toxin Producing Toxigenics
B1 in ng/ml
20000.0
18000.0
16000.0
14000.0
12000.0
10000.0
8000.0
6000.0
4000.0
2000.0
0.0
Atox 1
Atox 2
Atox 3
Atox 4
Atox 5
Atox 6
Atox 7
Atox 8
Atox 9
Atox 10
Atox 11
Atox 12
Atox 13
Atox 14
Atox 15
Atox 16
Atox 17
Atox 18
Atox 19
Atox 20
Atox 21
Atox 22
Atox 23
Atox 24
Atox 25
21882
K49
TX 9-8
Af 36
Control - Toxigenics Alone

2010 St. Joseph Biocontrol Test

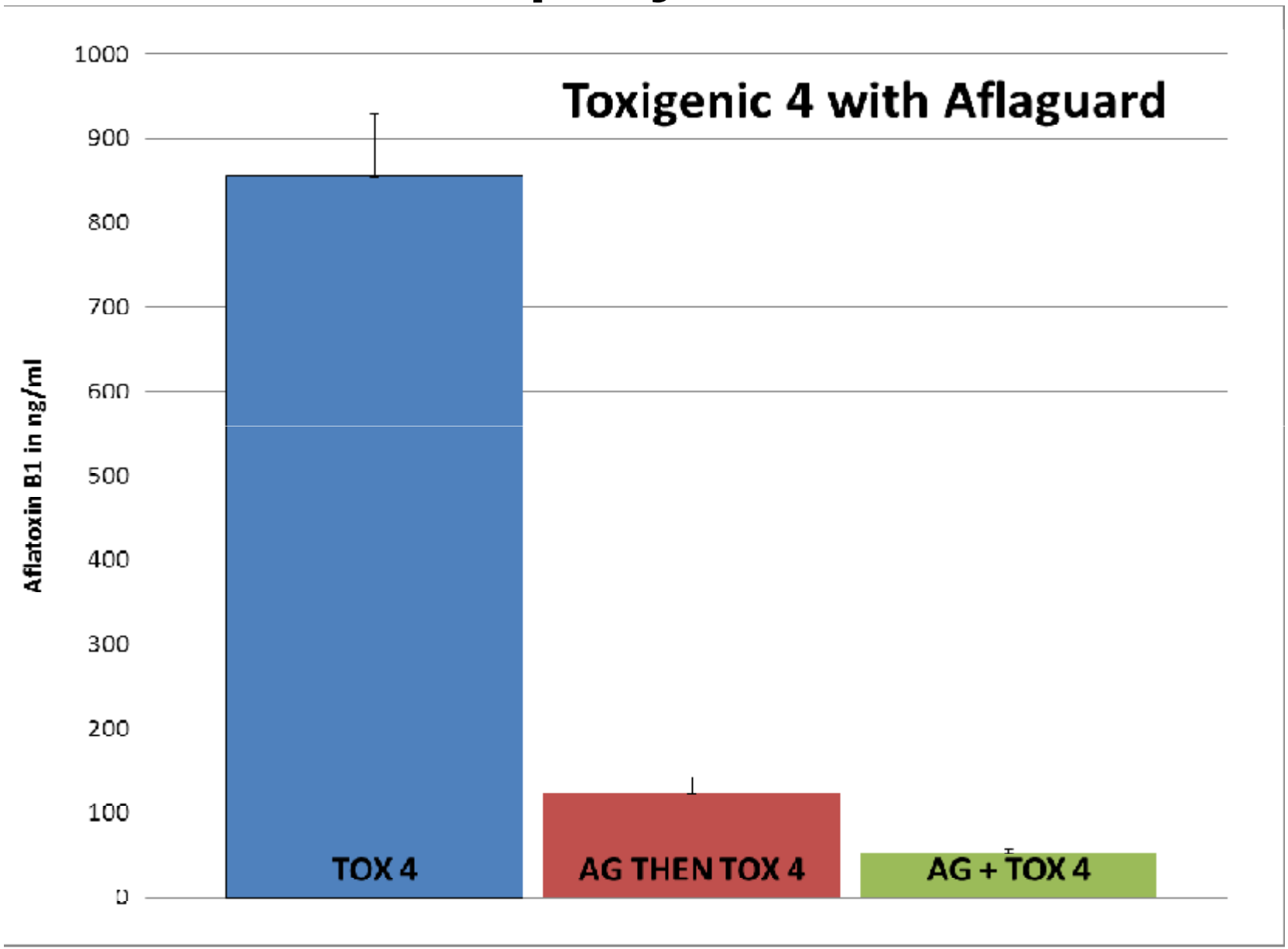


2011 St. Joseph Biocontrol Test




2012 ST. JOSEPH BIOCONTROL WITH TOX 4 (BARLEY DISPERSAL)
Aflatoxin B1 in ng/ml
130
110
90
70
50
30
10
-10
CHECK (TOX 4)
17
19
51
3
10
AG
AG + 17
AG + 19
AG + 51
AG + 3
AG + 10

