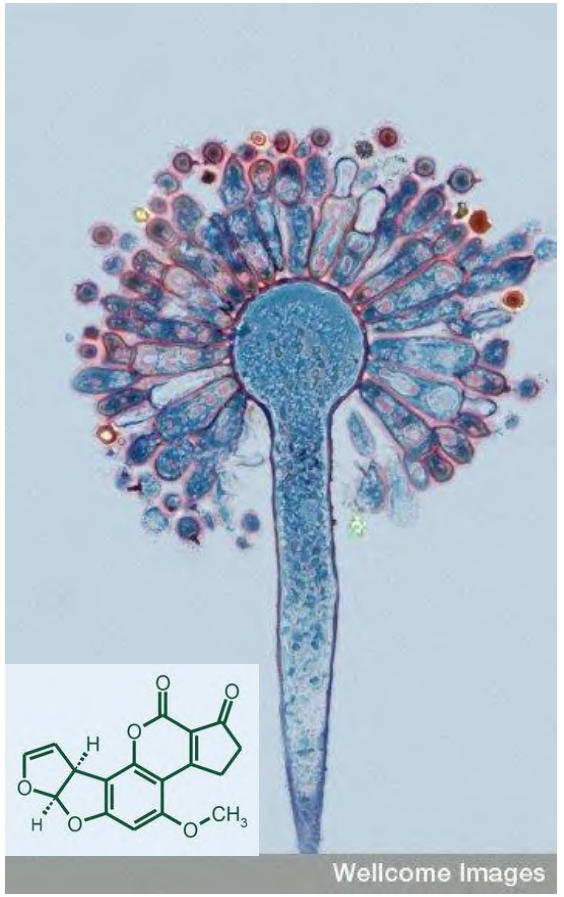
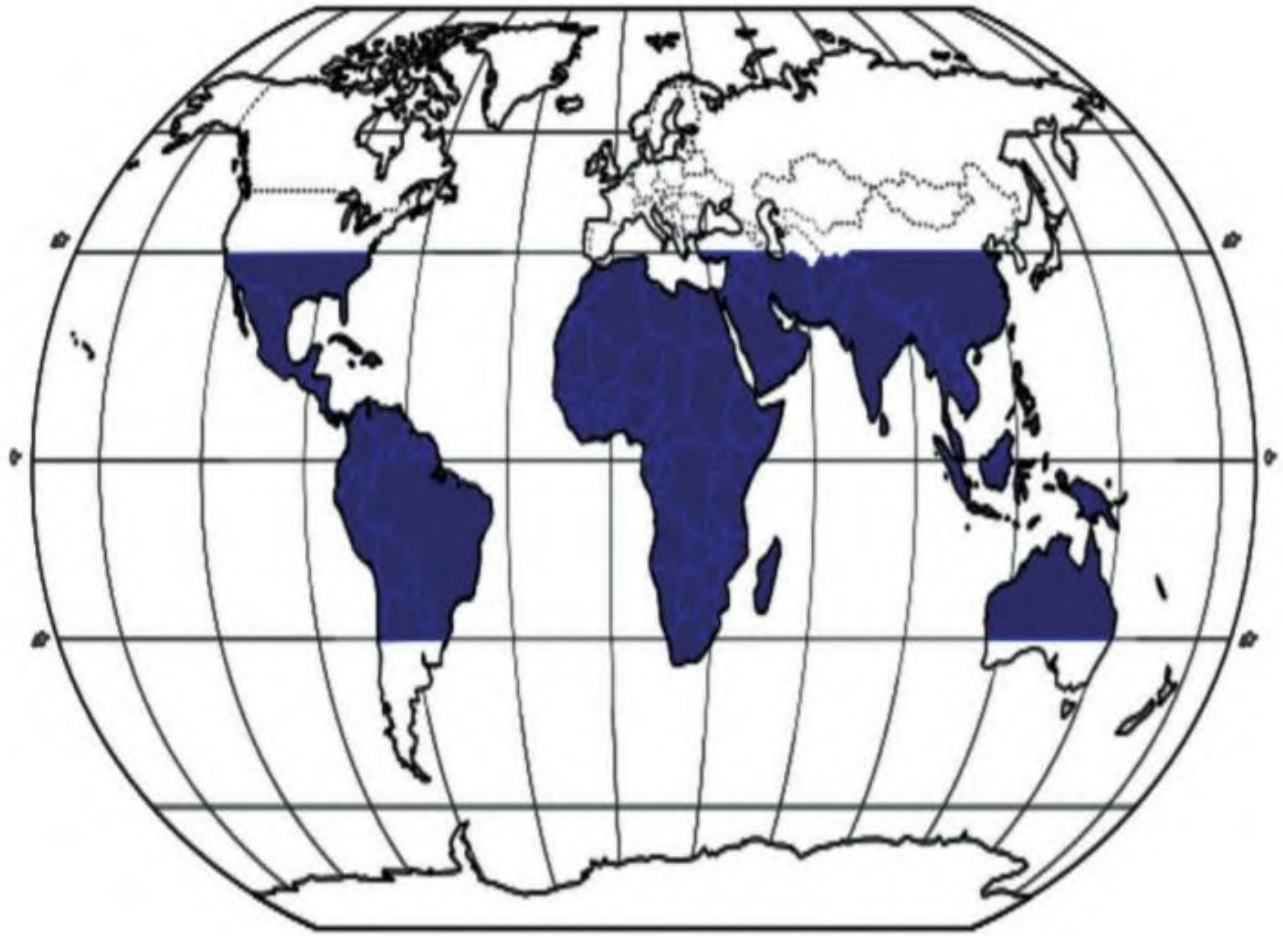
Breeding Peanuts Resistant to Preharvest Aflatoxin Contamination

What is Aflatoxin?

- “Aflatoxins are a family of toxins produced by certain fungi that are found on agricultural crops such as maize (corn), peanuts, cottonseed, and tree nuts.”
- “The main fungi that produce aflatoxins are *Aspergillus flavus* and *Aspergillus parasiticus*, which are abundant in warm and humid regions of the world.”
- “Aflatoxin-producing fungi can contaminate crops in the field, at harvest, and during storage.”



Where Is Aflatoxin a Problem?

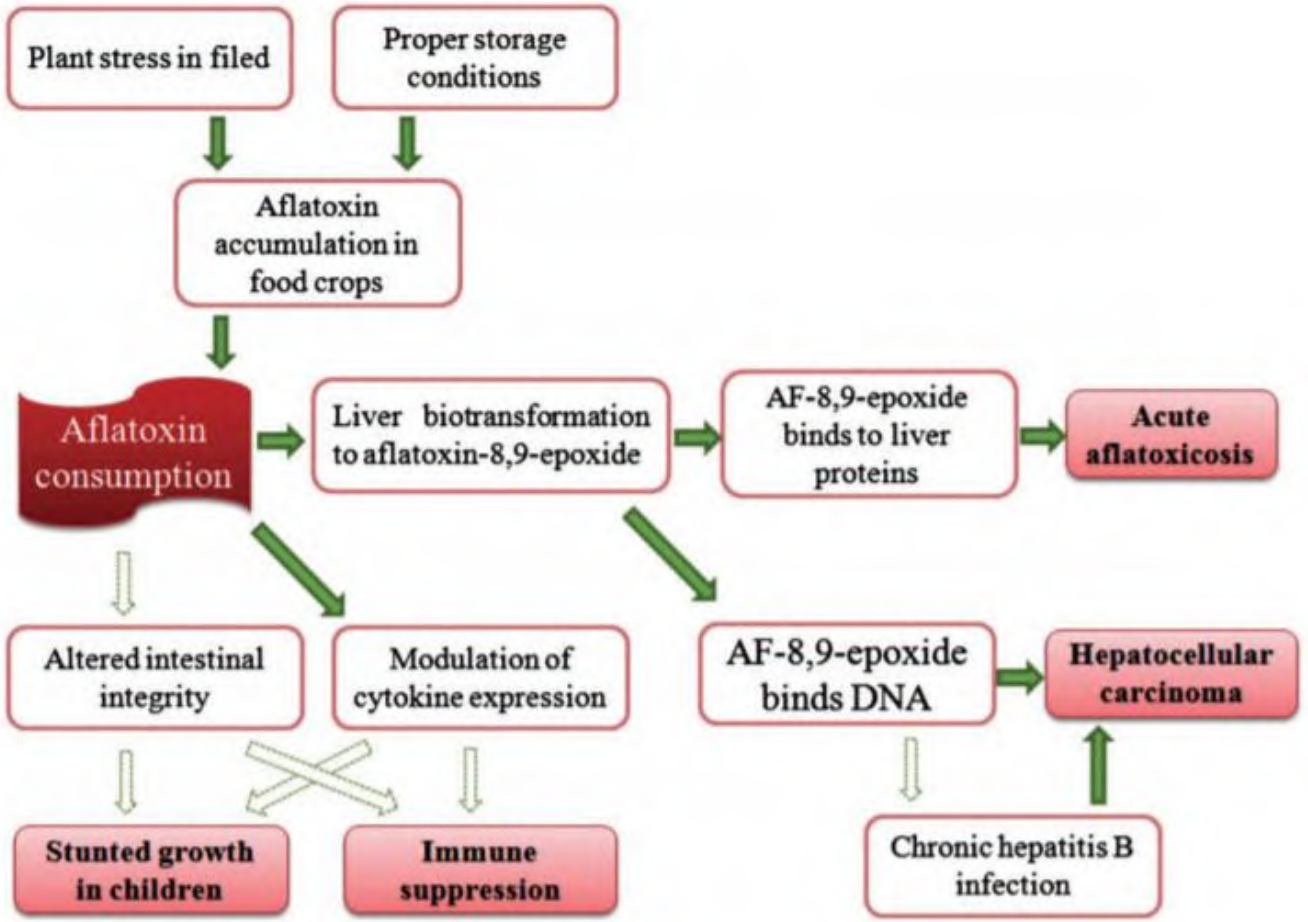


Umesha et al. 2017. J Sci Food Agric

What are the Consequences of Aflatoxin Exposure?

