

ETHANOL FUEL: THE IMPACT OF AFLATOXIN DEGRADATION

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ETHANOL

**National energy security
has never been more
important.**

Consider that:

- U.S. gasoline prices have surged to record highs and oil imports have increased to unprecedented levels;
- Fifty-five percent of the petroleum used in the United States is imported, and growing;
- U.S. drivers consume more than 140 billion gallons of gasoline per year, far more than any other country in the world.

**FACT
VS.
FICTION**



Bush pushes for green fuel

State of the Union includes an ethanol plug. Will it matter?

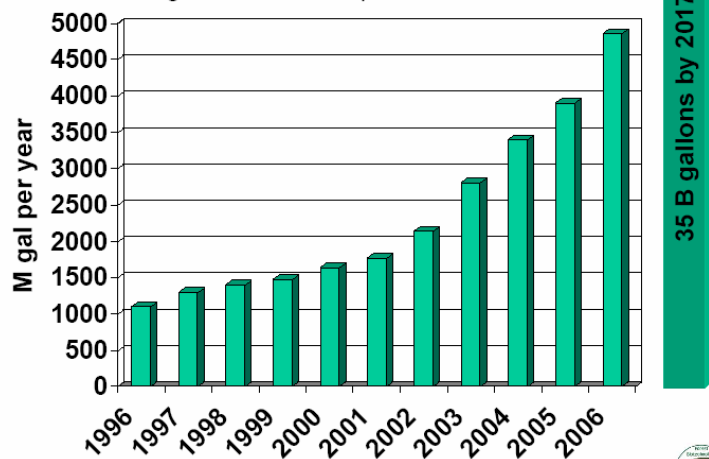


Last year's energy bill -- among other things -- mandated the use of 7.5 billion gallons of ethanol by 2012. Last year, Americans consumed about 4 billion gallons of ethanol, nearly all of it added to gasoline in a 90 percent gas/10 percent ethanol blend. In recent interviews, the president has been talking up the virtues of flexible fuel vehicles that can burn a blend of 85 percent ethanol called E85.

By [Adam Lashinsky](#), FORTUNE senior writer, February 1, 2006

Annual Ethanol Production in the U.S.

National goals for ethanol production are ambitious



Data source: Renewable Fuels Association (<http://www.ethanolrfa.org/>)

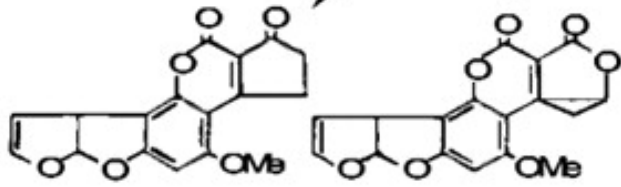




What Makes Aflatoxin?

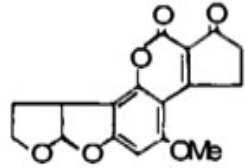
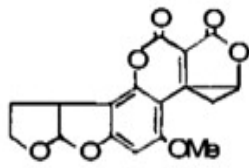
- *Aspergillus flavus*, *A. parasiticus*, and *A. nomius*.
- There are unverified reports of aflatoxins being produced by *Penicillium puberulum*, *Aspergillus wentii*, and *A. fumigatus*.

