

Understanding and Managing Molds, Mycotoxins and Allergens in Grain and Food

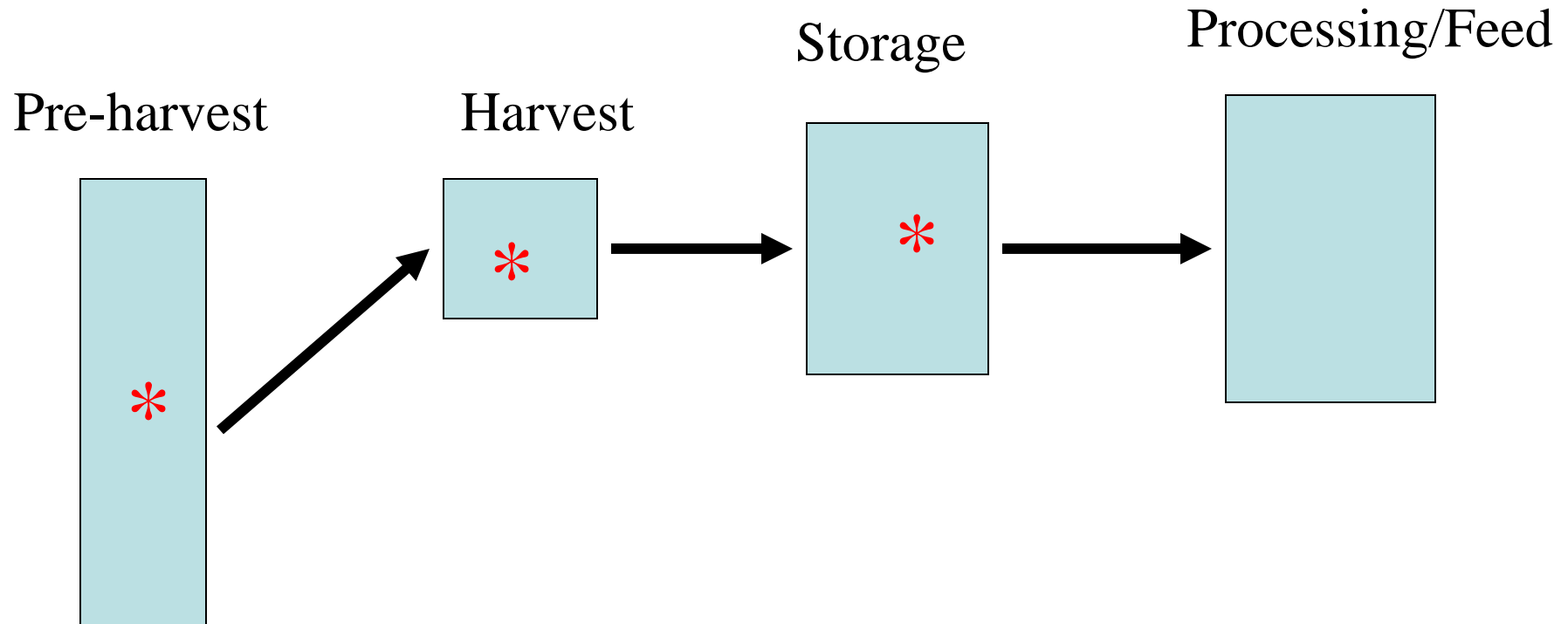
Charles Woloshuk
Purdue University

VIIth National Stored Product Integrated Pest Management
Training Conference
April 18, 2007
Stillwater, Oklahoma

Outline

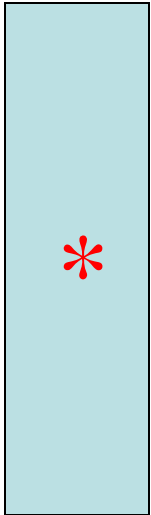
- Mycotoxins: Sources
- Pre-harvest Survey
- Maintaining Dry Storage Conditions
- Mycotoxin Analysis
- Purdue Research Highlights

Where are some Critical Control Points * for Molds & Mycotoxins?



Critical Control Points

Pre-harvest



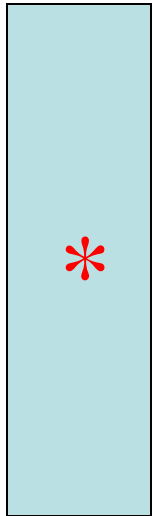
Weather

Insect Damage

Ear Rots

Critical Control Points

Pre-harvest



Weather

Insect Damage

Ear Rots

Surveillance

PREHARVEST

Ear Rots

Weather Extremes
Insect Damage



DON
Zearlalenone

Fumonisin
Aflatoxin



Rain during flowering
75-85 F

Drought
Insect damage

Gib Ear Rot Head Blight (Scab)

VOMITOXIN (DON) & ZEARALENONE



VOMITOXIN (DON)

FDA LIMITS

- **1 PPM** **HUMAN FOOD**
- **5 PPM** **SWINE, < 20% OF DIET (1 ppm)**
- **10 PPM** **CATTLE AND CHICKENS, < 50% OF DIET**
- **5 PPM** **ALL OTHER ANIMALS, < 40% OF DIET**
- No action, guidance or advisory levels for zearalenone have been established by the FDA

FUSARIUM EAR ROT

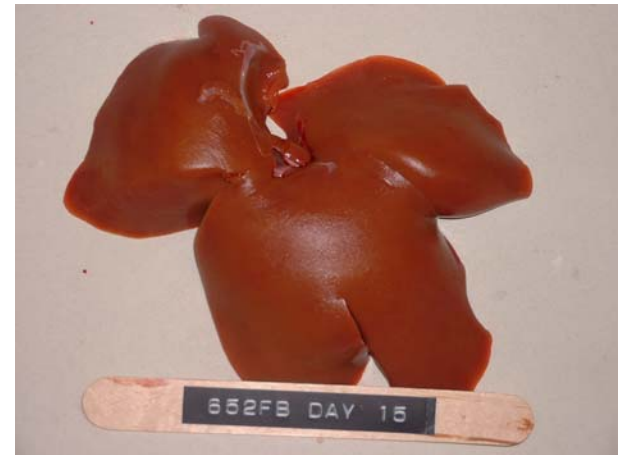


Toxicology of FUMONISIN (FB₁)

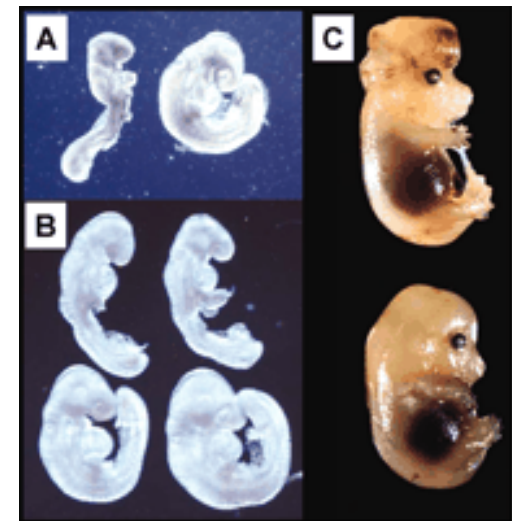


Horses: equine leukoencephalomalacia (ELEM)

www.vet.orst.edu/ppt/VM721



Pigs: liver damage and porcine pulmonary edema (PPE)