- 4.5 billion people in developing countries consume foods contaminated with aflatoxin
- Consumption leads to chronic and acute hepatocellular injury and child stunting
- Liver cancer is a frequent outcome of aflatoxin exposure, particularly in immunocompromised individuals
- In Kenya 2004, 317 cases of aflatoxin poisoning were reported with 125 deaths due to contaminated maize

What are the Consequences of Aflatoxin Exposure?



Umesha et al. 2017. J Sci Food Agric

FDA Action Levels for Aflatoxin

AFLATOXIN

Commodity	Action Level (ppb)	Reference
Animal Feeds		
Corn and peanut products intended for finishing (i.e., feedlot) beef cattle	300	CPG 683.100
Cottonseed meal intended for beef, cattle, swine, or poultry (regardless of age or breeding status	300	CPG 683.100
Corn and peanut products intended for finishing swine of 100 pounds or greater	200	CPG 683.100
Corn and peanut products intended for breeding beef cattle, breeding swine, or mature poultry	100	CPG 683.100
Corn, peanut products, and other animal feeds and feed ingredients but excluding cottonseed meal, intended for immature animals	20	CPG 683.100
Corn, peanut products, cottonseed meal, and other animal feed ingredients intended for dairy animals, for animal species or uses not specified above, or when the intended use is not known	20	CPG 683.100
Brazil nuts	20	CPG 570.200
Foods	20	CPG 555.400
Milk	0.5 (aflatoxin M1)	CPG 527.400
Peanuts and Peanut products	20	CPG 570.375
Pistachio nuts	20	CPG 570.500

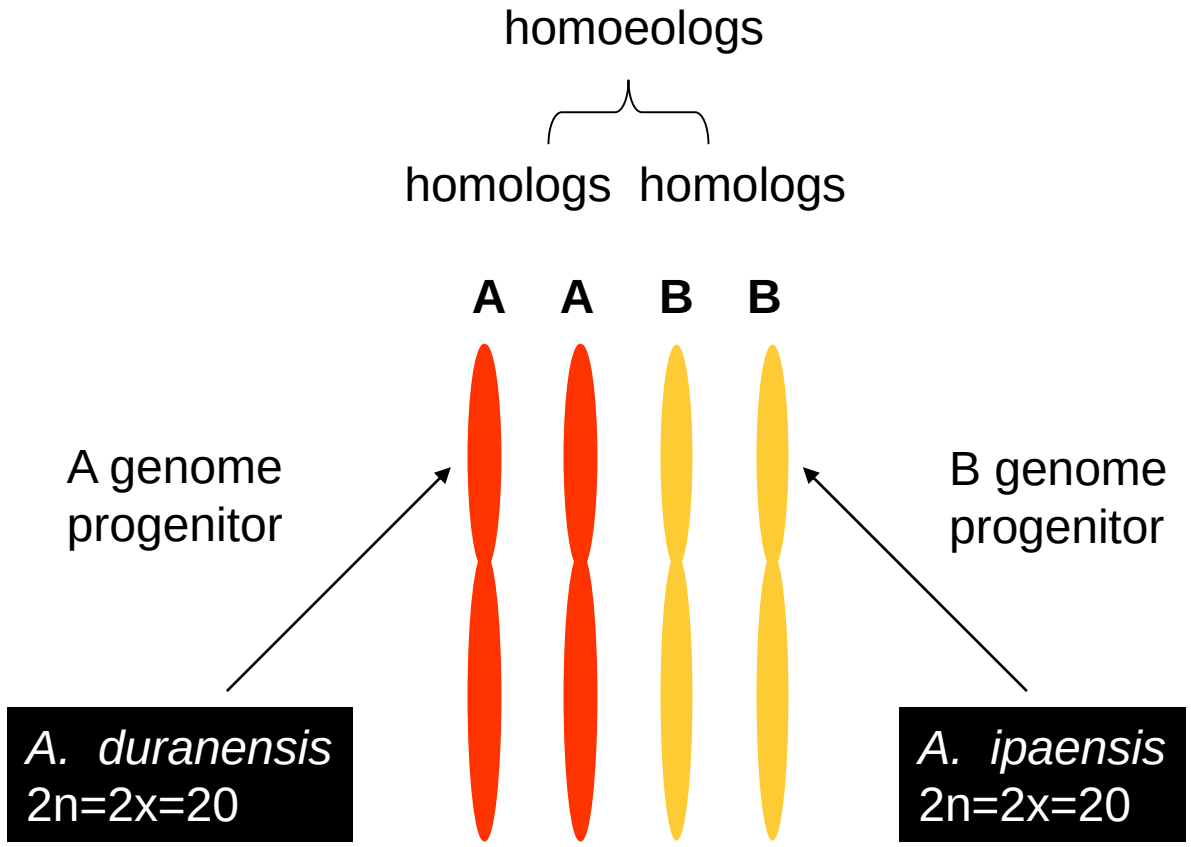
https://www.fda.gov/regulatory-information/search-fda-guidance-documents/guidance-industry-action-levels-poisonous-or-deleterious-substances-human-food-and-animal-feed#afla

Peanut Reproduction and Evolution

Peanut Reproduction and Evolution

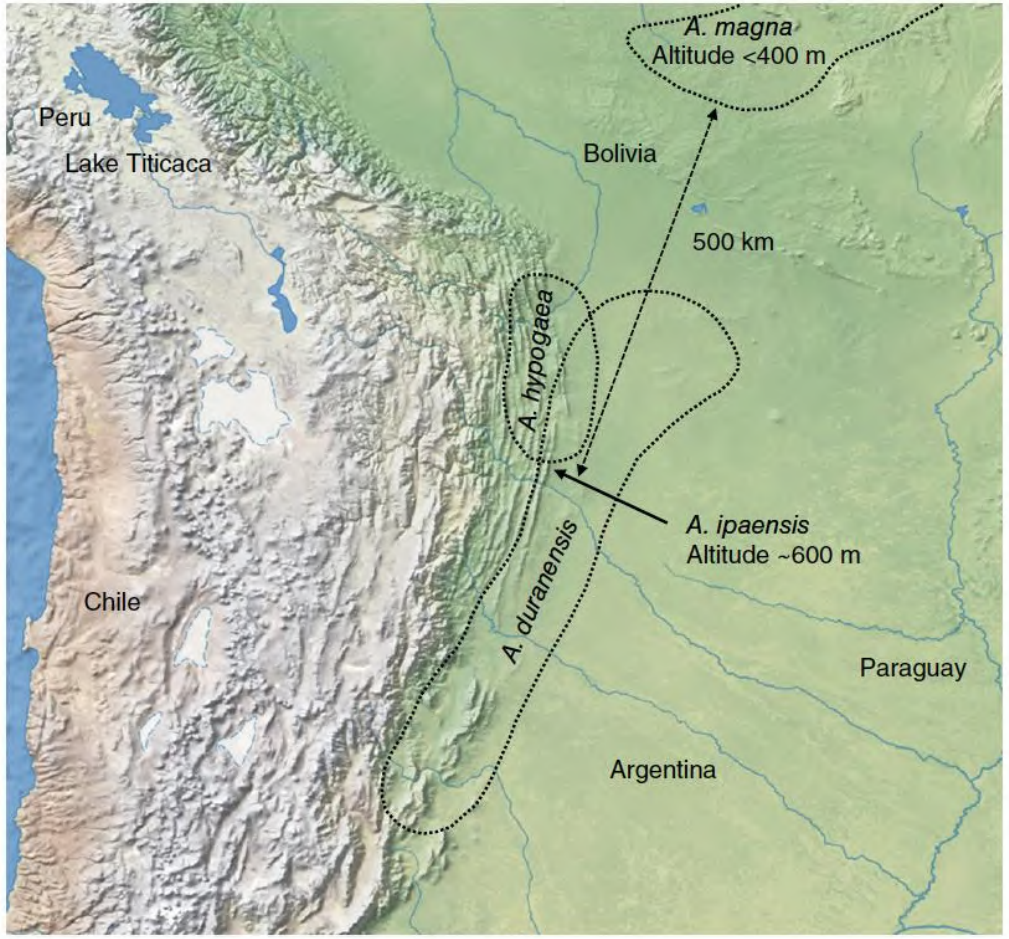
Ozias-Akins and Breiteneder. 2019. Allergy