Aflatoxin



AFB₁

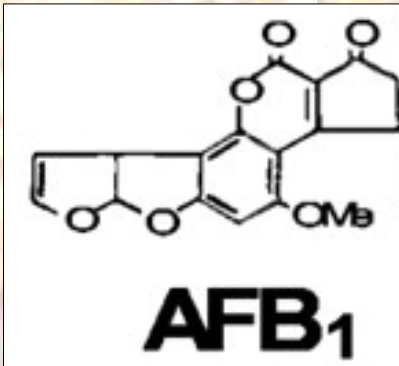
AFG₁



AFG₂

AFB₂

Aflatoxin



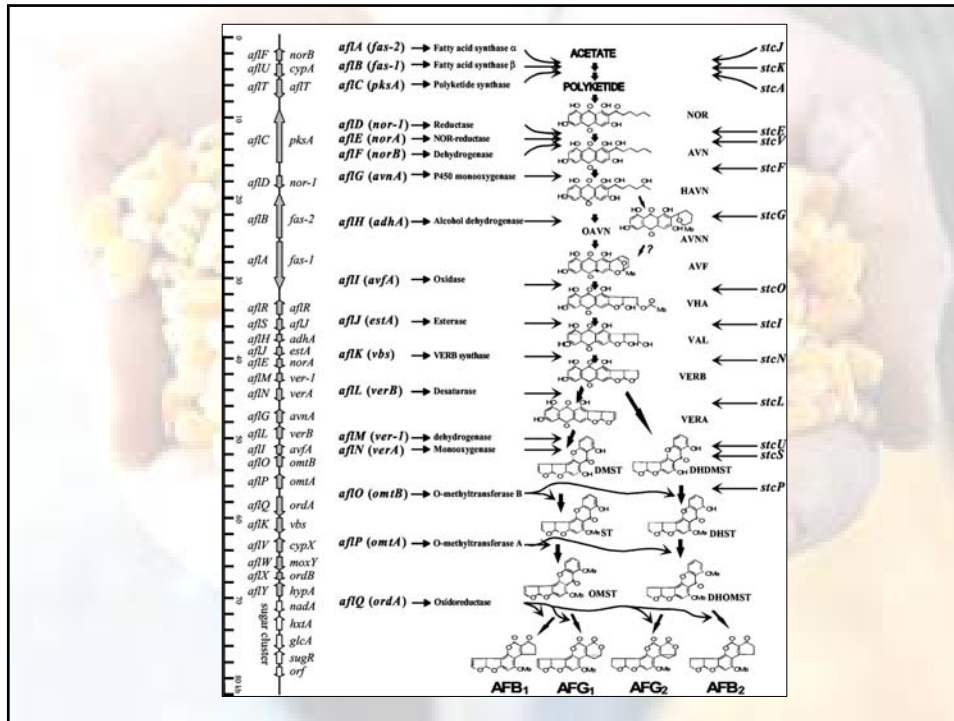


Table 1. Acute toxicity of aflatoxin B1 expressed as a single oral dose LD50 (FAO web library)

Species	LD50 mg kg ⁻¹ bodyweight
Rabbit	0.30
Duckling (11 day old)	0.43
Cat	0.55
Pig	0.60
Rainbow trout	0.80
Dog	0.50 - 1.00
Sheep	1.00 - 2.00
Guinea pig	1.40 - 2.00
Baboon	2.00
Chicken	6.30
Rat (male)	5.50 - 7.20
Rat (female)	17.90
Macaque (female)	7.80
Mouse	9.00
Hamster	10.20

LD50 is the term for "lethal dose at which 50% of the animals die".

Adult domestic pigs average weight varies between 90 to 260 pounds (40.8-118 kg)

So if it is 100 kg then 60 mg is sufficient to kill 50% of pigs weighing 100 kg or 220 pounds!