Marasas et al, 2004
Humans: esophageal cancer, nephropathy, neural tube defects (NTD)

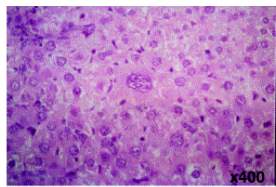


Fig. 3



Fig. 4

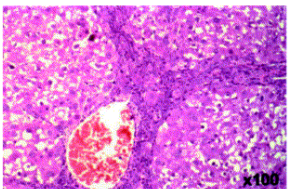
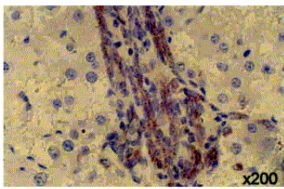


Fig. 5



Gelderblom et al, 2001

Rodents: liver damage and carcinogenesis; nephropathy

Fumonisin: FDA Advisory

Food 2-4 ppm

Equids 5 ppm , < 20% of diet

Swine 20 ppm, < 50% of diet

Ruminants 30-60 ppm, < 50% of diet

Poultry 100 ppm, < 50% of diet

ASPERGILLUS EAR ROT



Aflatoxin

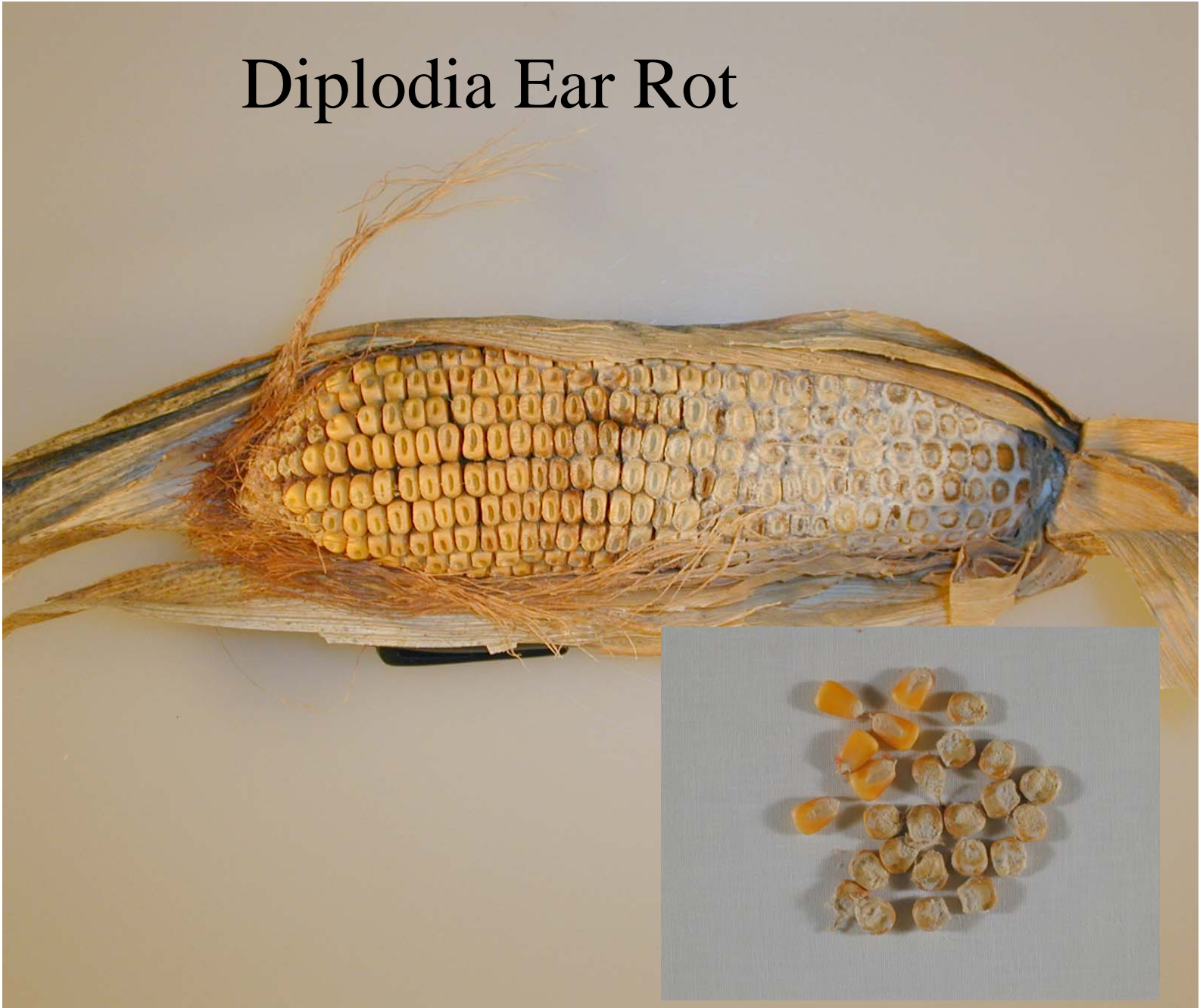


AFLATOXIN

FDA ACTION LIMITS

- **0.5 PPB** **MILK**
- **20 PPB** **GRAIN FOR INTERSTATE TRADE**
- **20 PPB** **HUMAN FOOD & DAIRY CATTLE**
- **100 PPB** **BREEDING CATTLE & SWINE**
- **100 PPB** **MATURE POULTRY**
- **200 PPB** **FINISHING CATTLE & SWINE (over 100 lb)**