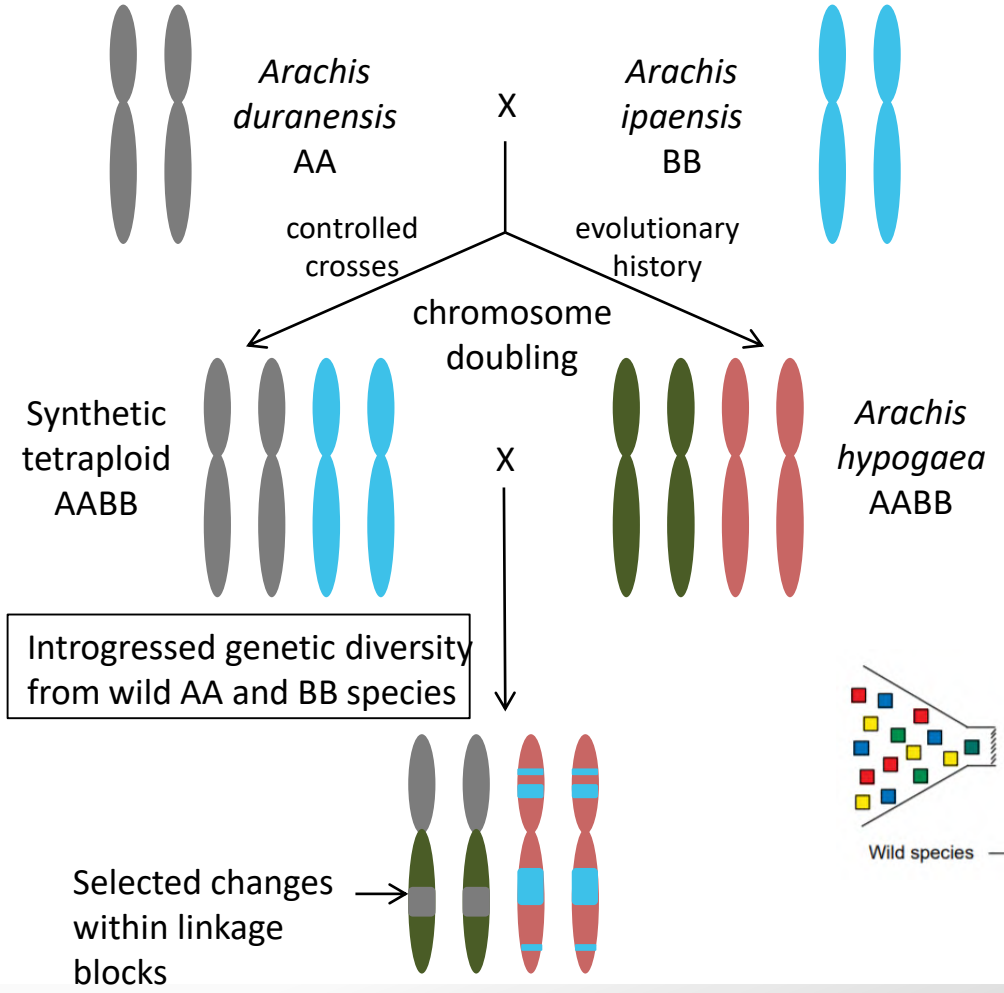
Peanut Reproduction and Evolution

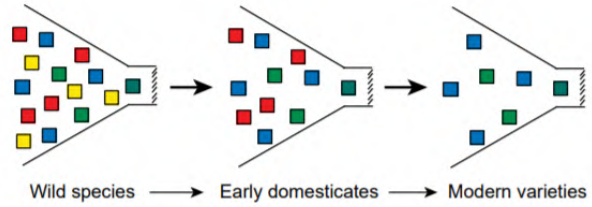


Arachis hypogaea
2n=4x=40; allotetraploid, inbred, homozygous

Peanut Reproduction and Evolution







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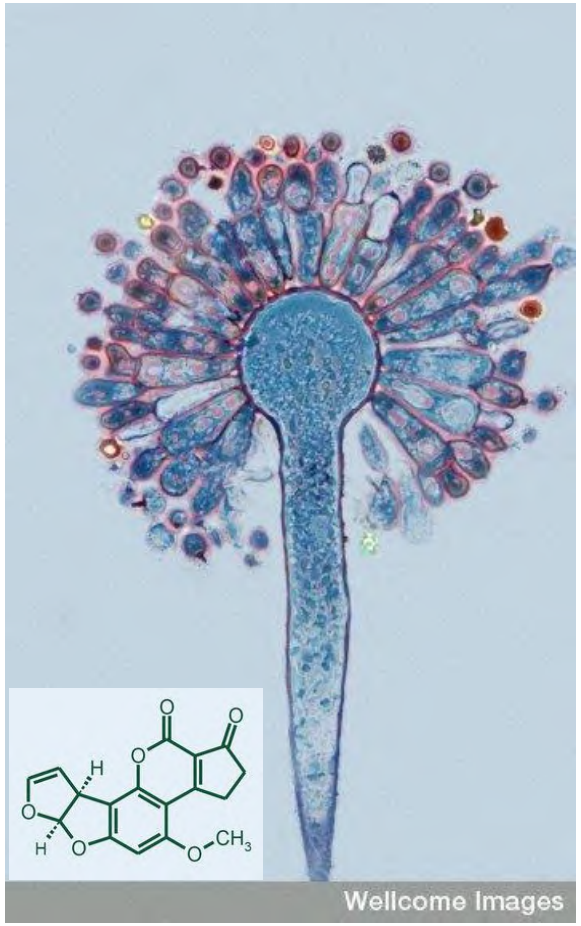
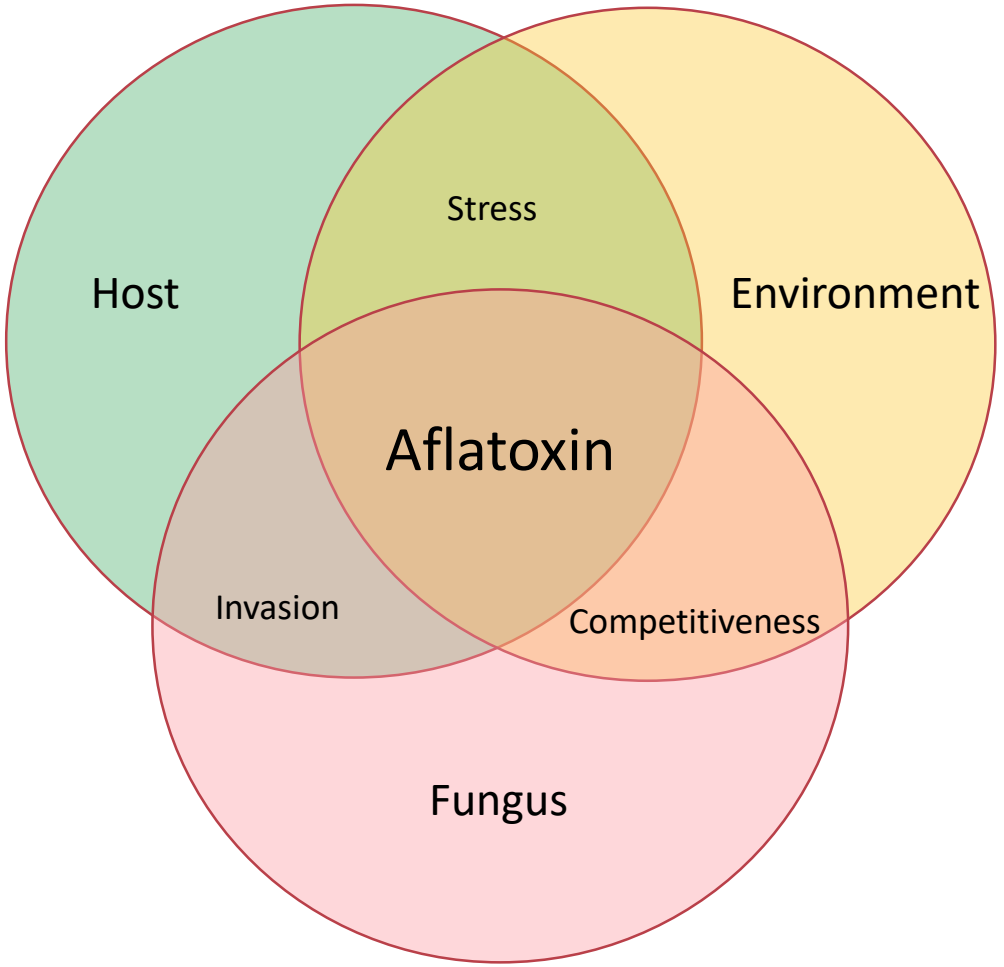
Bertioli et al. 2016. Nature Genet
Tanksley and McCouch. 1997. Science

Where Does Aflatoxin Accumulate in Peanut?

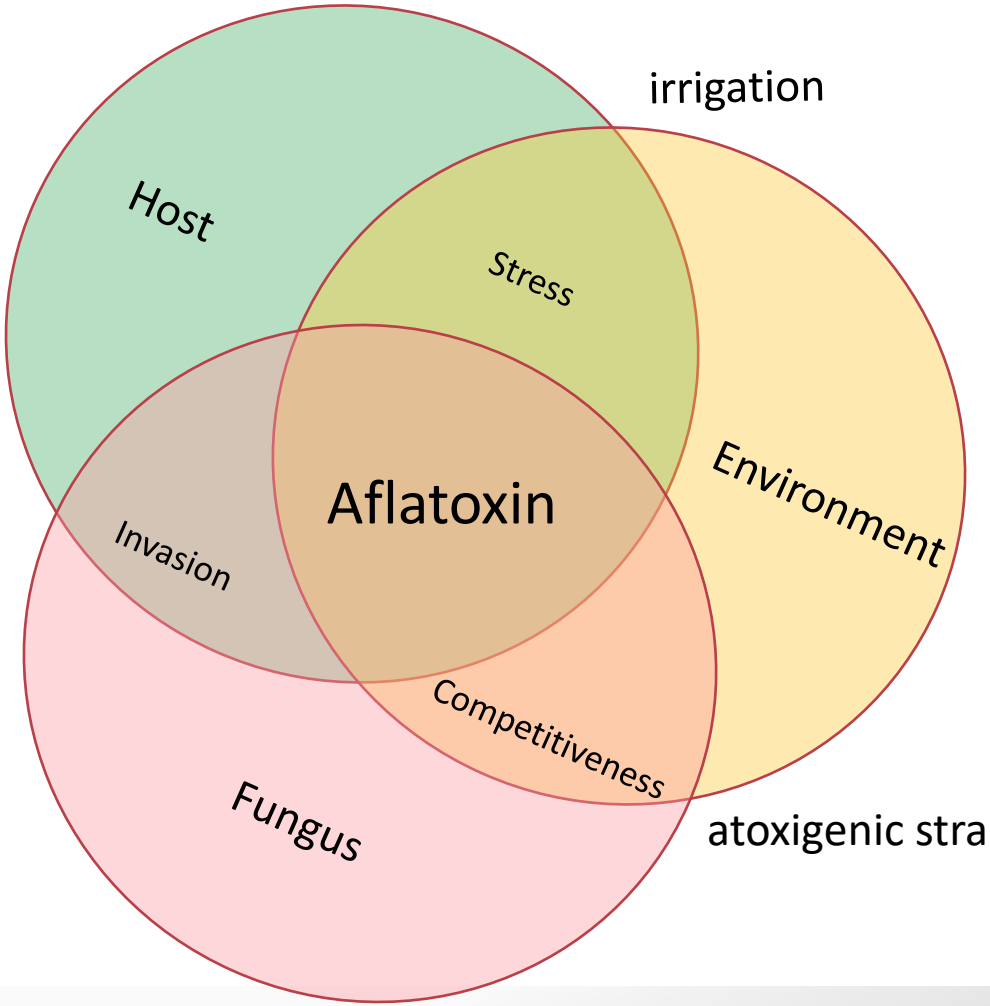


How Can Pre-harvest Aflatoxin Contamination Be Mitigated?