Regulatory limits

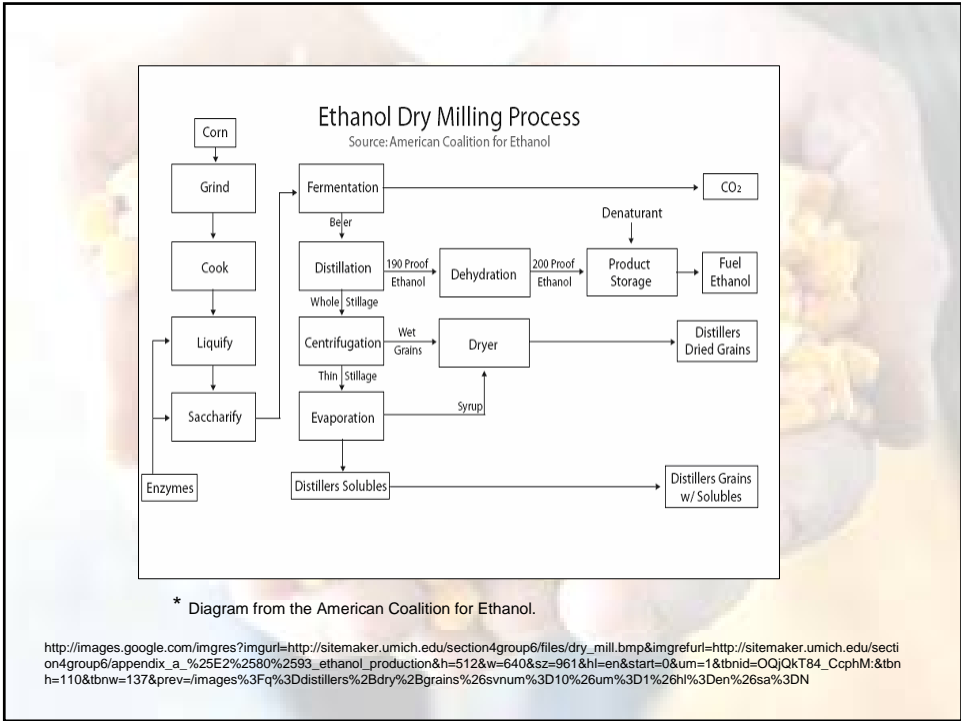
- **FDA ACTION LEVELS FOR AFLATOXIN CONTAMINATED CORN ARE:**

- **20 ppb:** food use by humans, feed for immature animals, dairy animals or unknown destination
 - (Japan 10 ppb, Europe 2 ppb!)
- **100 ppb:** feed for breeding cattle, breeding swine or poultry
- **200 ppb:** feed for finishing swine
- **300 ppb:** feed for finishing beef cattle



What about EtOH???

- No significant amounts of aflatoxin in EtOH.
- But, concentrates the aflatoxin 3-5X in the leftover products called distillers grains





<http://images.google.com/imgres?imgurl=http://www.biorenew.iastate.edu/typo3temp/pics/c4b3427b05.jpg&imgrefurl=http://www.biorenew.iastate.edu/research/thermochemicals/distillers-dried-grains.html&h=200&w=267&sz=12&hl=en&start=0&um=1&tbnid=AtVc0zf6alkApM:&tbnh=85&tbnw=113&prev=/images%3Fq%3Ddistillers%2Bdry%2Bgrains%2Bethanol%26svnum%3D10%26um%3D1%26hl%3Den>



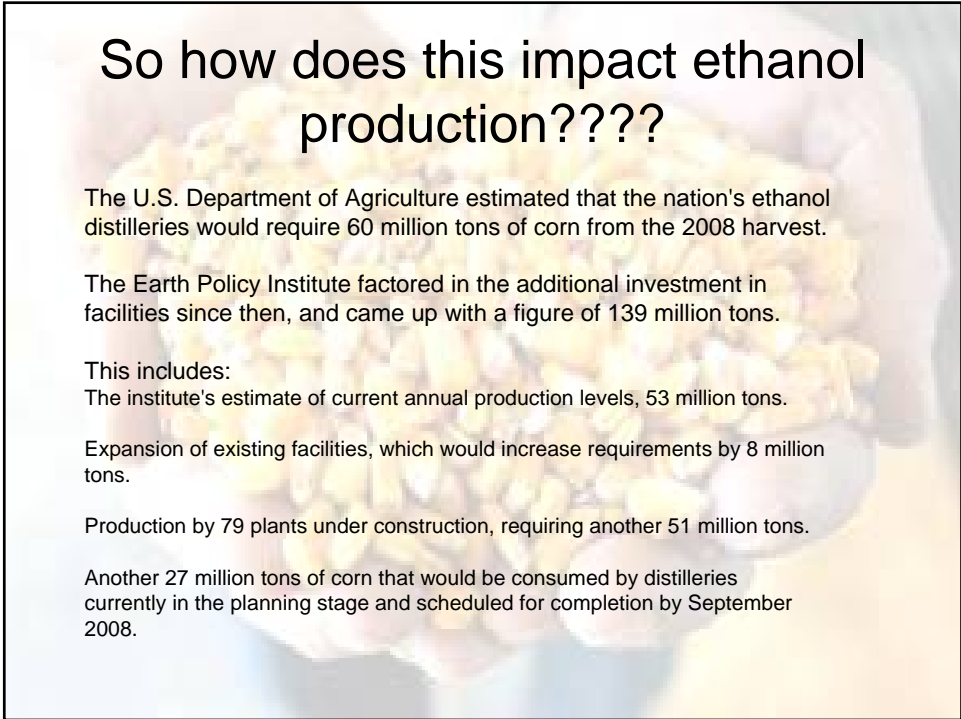


The Cost of Contaminated Corn!