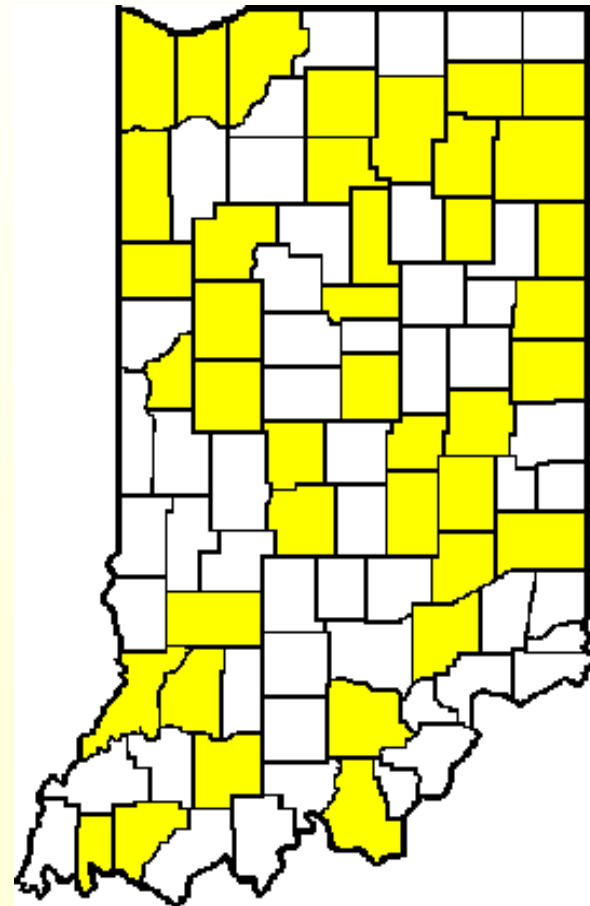
Diplodia Ear Rot



Indiana Corn Ear Rot and Mycotoxin Survey 1989-2006

1989 Objective: Develop a standardized survey and sampling protocol to assess ear rots and mycotoxins in Midwest

1995 Objective: Use the survey as one tool to assess quality of the Indiana corn harvest.



Sample Collection



Corn Field

1. Go to row #1 of Unit 1 & 2
2. Count to the 11th ear beyond each unit
3. Harvest the 11th-15th ears
4. Place ear sample in cloth mailing bag
5. Mail samples to Purdue University

Postage Cost: \$5 -7 per sample





Ear Rot Diseases

Fusarium ear rot
Gib ear rot
Aspergillus ear rot
Diplodia ear rot
“Minor” ear rots