How Can Pre-harvest Aflatoxin Contamination Be Mitigated?

insect resistance
fungal resistance
aflatoxin biosynthesis resistance



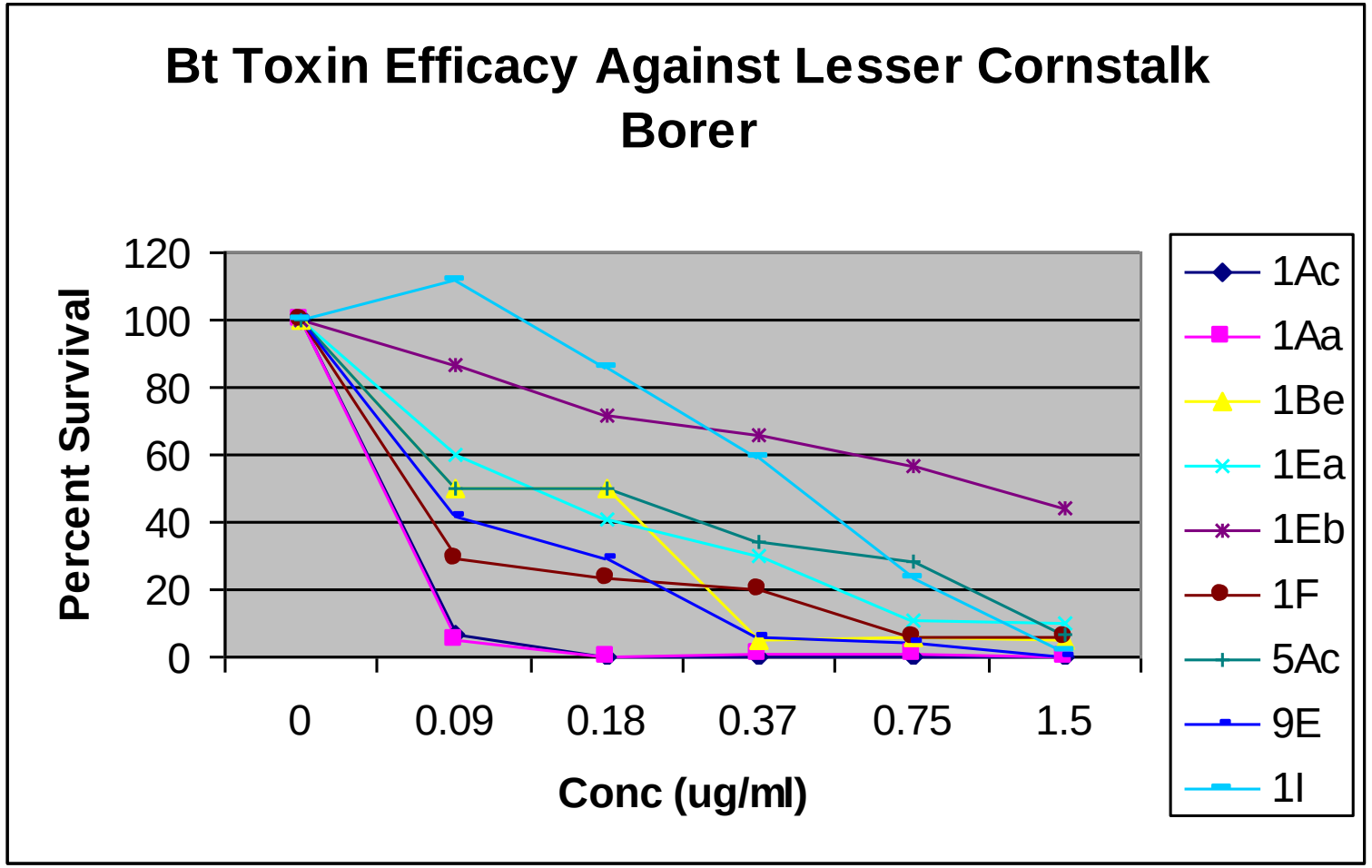
Aflatoxin Mitigation Through Insect Control

- Lesser cornstalk borer damages pods during drought stress
- LCB damage is associated with increased aflatoxin
- Drought management (irrigation) can reduce risk
- Insecticides (Lorsban) can reduce risk but irrigation also needed for pesticide to be effective
- Lorsban is detrimental to predatory insects (beneficials) resulting in outbreaks of other insect pests such as spider mites

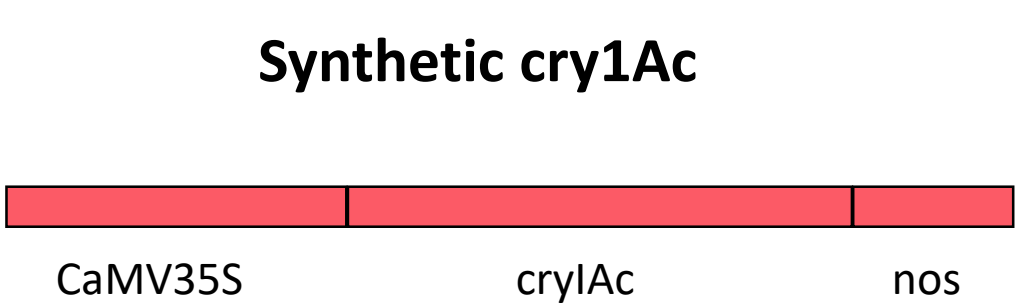
Insects and Aflatoxin

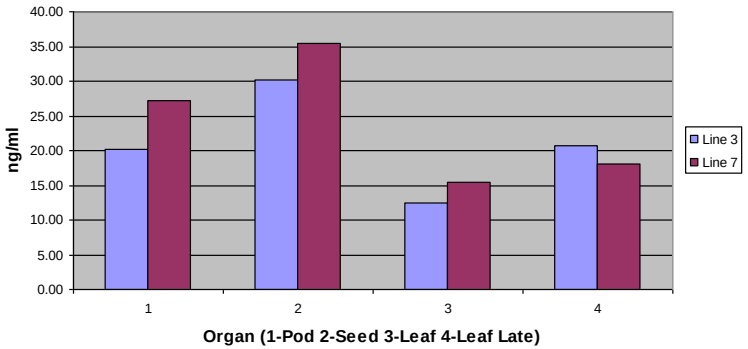
- Lesser cornstalk borer is difficult to monitor and control with chemicals because the larvae feed on the underground pods
- Lesser cornstalk borer – limited genetic resistance in cultivated peanut gene pool
- Alternative sources of genetic resistance
  - Transgenes (Bt)
  - Wild relatives

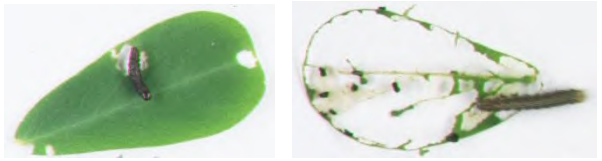
Aflatoxin Mitigation Through LCB Control



Aflatoxin Mitigation Through LCB Control







- Introduced into MarcI in 1995
- Lines with resistance to lesser cornstalk borer (LCB) selected
- Field tests for insect resistance conducted in ‘97, ‘98, ’99
- Significant reduction in LCB damage and aflatoxin contamination

Peanut Cultivation With Temperature and Water Stress

Imposing stress with rainout shelters

Peanut Cultivation With Temperature and Water Stress

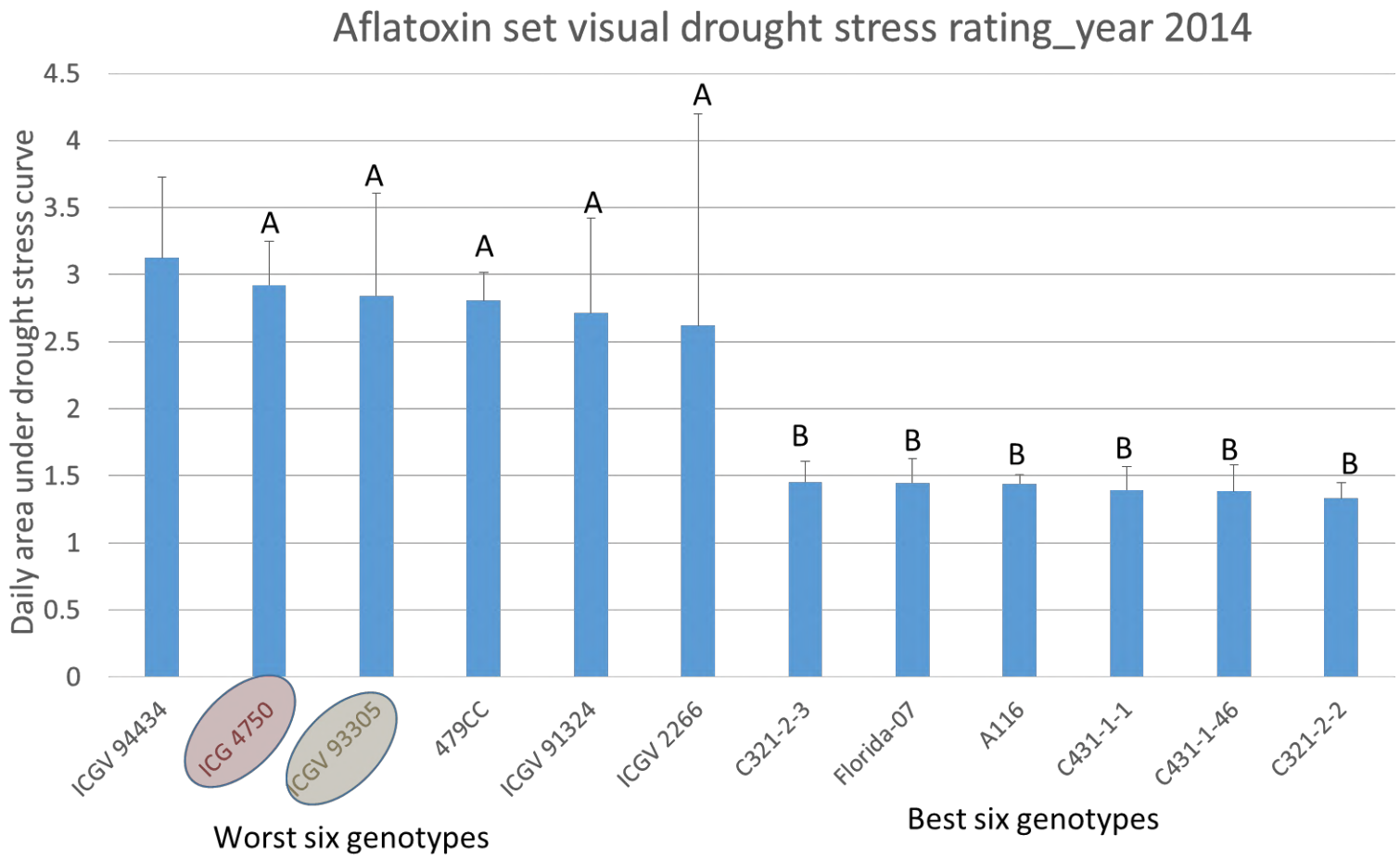
Table 3. Correlation coefficients of aflatoxin contamination with leaf temperature and visual drought stress ratings from plots in Tifton, GA in 1992.