Corn: US corn production, worth \$30 billion annually Source: SeedQuest.com
28 February 2005

Corn: Estimates of direct losses in U.S. is \$250 to 500 million annually (Vardon et al. 2003).

These losses are not distributed equally!



So how does this impact ethanol production???

The U.S. Department of Agriculture estimated that the nation's ethanol distilleries would require 60 million tons of corn from the 2008 harvest.

The Earth Policy Institute factored in the additional investment in facilities since then, and came up with a figure of 139 million tons.

This includes:

The institute's estimate of current annual production levels, 53 million tons.

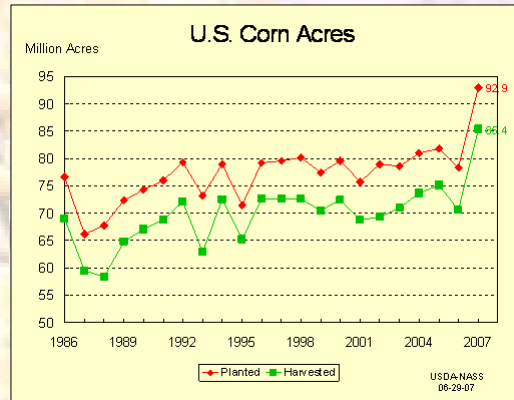
Expansion of existing facilities, which would increase requirements by 8 million tons.

Production by 79 plants under construction, requiring another 51 million tons.

Another 27 million tons of corn that would be consumed by distilleries currently in the planning stage and scheduled for completion by September 2008.

Top Maize Producers in 2005	
(million metric tons)	
 United States	280
 China	131
 Brazil	35
 Mexico	21
 Argentina	20
 Indonesia	15
 France	13
 India	12
 South Africa	12
 Italy	11
World Total	692

Source:
UN Food & Agriculture Organisation
(FAO)[1]