Mycotoxin Analyses

Purdue University Animal Disease and Diagnostics Lab

Aflatoxin

Zearalenone

DON

Ochratoxin

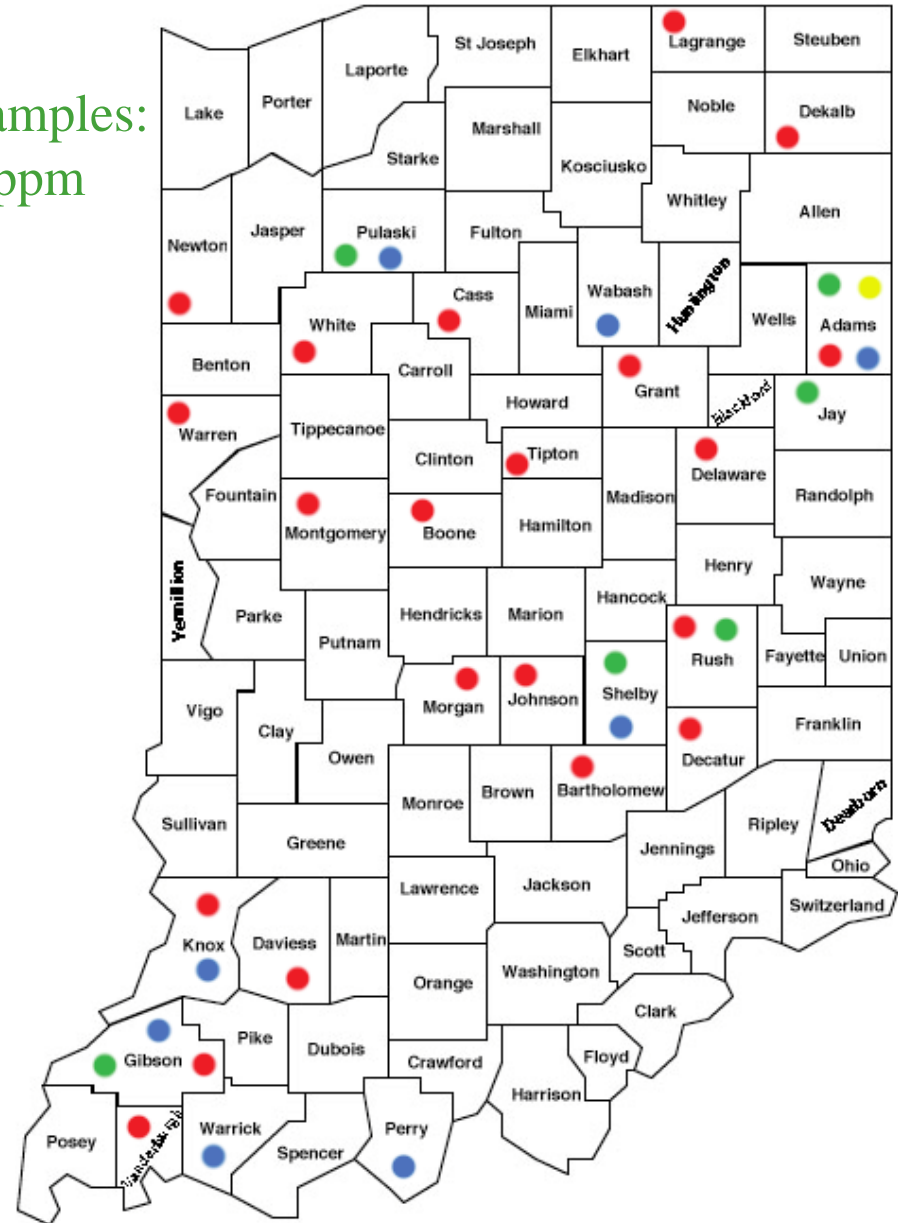
Fumonisin

Cost: \$ 45 per sample



- 2006
- 316 samples from 158 fields
 - 20 analyzed for mycotoxins

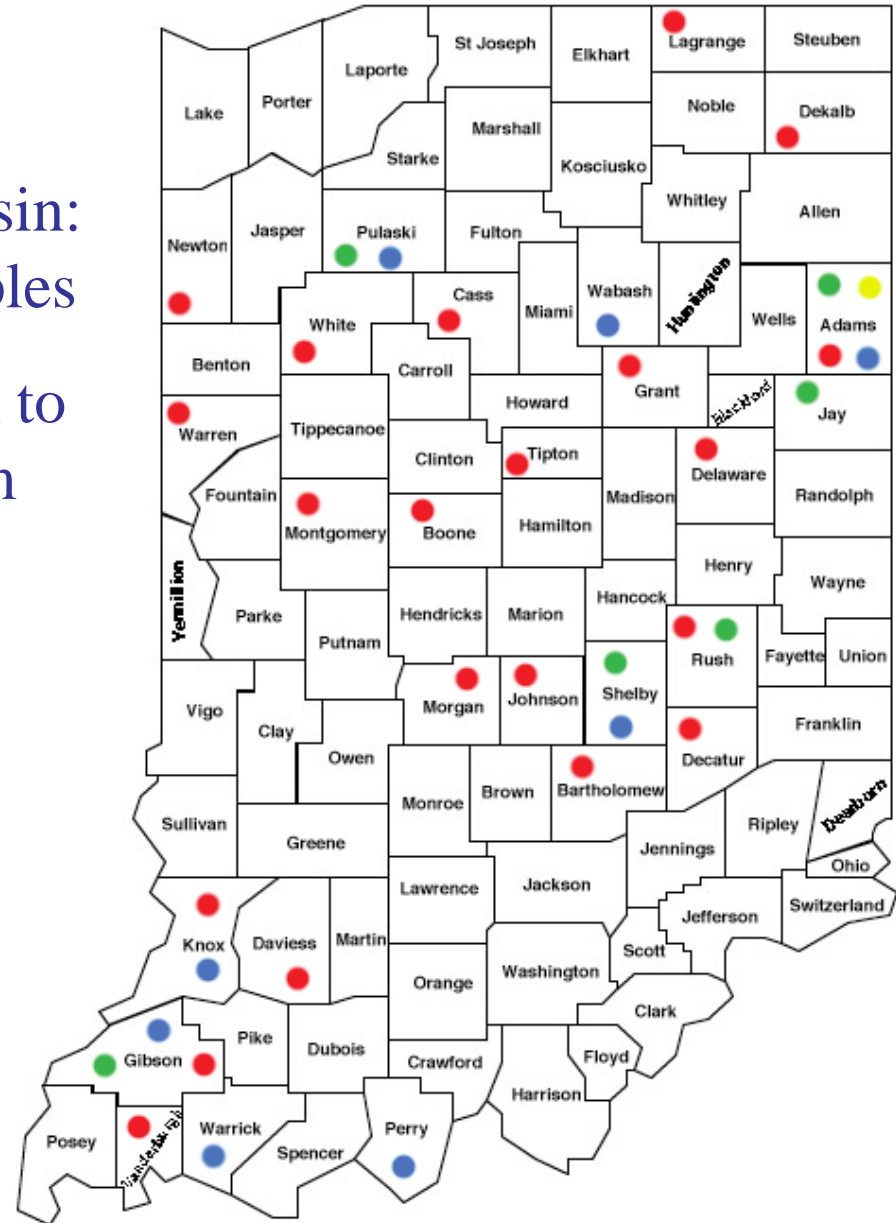
DON: 6 samples:
trace to 8 ppm



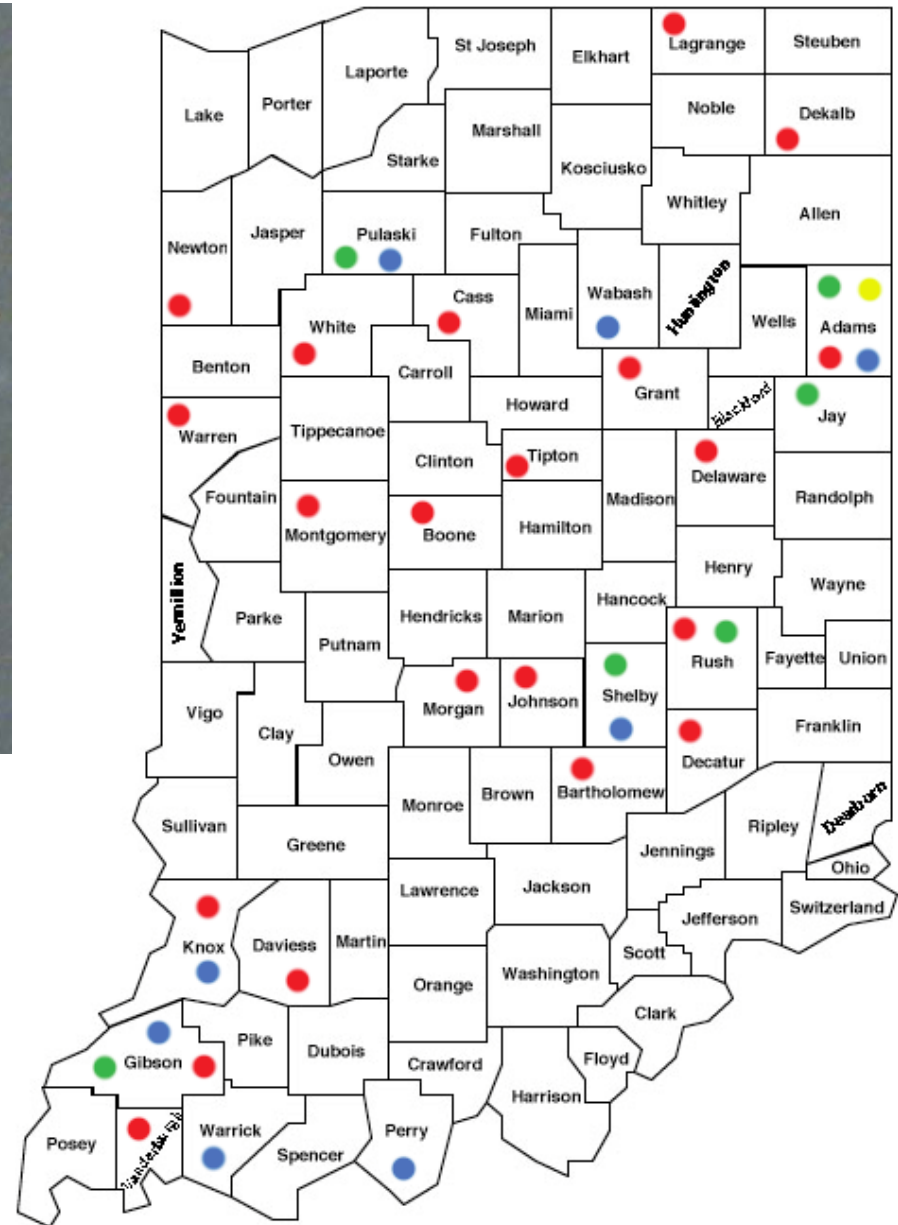


Fumonisin:
10 samples
0.8 ppm to
175 ppm

- 2006
- 316 samples from 158 fields
- 20 analyzed for mycotoxins



- 2006
- 316 samples from 158 fields
 - 20 analyzed for mycotoxins



Aflatoxin: one sample with 10 ppb

Disease was not recognized. Sample had 9 ppm fumonisin