	Measurement date								
	Aug.				Sept.				
	20	24	28	30	4	8	10	15	21
Aflatoxin & leaf temp.	.19*	.25**	.26**	.21*	.21*	.22*	.25**	.23*	.22*
Aflatoxin & visual rating	-.01	.04	.04	.35**	.09	.16	.21*	.19*	.21*

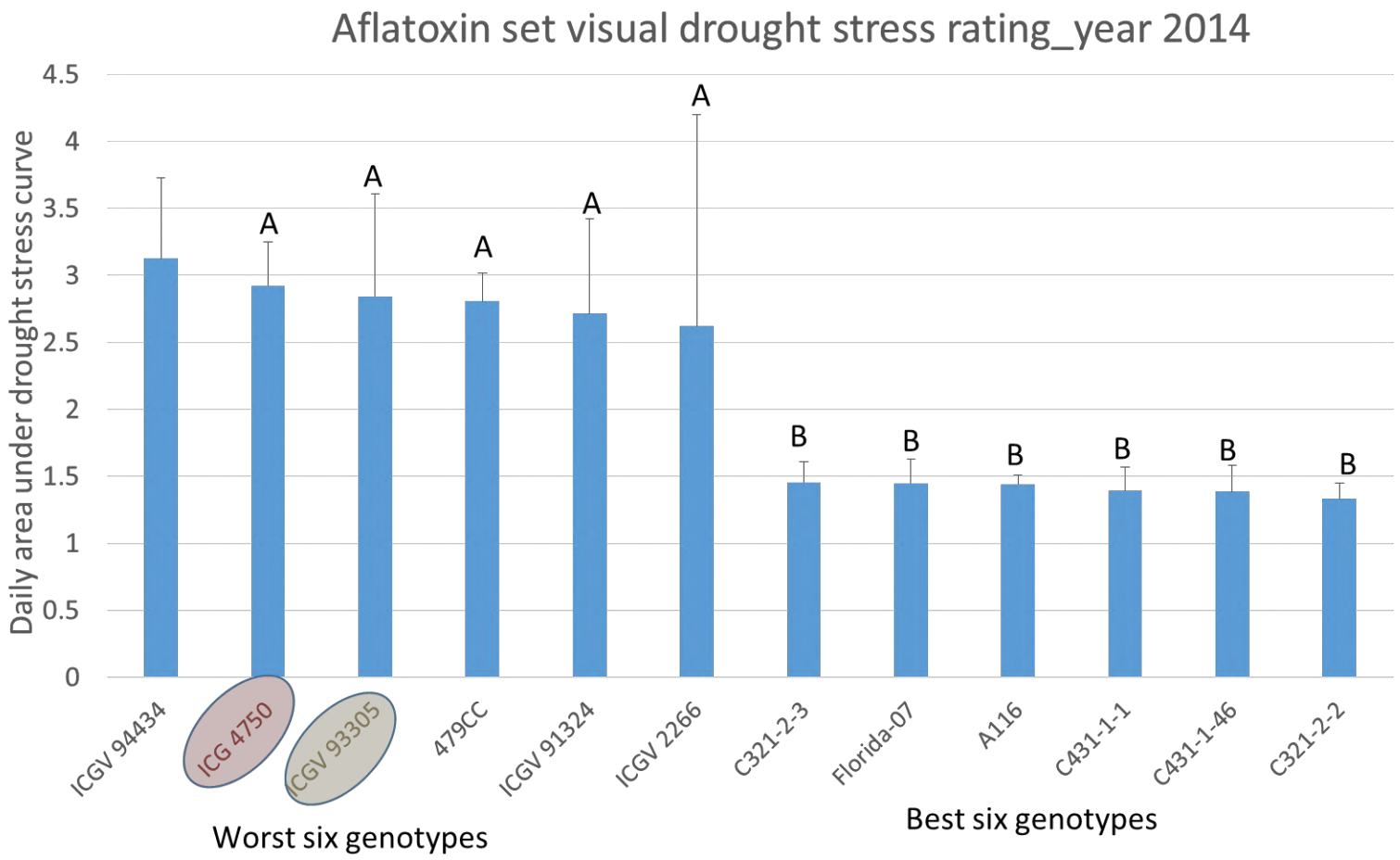
*,** = significant at P = 0.05 and 0.01, respectively.

Holbrook et al. 2000. PeanutSci

Peanut Cultivation With Temperature and Water Stress

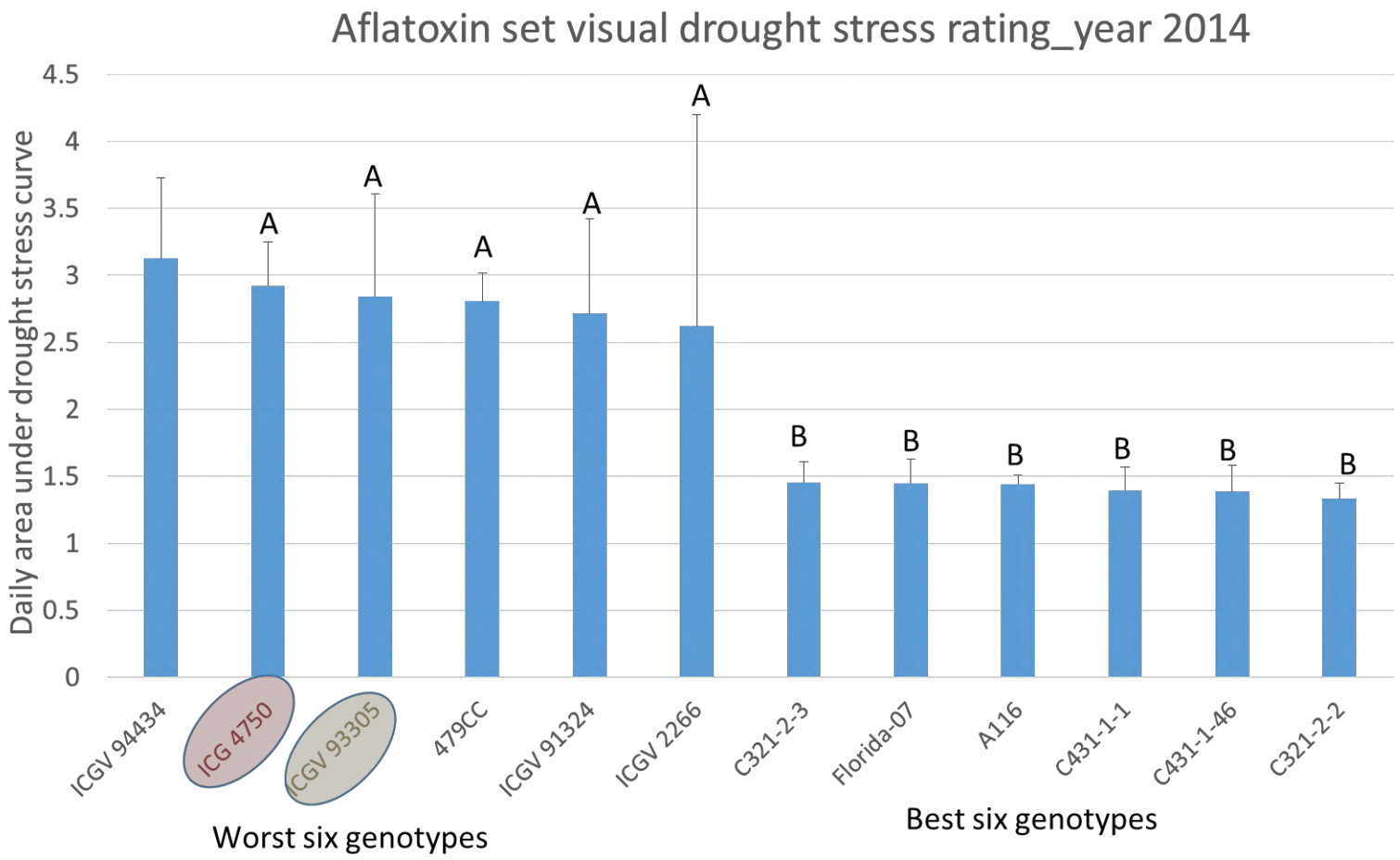


Peanut Cultivation With Temperature and Water Stress



Entry	N	Mean Relative Toxin
ICGV2184	3	0.2
ICGV93280	3	0.2
A116	4	0.2
ICG1448	3	0.2
ICGV2207	3	0.2
ICG2800	3	0.2
A104-12	4	0.2
ICGV87110	3	0.3
A91	4	0.3
ICG10094	3	0.3
*C321-2-3	4	0.3
*C321-2-2	6	0.3
A103	4	0.4
C431-1-4	5	0.4

Peanut Cultivation With Temperature and Water Stress



Entry	N	Mean Relative Toxin
ICGV93305	3	7.1
A69	3	4.2
ICG4750	3	3.9
ICGV1124	3	3.8
A47	4	3.6

Peanut Cultivation With Temperature and Water Stress

Peanut Cultivation With Temperature and Water Stress

Table 3. Correlation between aflatoxin contamination, yield and drought stress evaluation methods of the peanut genotypes planted at the Gibbs Farm shelter.