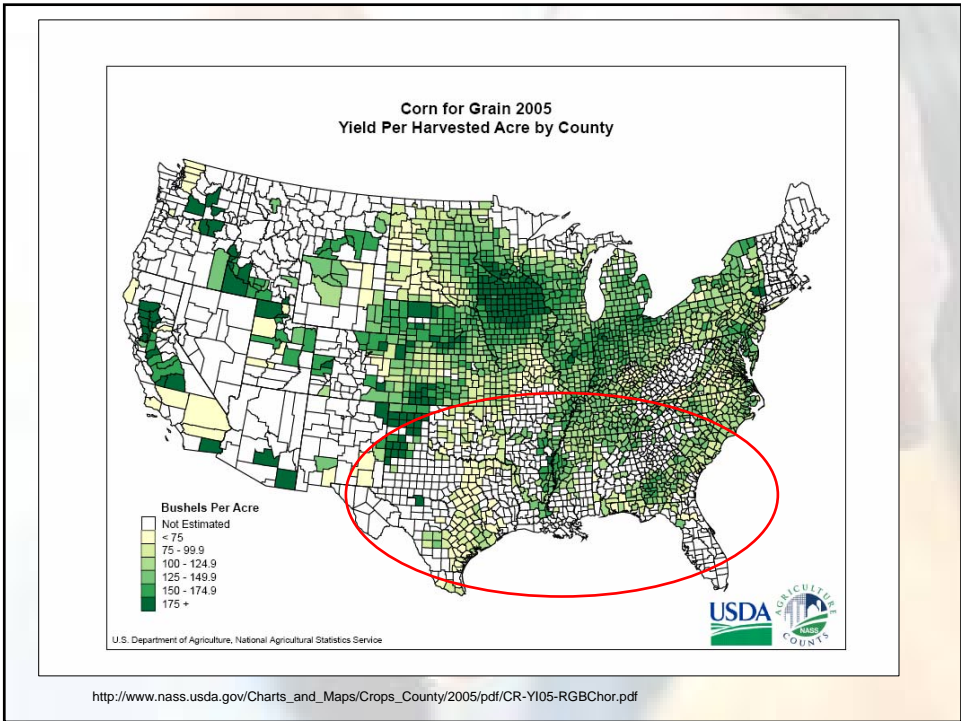
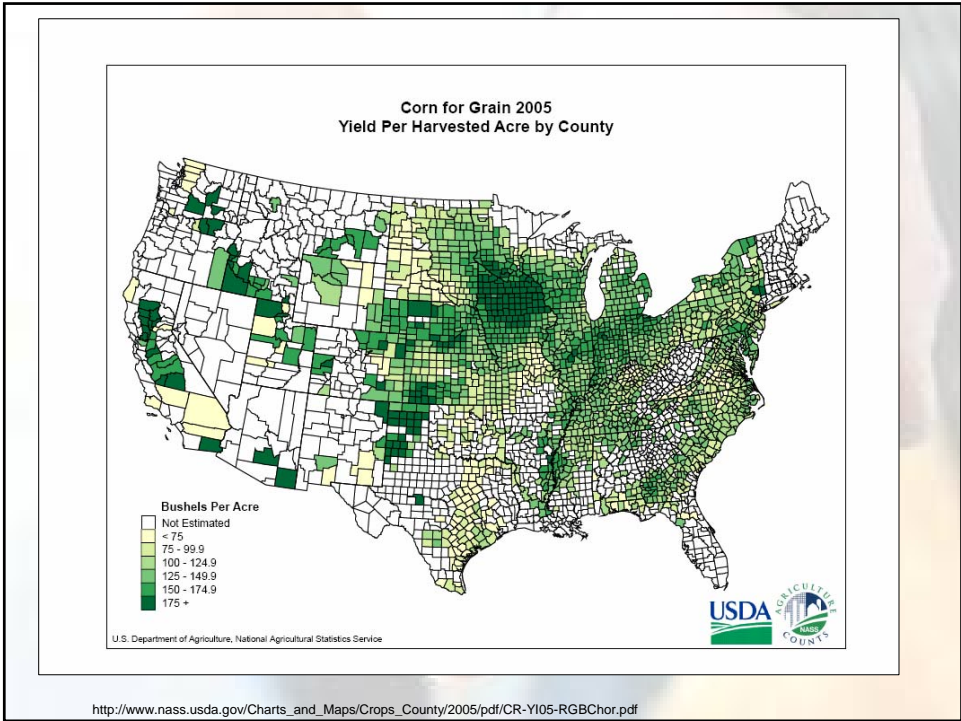
http://www.nass.usda.gov/Charts_and_Maps/Field_Crops/cornac.asp

Food VS. Fuel



- Corn is caught in a tug-of-war between ethanol plants and food,
- Greg Boerboom raises 37,000 pigs in Marshall, Minn. Those hogs eat a lot of corn—10 bushels each from weaning to sale. In past years he has bought feed for about \$2 a bushel. But since late summer, average corn prices have leapt to nearly \$4 a bushel. To reduce feed costs, he sells his pigs before they reach the normal 275 pounds, and keeps them warmer so they don't devour more food fighting off the cold. "The next eight months will be really tough."
- In the U.S., last year's harvest was 10.5 billion bushels, the third-largest crop ever. But instead of going into the maws of pigs or cattle or people, an increasing slice of that supply is being transformed into fuel for cars. The roughly 5 billion gallons of ethanol made in 2006 by 112 U.S. plants consumed nearly one-fifth of the corn crop. If all the scores of factories under construction or planned go into operation, fuel will gobble up no less than half of the entire corn harvest by 2008.