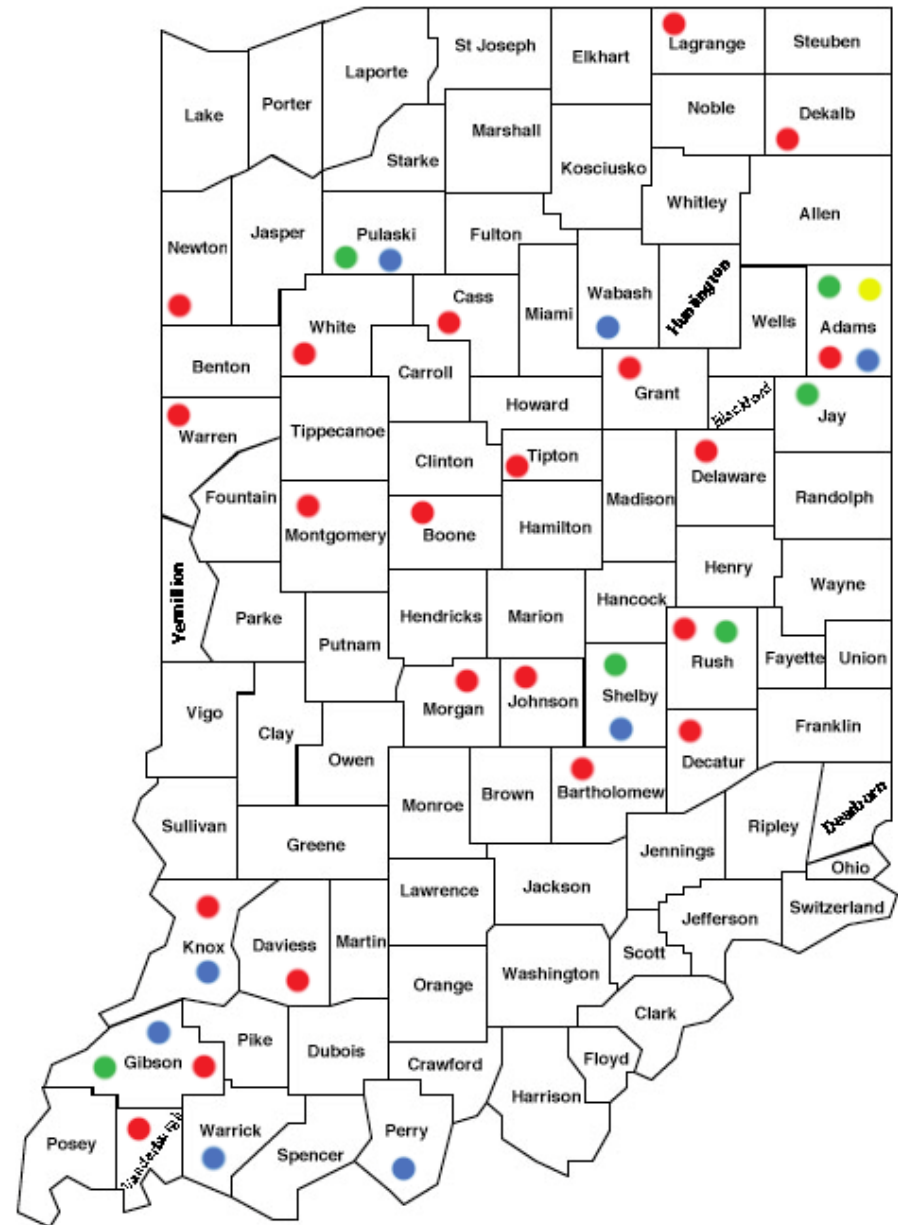
2006 • 316 samples from 158 fields



Diplodia Ear Rot:

23 samples

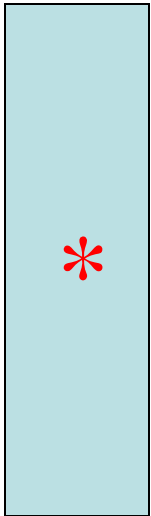
7%



Critical Control Points

Control Action

Pre-harvest



Hybrid Selection

Tillage

Crop Rotation

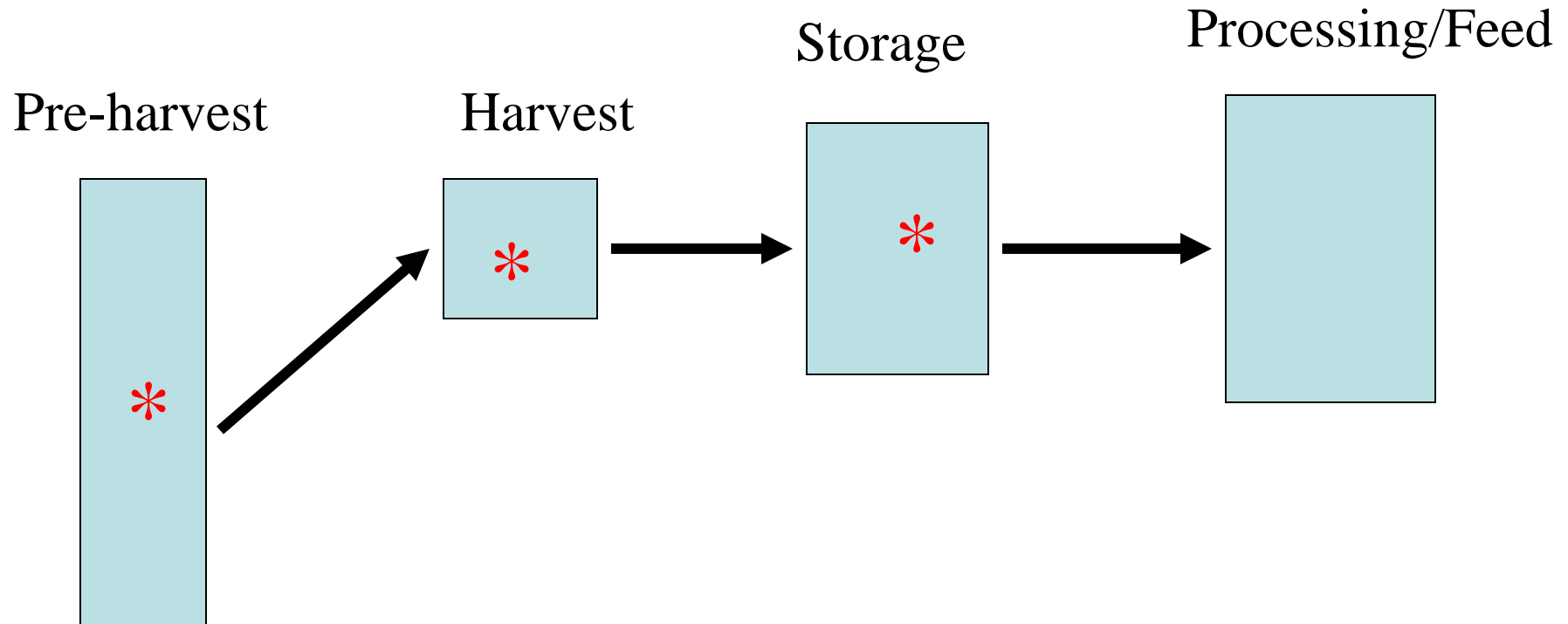
Harvest Diseased Crop First

Segregate Grain

What should you do if you have pre-harvest mycotoxin contamination?

- Harvest early and dry grain immediately
- Test for mycotoxins to determine level
- Clean grain before storage
- Do not hold grain into summer months

Where are some Critical Control Points * for Molds & Mycotoxins?