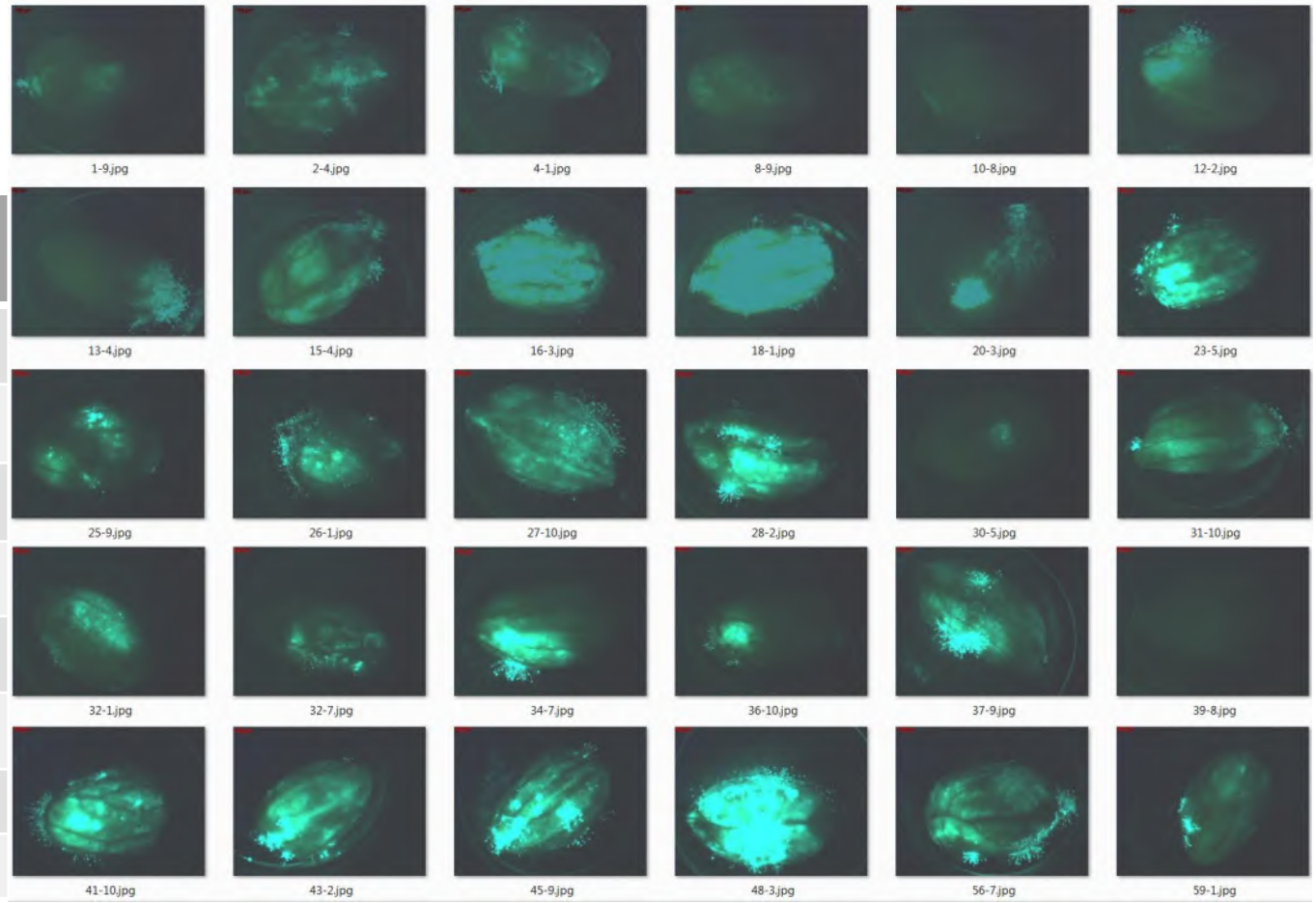
			– Chlorophyll fluorescence[c] –			— Visual rating[d] –				
	Aflatoxin[a]	Pod Yield[b]	PI_{ABS}	F_v/F_m	ϕ_{EO}	AM	PM	SCMR[e]	CT[f]	CTD[g]
Pod yield	−0.44**[h]									
PI_{ABS}	−0.27	0.26								
F_v/F_m	−0.62**	−0.12	0.56**							
ϕ_{EO}	−0.26	0.25	0.98**	0.59**						
Visual rating AM	0.85**	−0.40	−0.29	−0.41*	−0.25					
Visual rating PM	0.85**	−0.26	−0.24	−0.50**	−0.21	0.98**				
SCMR	−0.57**	0.32	0.22	0.43*	0.21	−0.60**	−0.62**			
CT	0.73**	0.01	−0.09	−0.64**	−0.10	0.73**	0.84**	−0.50**		
CTD	−0.81**	0.19	0.15	0.42*	0.15	−0.79**	−0.85**	0.37	−0.92**	
NDVI	−0.79**	0.17	0.25	0.62**	0.24	−0.84**	−0.87**	0.75**	−0.81**	0.70**

Luis et al. 2016. Peanut Sci

Pre- and Post-harvest Resistance

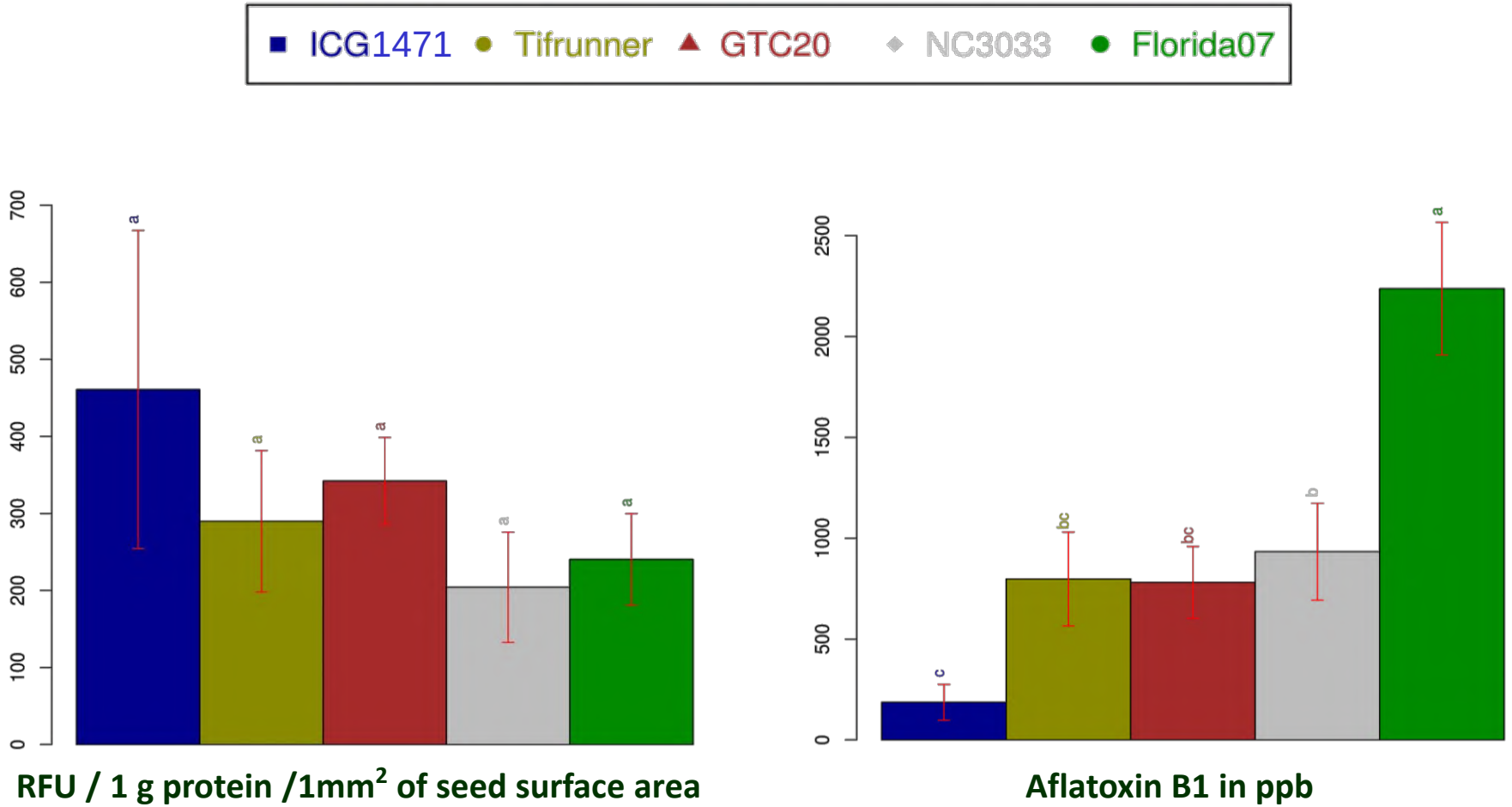
Entry	Relative Toxin
ICG 1471 (55-437)	0.5
ICGV 88145	0.5

Entry	Relative Yield (wd/ww)	Relative Toxin (log10)
ICG 862	0.74	1.06
ICG 8285	0.65	1.25
ICG 1703	0.41	1.12
ICG 4729	0.58	0.97
ICG 6667	0.32	2.34
ICG 6766	0.43	2.71
ICG 1471	0.48	1.18
Fleur 11	0.41	1.42