BusinessWeek, February 5, 2007



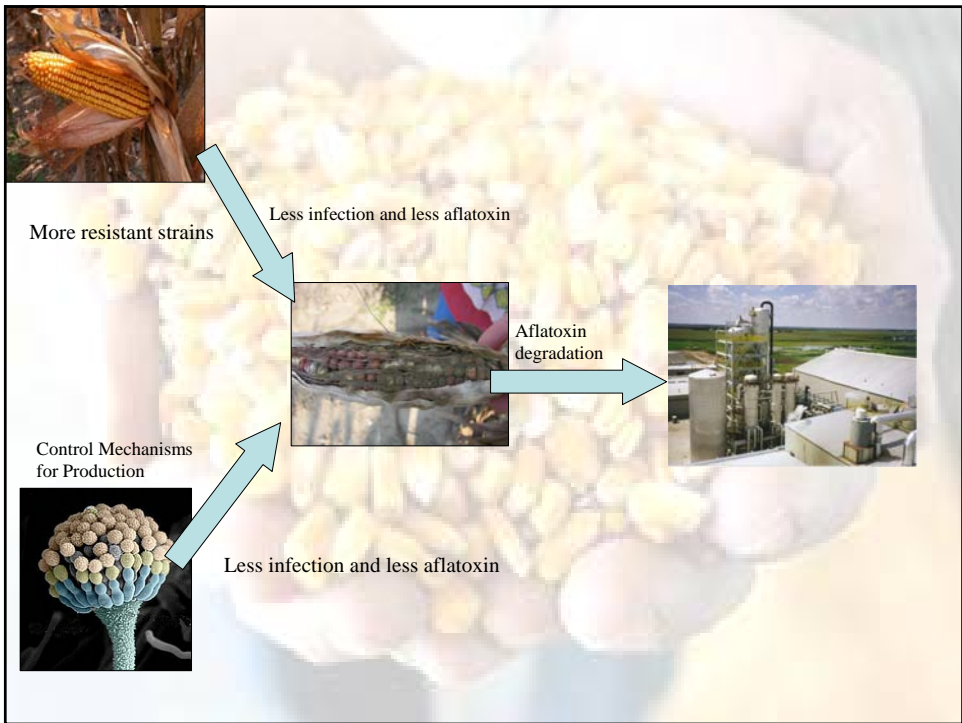
Existing and planned U.S. corn processing plants.



<http://images.google.com/imgres?imgurl=http://www.ipm.iastate.edu/ipm/icm/files/images/USAEthanolMap20070212.gif&imgrefurl=http://www.ipm.iastate.edu/ipm/icm/2007/2-12/ethanol.html&h=318&w=504&sz=63&hl=en&start=0&tbid=m0Js0nLFGTKiZM:&tbh=82&tbw=130&prev=/images%3Fq%3Dmap%2B%2Bcom%2Bproduction%2Bsvnum%3D10%26h%3D0en>

Can we remove the aflatoxin?

- Resistant to Heat
- Can be removed by ammoniation procedure.
 - Not Approved by FDA
 - Metabolites as toxic as Aflatoxin remain
 - Expensive
- Not Harvest it?!



Quality Control and Testing



- FDA/AAFCO Annual Briefing and the Enforcement Issues Committee
- Distiller by-products from ETOH production
 - Antibiotic residue, mycotoxins, high sulfur, high salt
- Industry has complained of false positives with mycotoxin test kits

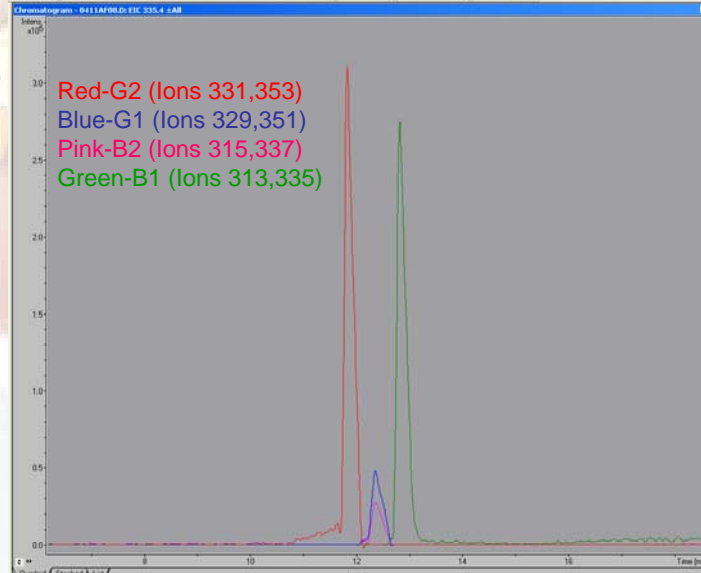
August 2007 Memo to FDA/AAFCO Committee Members