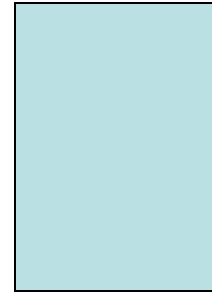
Critical Control Points

Harvest



Excessive Breakage
Trash
Dryer Breakdown

Storage



Hot Spots
Leaks
Condensation
Insects

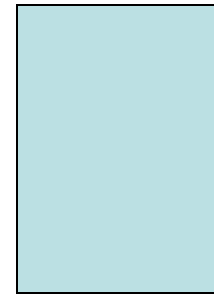
Critical Control Points

Harvest



Control Action

Storage



Diligence: “conscientiousness in paying proper attention to a task; giving the degree of care required in a given situation”

Sanitation **L**oading **A**eration **M**onitoring

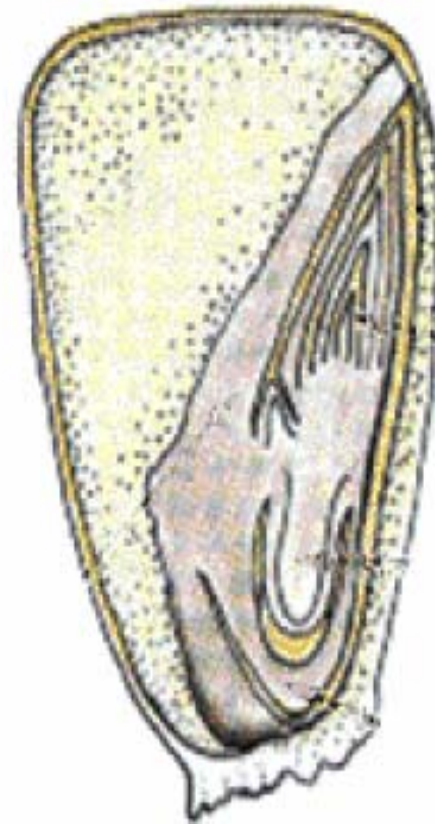
Conditions that Contribute to Spoilage of Grain

- Moisture content of the grain
- Temperature of the grain
- Amount of broken kernels and foreign material
- Degree which the grain is invaded by mold

Deterioration of Grain in Storage

How is it measured?

1. Loss of seed germination
2. Loss of dry matter
3. Increase in fat acidity
4. Grain heating
5. Sprouting



Impact of Mold

Each fungal species has its own moisture preference

- **Field Fungi**
 - Grow best under **high moisture** conditions
 - Do not grow well or compete well under dry storage conditions
- **Storage Fungi**
 - Adapted to grow under **low moisture** conditions
 - Normally invade kernels after harvest
 - Generate heat and moisture as they grow

Minimum Water Activities Supporting Field and Storage Fungi

Fungal Strain	Minimum water activity
<i>Stenocarpella maydis</i> (<i>Diplodia</i>)	0.96
<i>Fusarium graminearum</i>	0.95
<i>Fusarium verticillioides</i>	0.87
<i>Aspergillus flavus</i>	0.78
<i>Aspergillus candidus</i>	0.75
<i>Eurotium</i> spp. (<i>A. glaucus</i>)	0.72

J.E.L. Corry, 1986

Water Activity

High

Low

Very Low

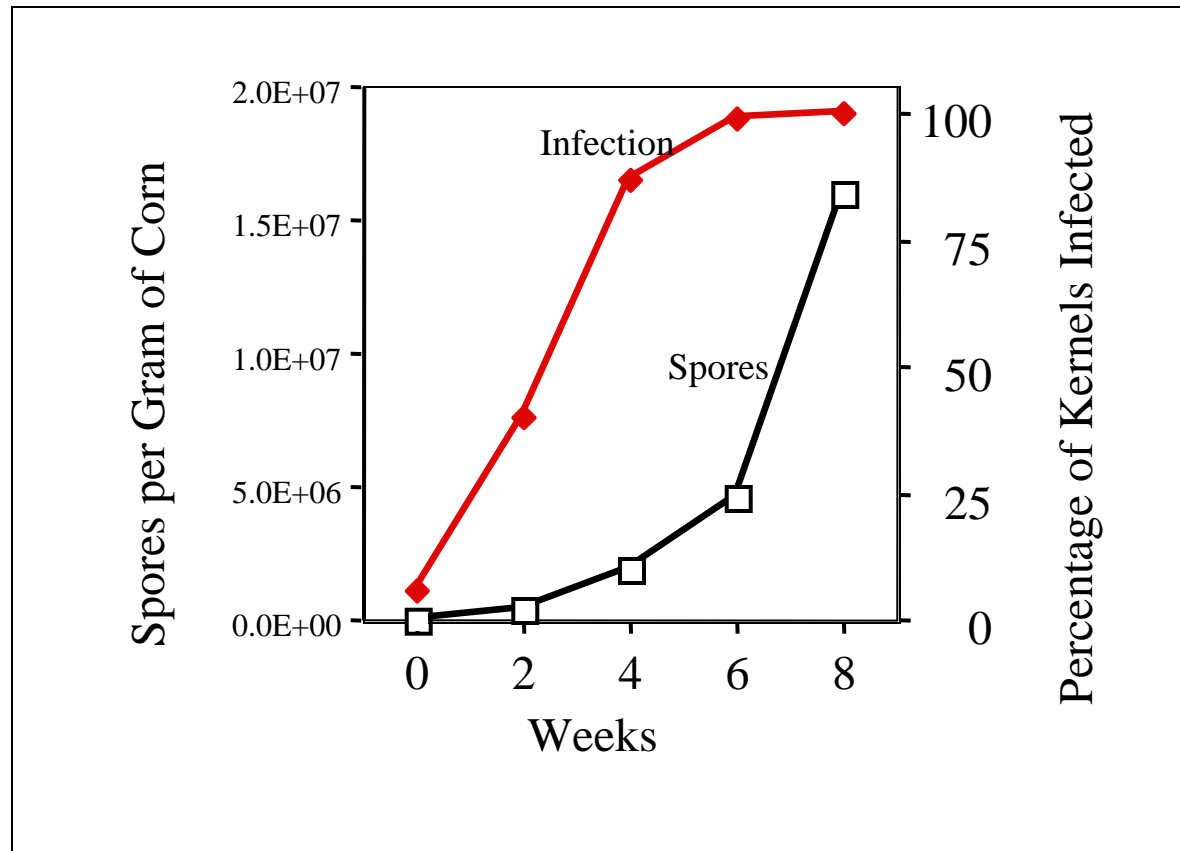
Eurotium spp.
Storage Mold

Fusarium graminearum
Field Mold

Aspergillus flavus
Field/Storage Mold



Growth of *Eurotium sp.*
in 15 % Moisture Corn Stored at 90 ∇ F



Temperature

- At 40 F (5 C) growth is minimal
- At 85 F (29 C) is ideal if moisture is favorable

Time of Storage (Corn)


Temperature (F)	Grain Moisture (%)			
	14	15	16	17
	Months			
40	58	28	14	9
50	34	16	9	5
59	21	10	5	3
68	13	6	3	2
77	8	4	2	1