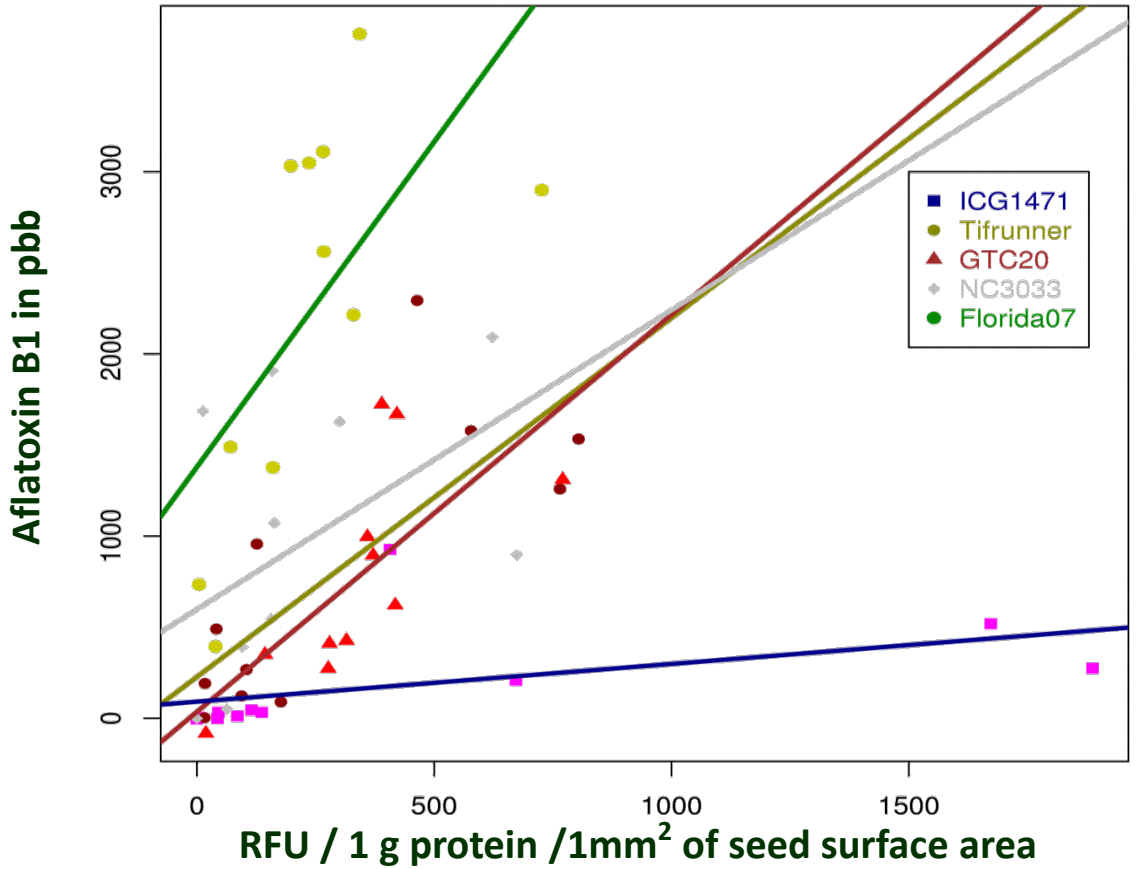


Hamidou et al. 2014. Field Crops Res
Korani et al. 2017. Toxins

Post-harvest Resistance

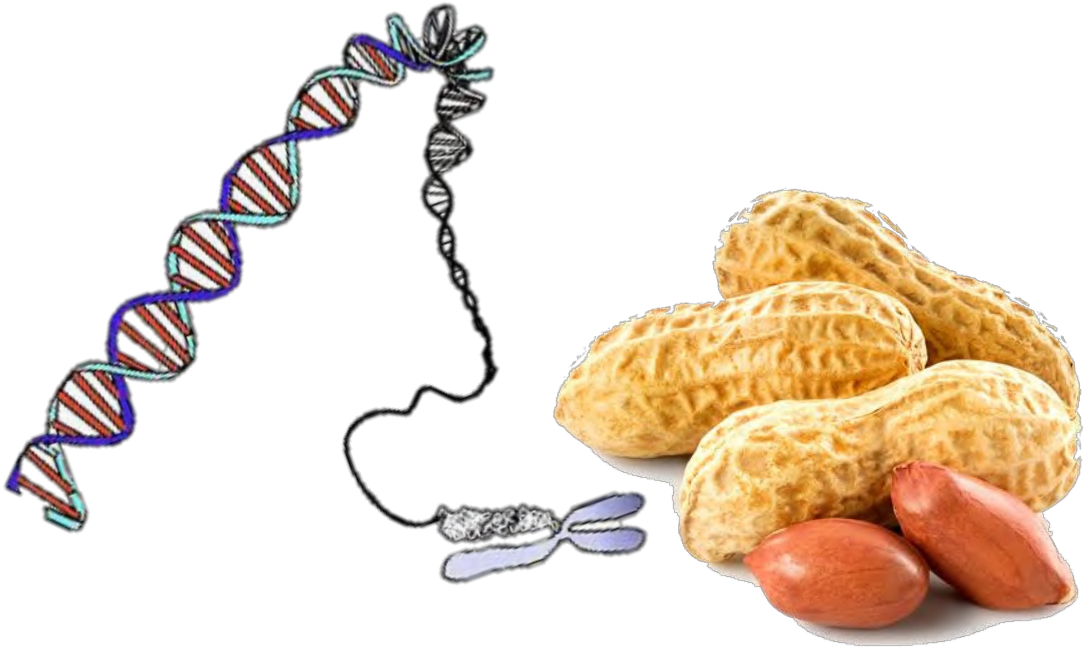


Post-harvest Resistance



Genetic Factors in ICG 1471 (55-437) for Aflatoxin Reduction

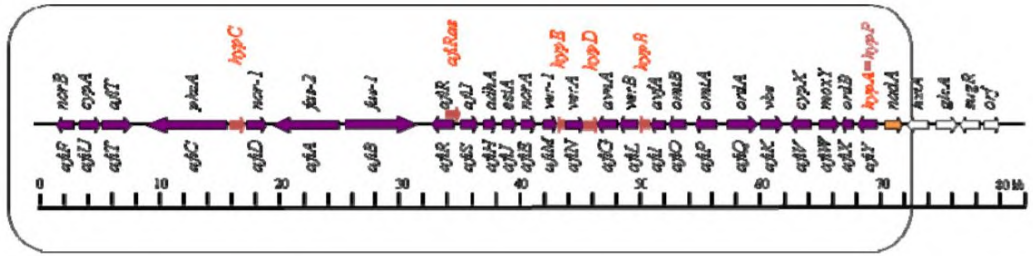
- Improvement of aflatoxin resistance in peanut by identifying genomic regions controlling post-harvest aflatoxin production that may also contribute to pre-harvest reduction in contamination



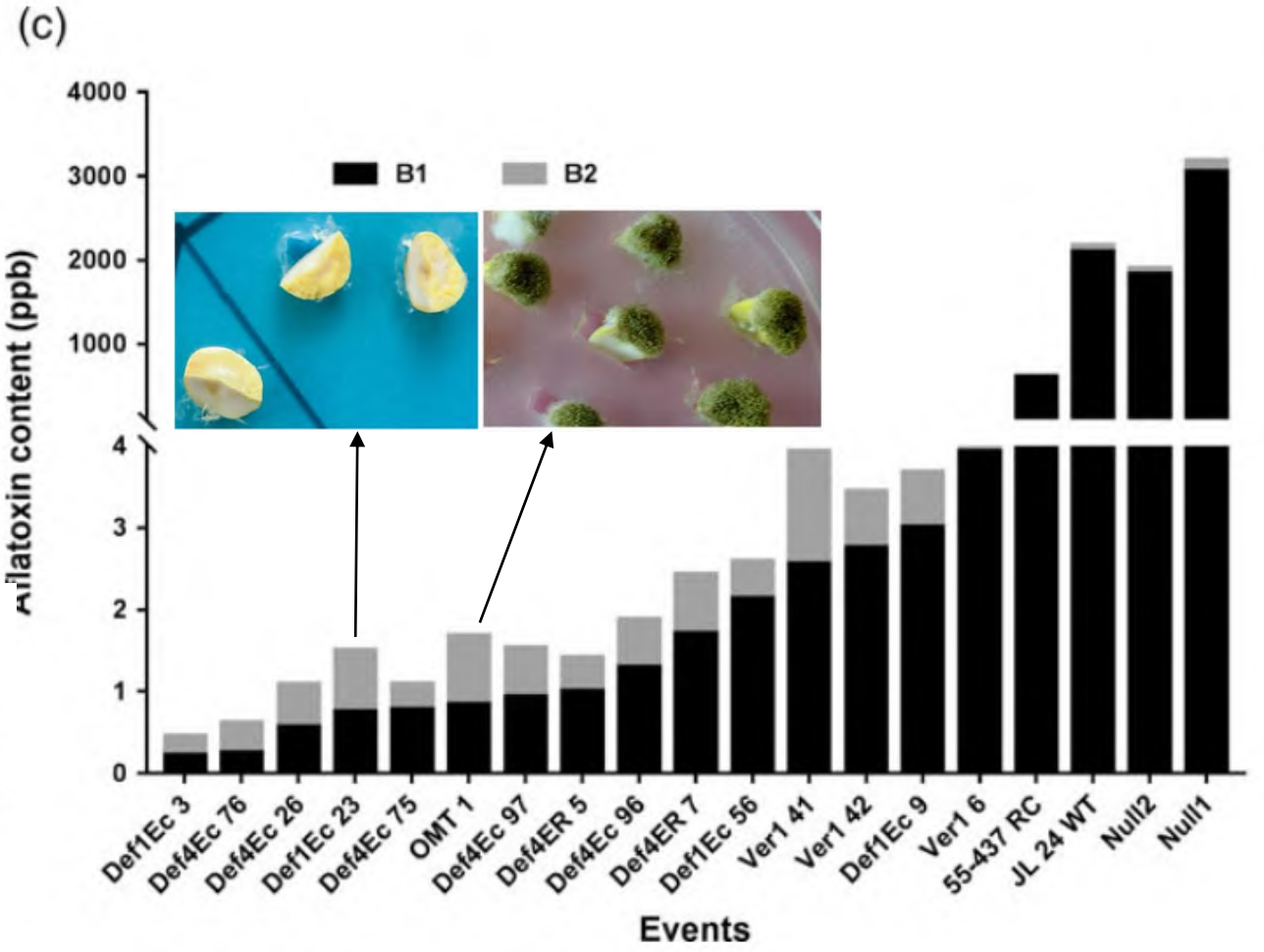
Transgenic Approaches

Combine existing host resistance with transgene resistance

- Insect resistance
- Defensins
- Reactive oxygen scavenging enzymes
- Host-induced gene silencing