Analytical Method

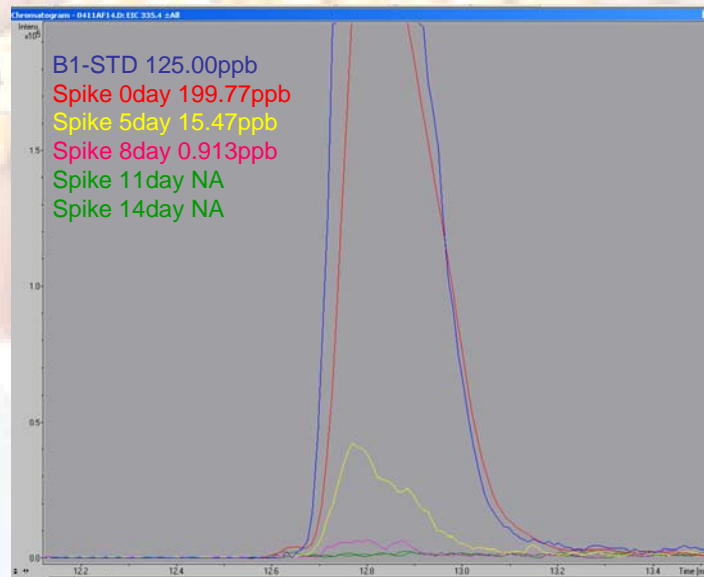
Liquid Chromatography/Mass Spectrometry

- Column: Restek Pinnacle II C18 5um 150x2.1mm
- HP 1100 Solvent Delivery System
 - Flow rate of 300uL/min.
 - Water +0.1%FA: ACN+0.1%FA
 - 95:5 hold for 2 min programmed gradient to 5:95 over 16 min and hold for 7 min.
- Bruker Esquire Mass Spectrometer
 - ESI positive ion isolation
 - m/z 353.5 G2
 - m/z 351.4 G1
 - m/z 337.4 B2
 - m/z 335.4 B1

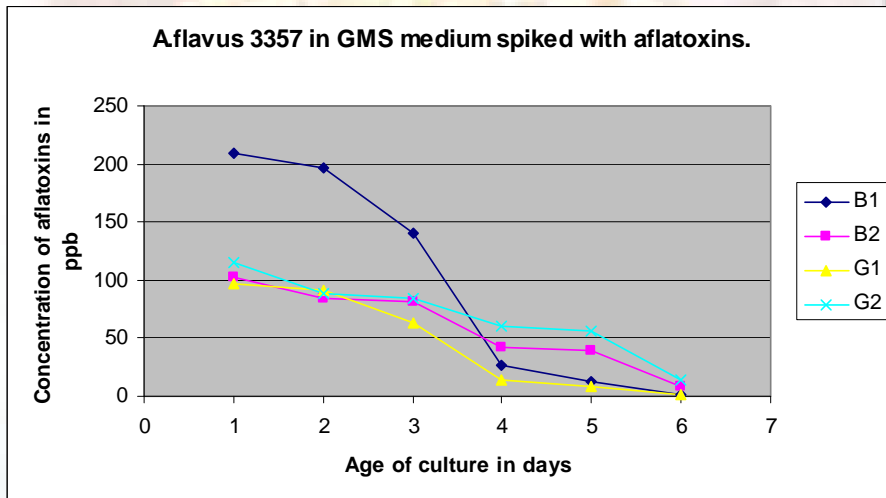
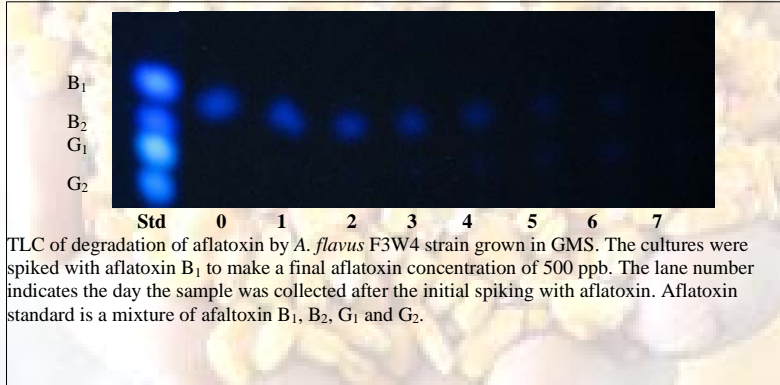
Extracted Ion Chromatogram of Standard