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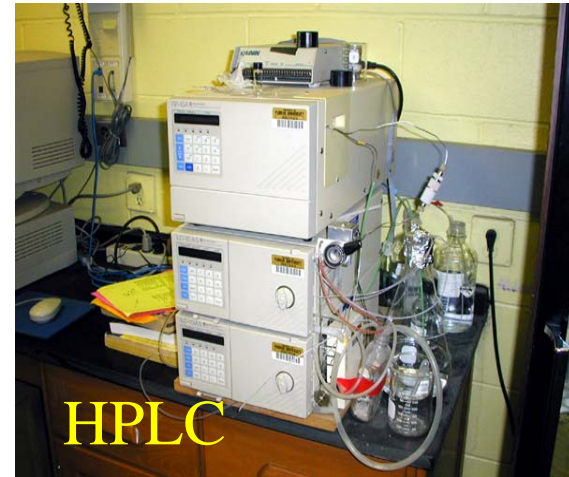
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Testing for Mycotoxins



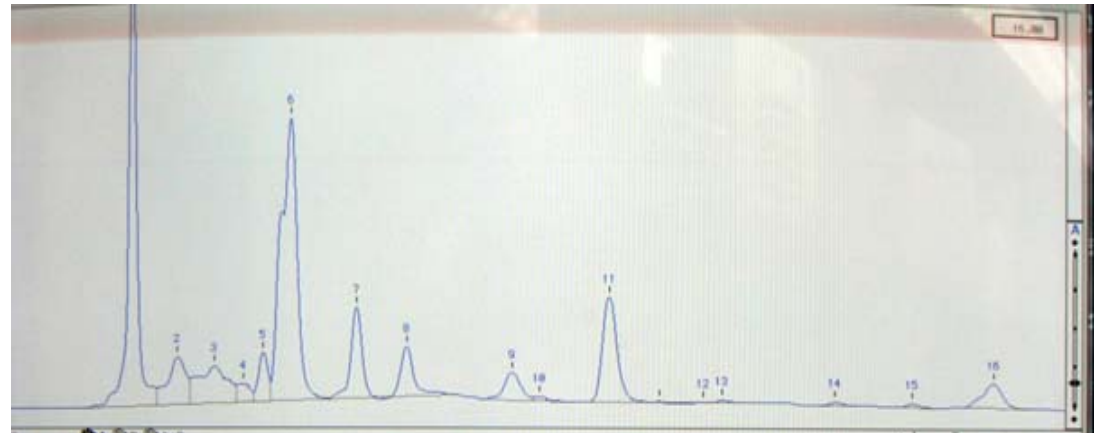
Test Kits



HPLC

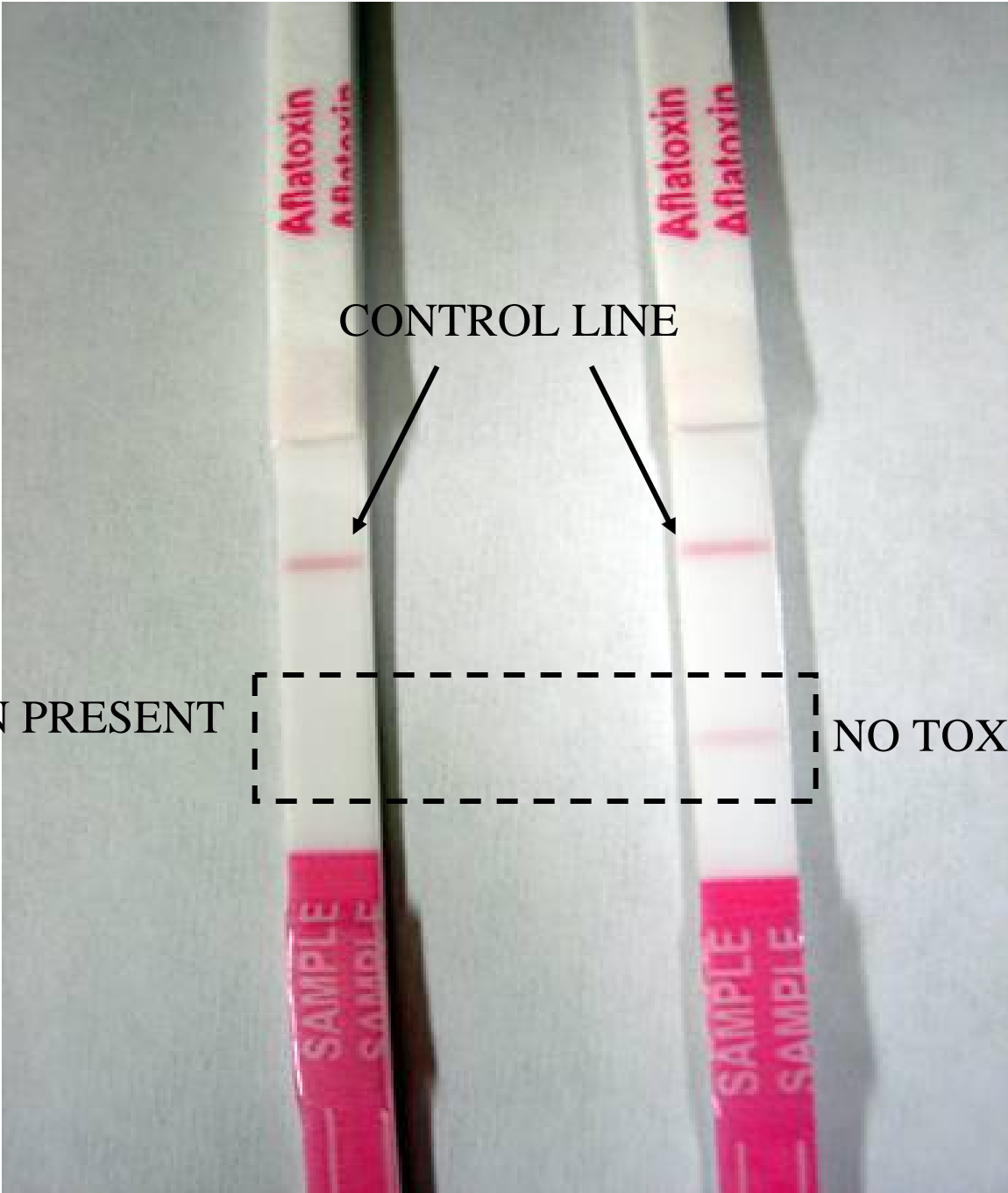


ELISA



HPLC Trace





CONTROL LINE

TOXIN PRESENT

NO TOXIN PRESENT





Research

Effect of fungal species on the development of Hairy Fungus Beetle



	Development Time
Aspergillus	9 - 15 days
Eurotium	11 - 22 days
Penicillium	11 - 25 days