Afla-Guard Spray –Ben Hur 2012



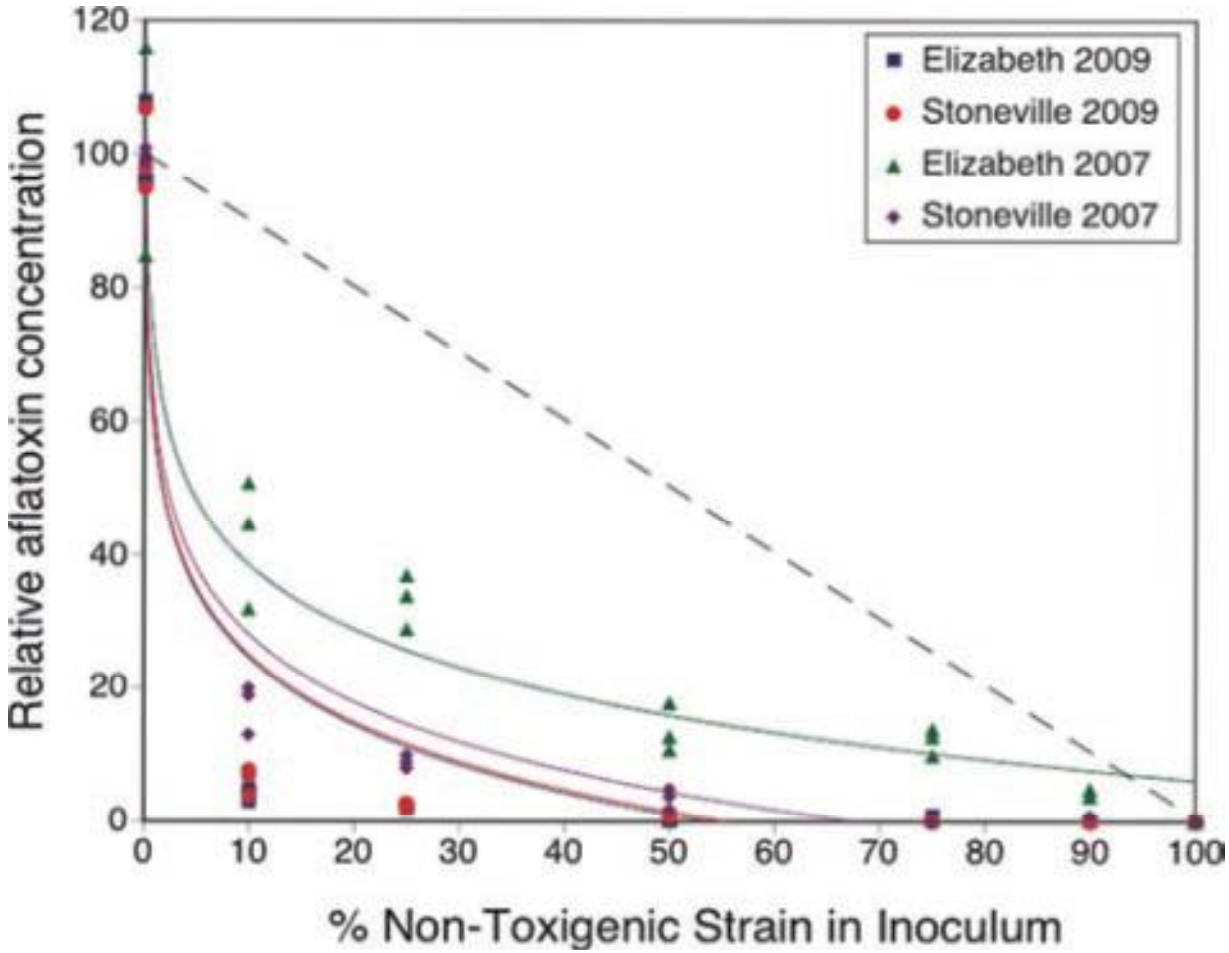


Figure 3. Effect on aflatoxin levels in harvested corn kernels after co-inoculating corn ears by the pin-bar method with varying ratios of non-aflatoxigenic *Aspergillus flavus* strain K49 and aflatoxigenic strain F3W4 for two years at two field sites. Aflatoxin values were normalized to 100 for the maximum observed aflatoxin concentration for each site-year. The dashed line indicates the anticipated value based on an equally competitive, two-strain model.

Abbas, H.K.; Weaver, M.A.; Horn, B.W.; Carbone, I.; Manacell, J.T.; Shier, W.T. Selection of *Aspergillus flavus* isolates for biological control of aflatoxins in corn. *Toxin Reviews* **2011,***30,*59-70

Aspergillus flavus disease/ biocontrol cycle on corn

Parasitic biocontrol = "competitive inclusion" or "competitive phenotype conversion" by "touch inhibition" or intraspecific aflatoxin inhibition by thigmo-down regulation of aflatoxin synthesis which is strain specific.

Saprophytic biocontrol = "competitive exclusion" by lowering inoculum potential is epidemiology based. This provides sustainability.