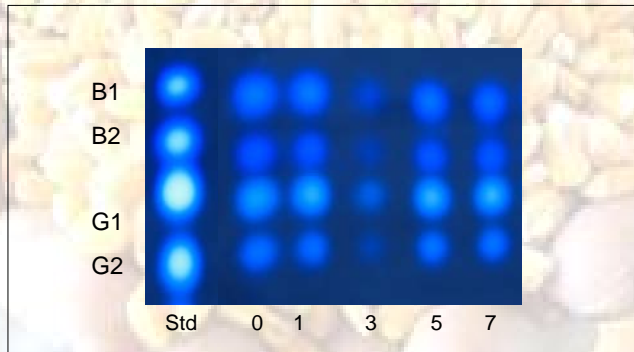
Chromatogram Analysis of Degradation



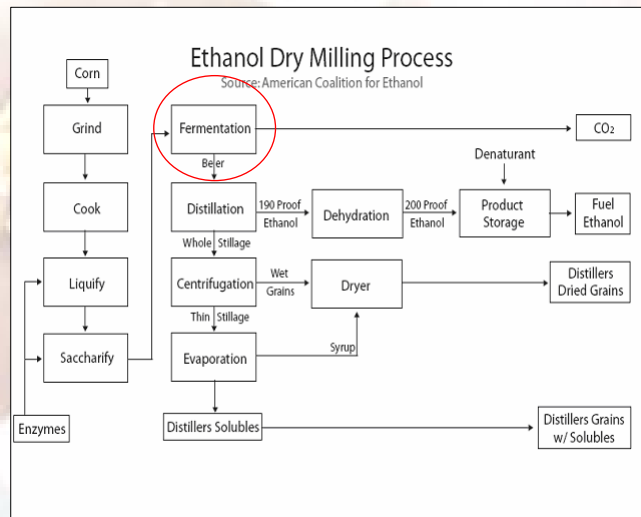
Enzymatic Degradation!



TLC profile of *A. flavus* F3W4 strain grown in Corn slurry medium spiked with 500 ppb B1 and G1, and 100 ppb of B2 and G2.



Lane number indicates the day the sample was collected. Aflatoxin standard is mixture of B1, B2, G1, G2. There is no degradation in this media.



* Diagram from the American Coalition for Ethanol.

http://images.google.com/imgres?imgurl=http://sitemaker.umich.edu/section4group6/files/dry_mill.bmp&imgrefurl=http://sitemaker.umich.edu/section4group6/appendix_a_%25E2%2580%2593_ethanol_production&h=512&w=640&sz=961&hl=en&start=0&um=1&tbid=OQjQkT84_CephM:&tbnh=110&tbnw=137&prev=/images%3Fq%3Ddistillers%2Bdry%2Bgrains%26svnum%3D10%26um%3D1%26hl%3Den%26sa%3DN

So How will this effect EtOH?

- Corn sales at \$4.00 per bushel in 2007.
- High aflatoxin levels can cause deep discounts resulting in crops selling for as low as \$0.22 per bushel.
- Approximately 50% of the cost of ethanol production results from cost of the feedstock itself (Pimentel, 2003).
- Even if contaminated stocks sold at \$0.50 a bushel EtOH production in the south becomes more economically viable with the utilization of these contaminated stocks.



Collaborators and Contributors

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