# Immunocapture Real-Time PCR to Detect Mycotoxigenic Mold Spores in Grains and Foods

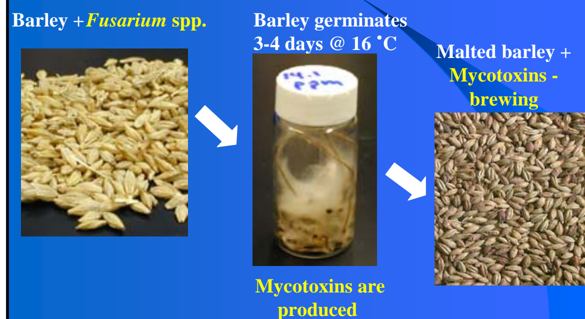
M. A. Cousin
Department of Food Science
C. P. Woloshuk
Department of Botany and Plant Pathology
Purdue University

## Mycotoxigenic Molds

- *Aspergillus* species: aflatoxins ($B_1$ and $M_1$), ochratoxin A
- *Fusarium* species: deoxynivalenol (DON), fumonisins ($B_1$ and $B_2$), zearalenone
- *Penicillium* species: ochratoxin A
- Action levels for aflatoxins
- Advisory levels for DON, fumonisins, zearalenone

## Detect Molds or Mycotoxins?

Mycotoxins can be produced during some food manufacture.




## Previous Research

- Develop immunocapture real-time PCR method for *Fusarium* species
- Limitation on PCR due to inability to release DNA from spores

## Objective of Research

- To study methods to break mold spores to release DNA
- To incorporate the best method into immunocapture real-time PCR to detect *molds* in foods and grains
- To develop a PCR library to mycotoxigenic molds

## Methods to Study for Spore Breakage

- Enzymatic - lyticase digestion
- Physical methods - heat or cold
- Mechanical methods – ball mill, FastPrep™
- Partial spore germination
- Combination of methods

## Breakage of Spores

- **Prelimary research**

  **1. Lyticase can break some *Fusarium* spores**

  **2. Some *Fusarium* spores germinate in 2-3 h**

  **3. FastPrep™ variable for *Fusarium* species**

- **Physical methods – just beginning**

## PCR Library For Detection of Mycotoxigenic Molds

- Current Activities
  - –Multiplex Genus-specific Assay: Developing TaqMan primers and probes to detect the presence of Penicillium, Fusarium, and Aspergillus species
  - –Multiplex Toxin-specific Assay: Assembling TaqMan primers and probes to detect pathway genes for DON, fumonisin, ochratoxin, and aflatoxin

## Future Research

- **Incorporate procedures to break spores into real-time PCR**
- **Simplify immunocapture before real-time PCR**
- **Test in industry to determine cost effectiveness**