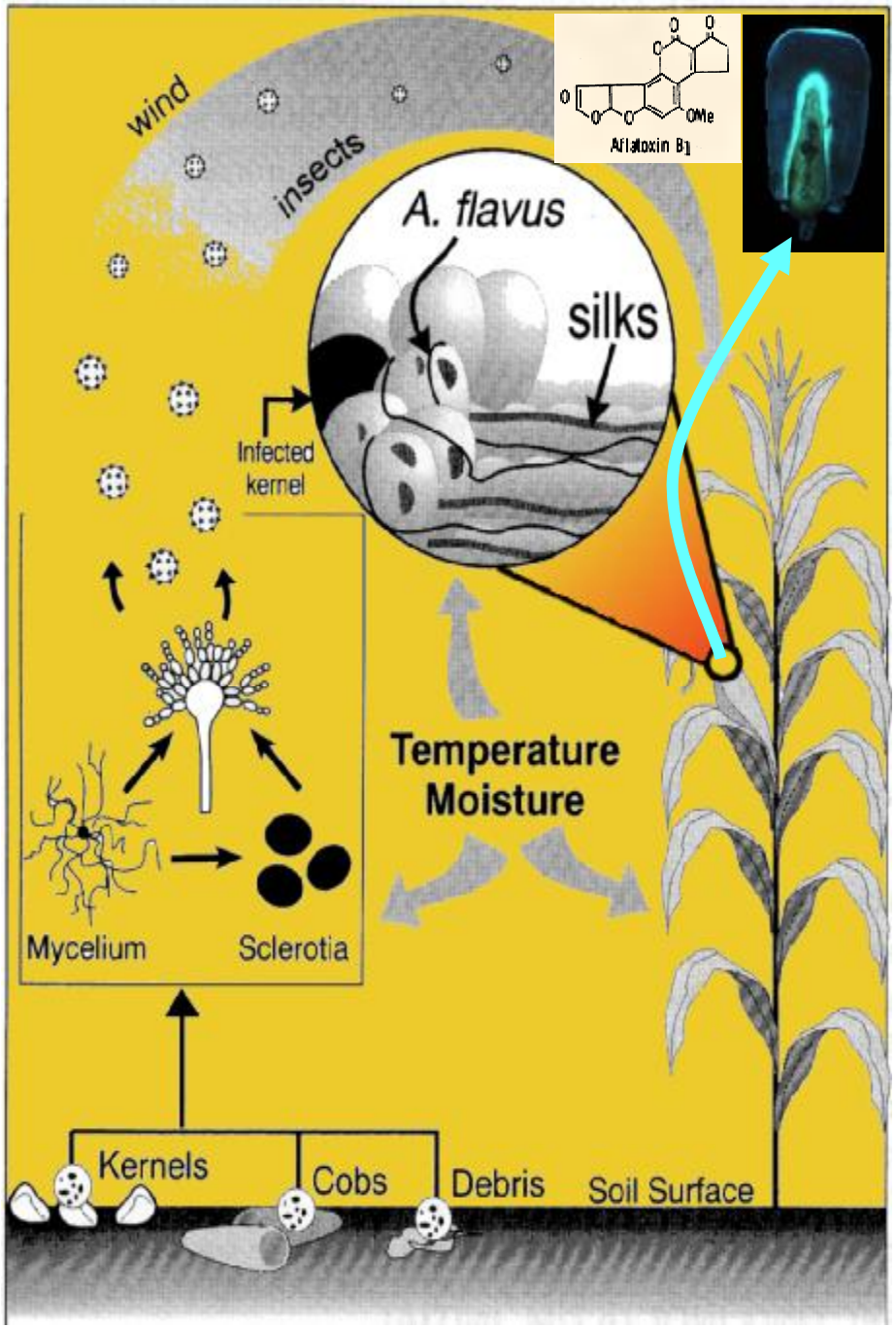
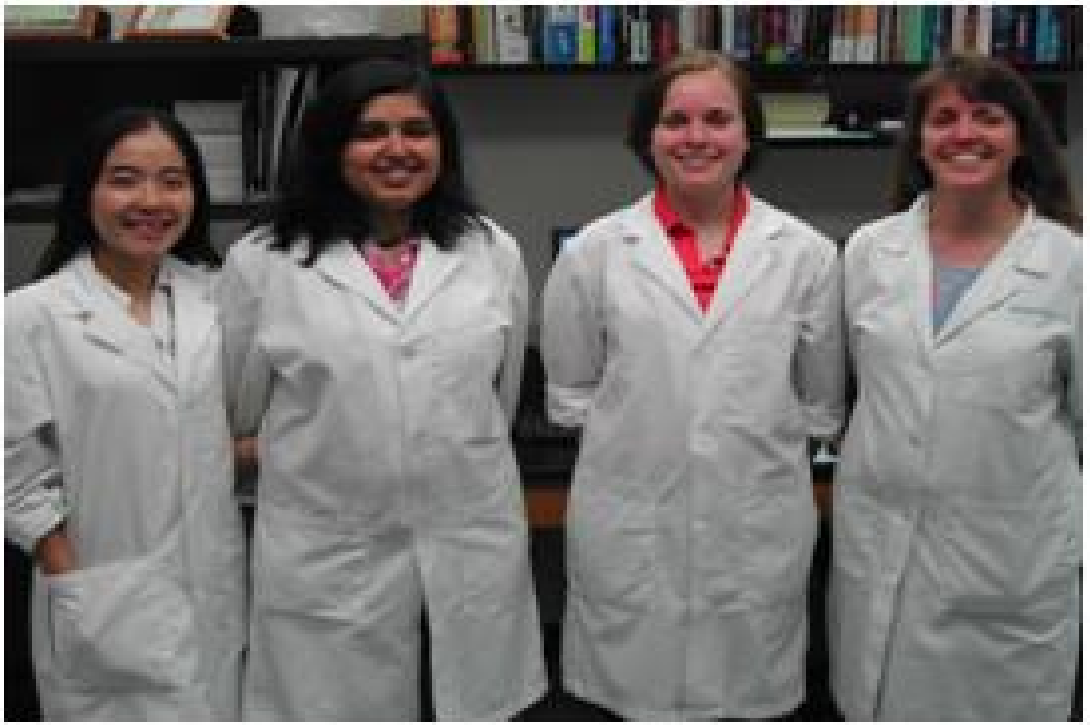


Photo: Gary Payne, N. C. State Univ.

Acknowledgement



Louisiana Soybean & Grain Research & Promotion Board,
Syngenta Crop Protection, NCGA & LSU AgCenter

The Mission:
To Find the Best Atoxigenic *Aspergillus flavus* Biocontrol for Louisiana