Moldy Grain Attracts Mold-Feeding Insects



Hairy Fungus Beetle
Foreign Grain Beetle
Rusty Grain Beetle

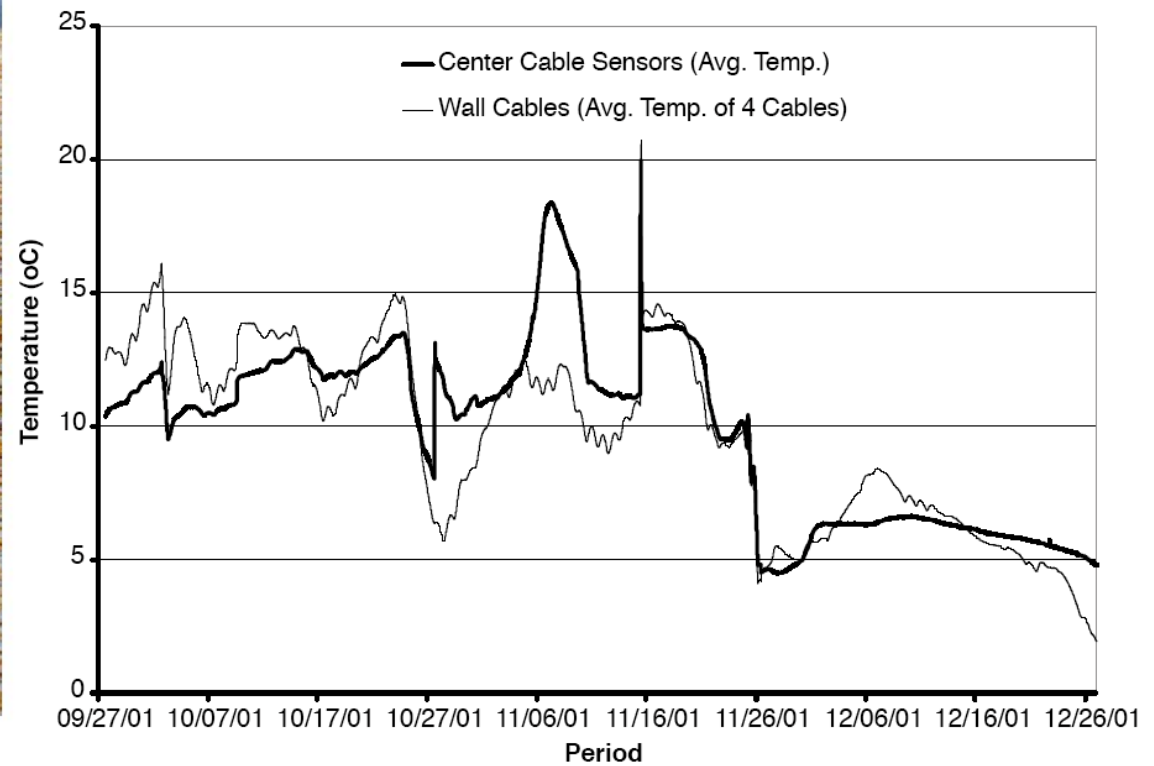
Ozone Treatment

Raj Hulasare

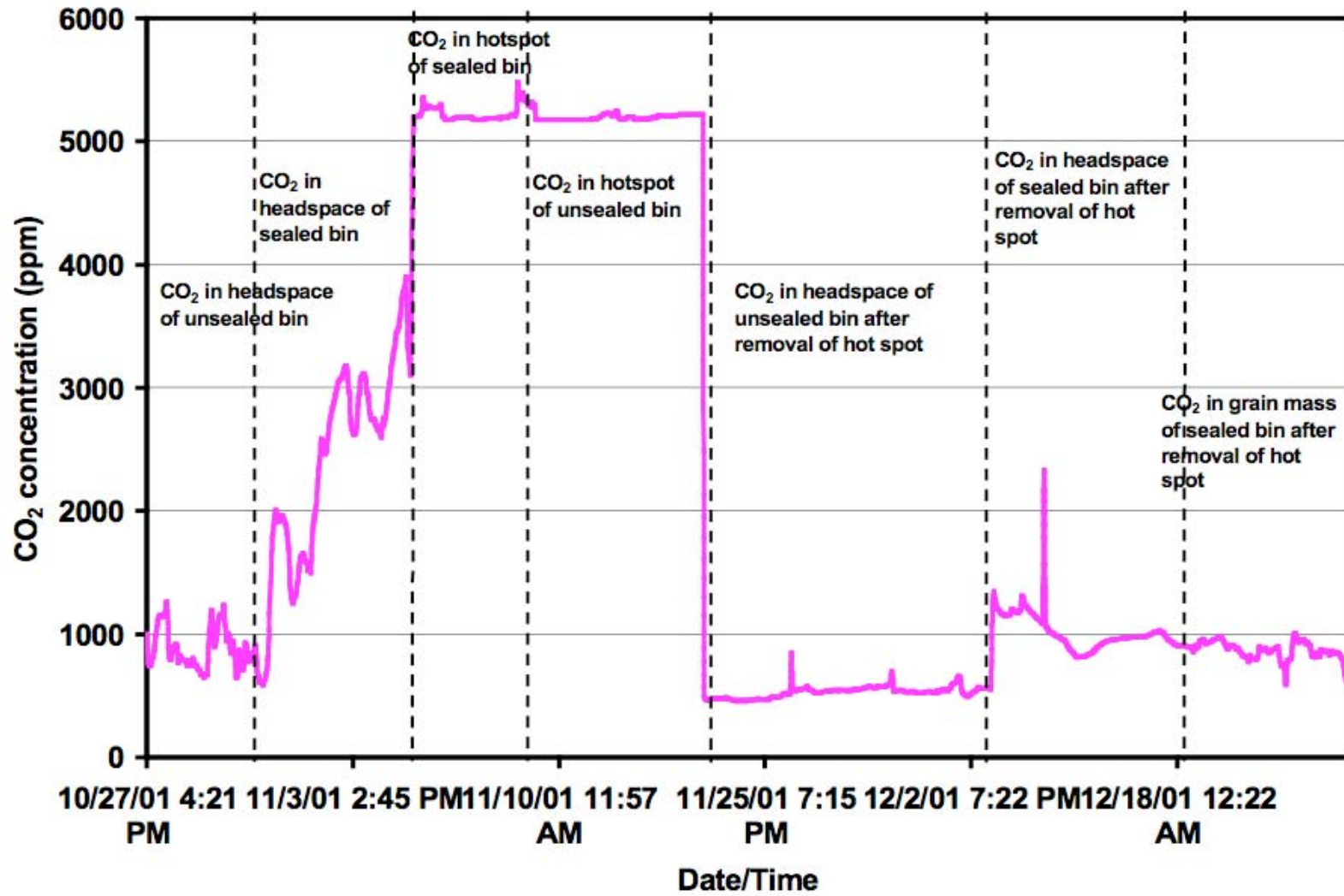


CO₂ SENSORS

Klein Ileleji et al., Applied Engineering in Agriculture 2006



CO2 SENSORS



Summary

- Surveillance
- Diligence
 - SLAM program
- Maintain dry conditions