Aflatoxin biosynthesis gene cluster



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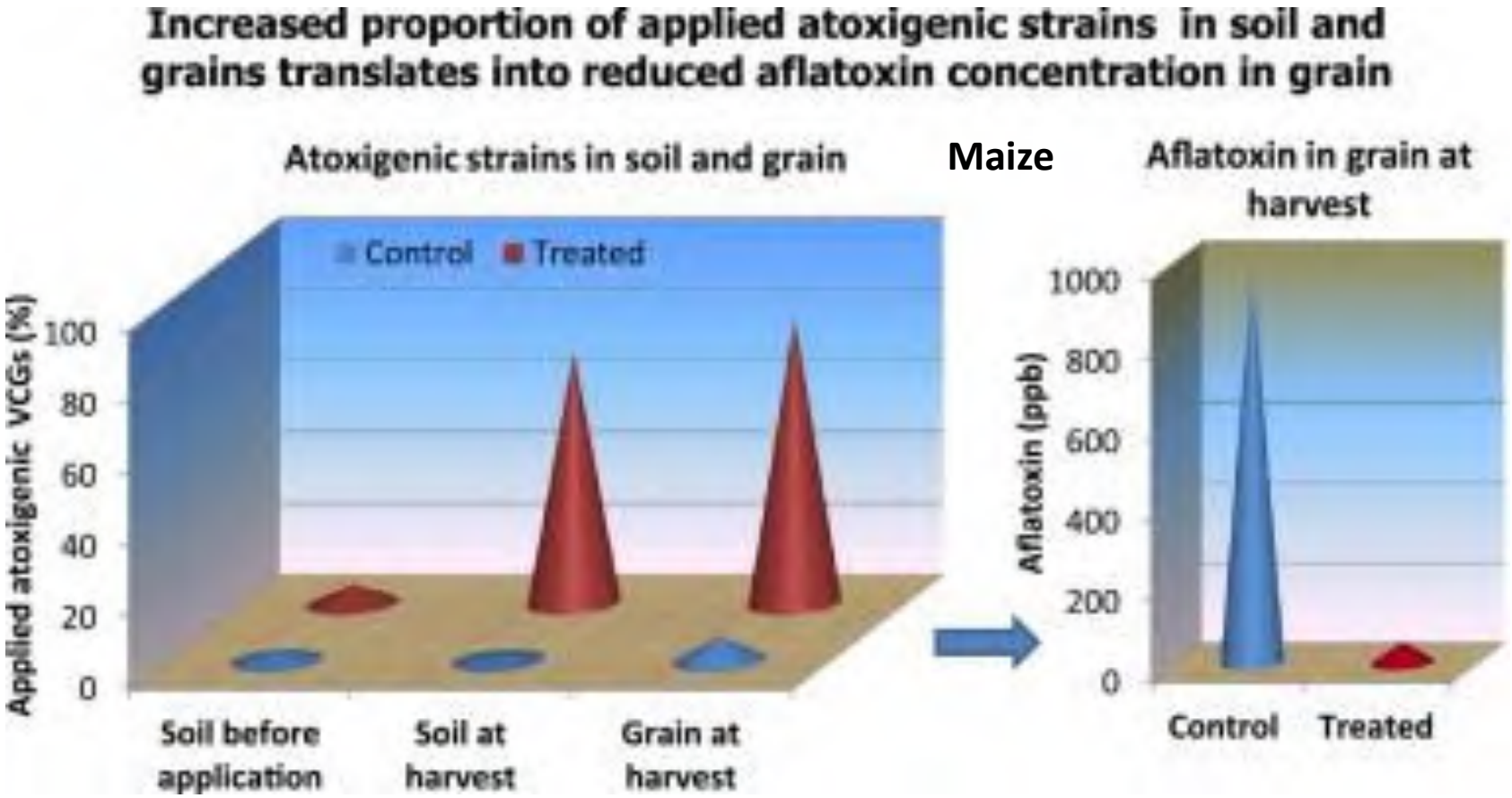
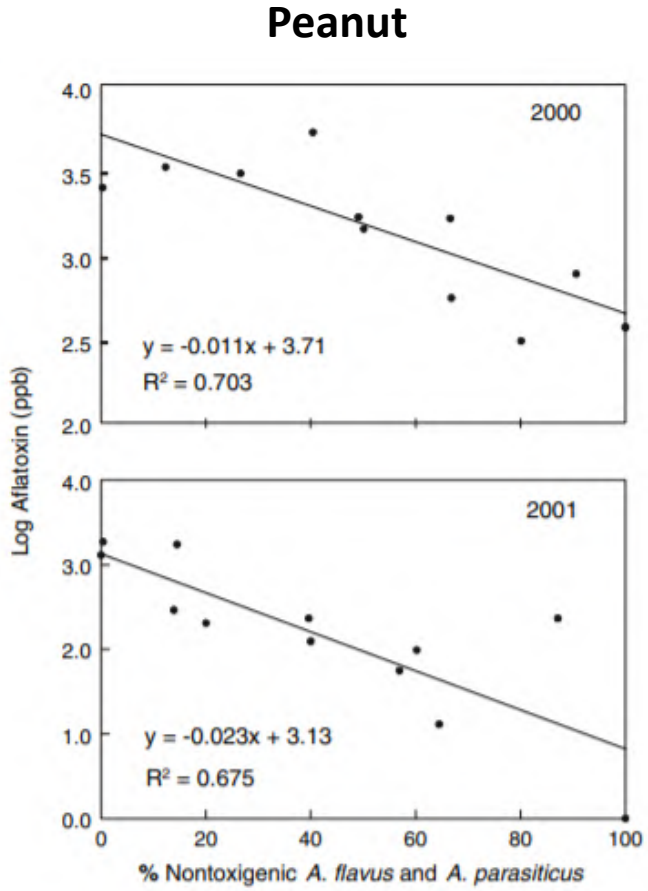
Yu. 2012. Toxins
Sharma. 2018.Plant Biotech J

Biological Control Through Competition

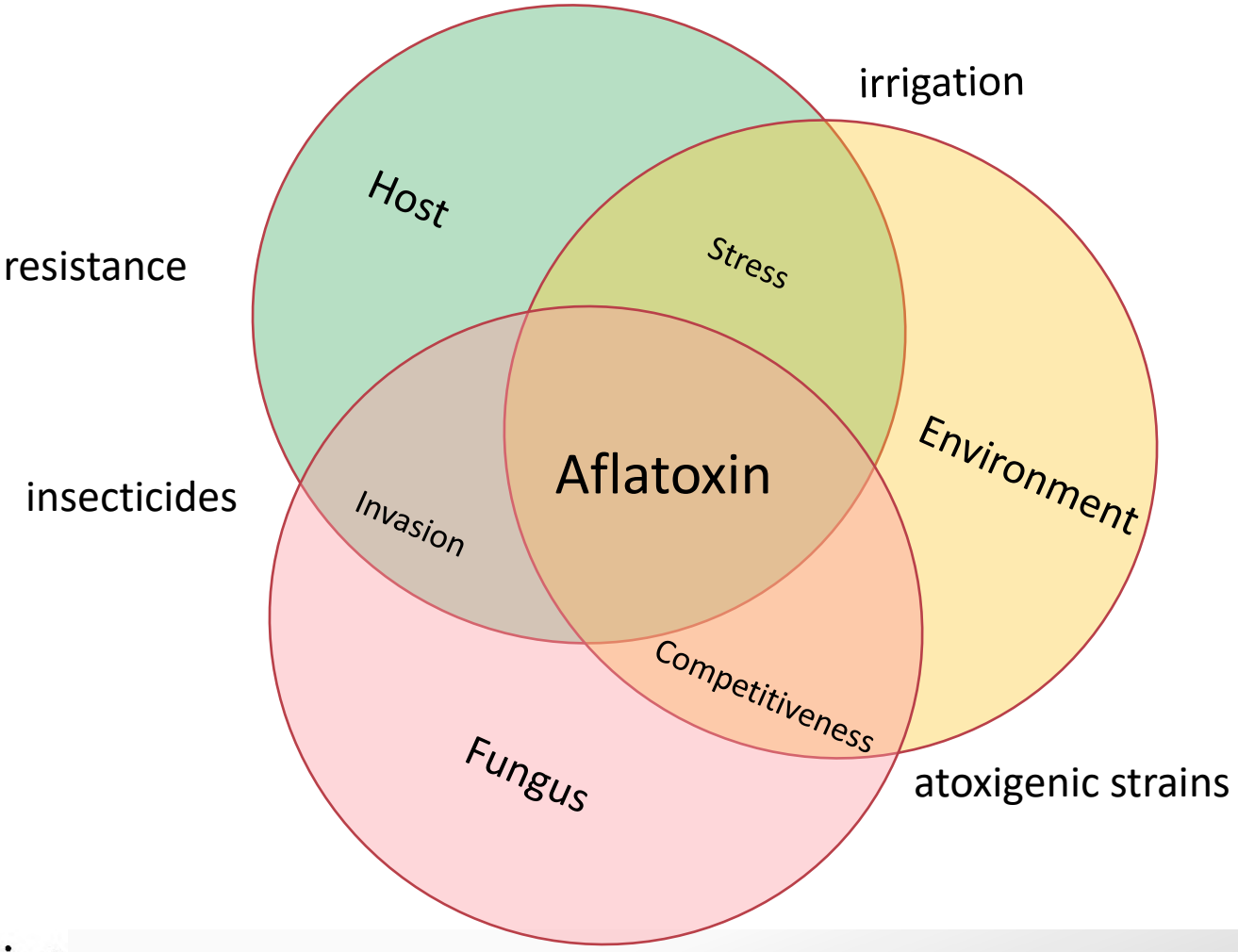




Atehnkeng et al. 2014. Biol Control
Dorner and Horn 2007. Mycopathologia

Conclusion – All Technologies Must Be Made Available To Combat Aflatoxin Contamination

insect resistance
fungal resistance
aflatoxin biosynthesis resistance



insecticides

atoxigenic strains

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- Soraya Bertioli
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- Dongying Gao
- Carolina Chavarro

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