The Environment and Azole Resistance in *Aspergillus*
The Ohio State University

• Public land grant university in Columbus, OH
  – Founded in 1870
  – More than 50,000 students
  – Ohio Agricultural Research and Development Center (OSU Wooster campus)

• College of Food, Agricultural and Environmental Sciences

• College of Veterinary Medicine
  – Teaching, research and extension in agriculture and the environment
    • AMR research and outreach in animal and plant pest/disease management
Aspergillus spp. Causing Invasive Aspergillosis

- *Aspergillus fumigatus* (~80-90% of cases)
- *A. flavus*
- *A. niger*
- *A. terreus* and others
- Taxonomic separation into Sections
  - Morphology, colony color, genetic variation (MLST)
  - Asexual stage (anamorph) common in environment
  - Sexual stage (teleomorph) in *A. fumigatus, A. flavus* – heterothallic
    - *Neosartorya* (A. fumigatus); *Petromyces* (A. flavus)

https://www.ppdictionary.com/mycology/fumigatus.htm
MLST = Multilocus sequence typing, a molecular method by which certain fungal genes, often housekeeping genes, are sequenced and compared.

Asexual stage morphology (pictured): stalk ending in vesicle with phialides generating unbranched chains of conidia (oldest most exterior).

Sexual structure is cleistothecium containing asci w/ eight ascospores each
Biology of *Aspergillus*

- Vegetation decomposers critical to carbon and nitrogen cycling

- Some are weak but important plant pathogens
  - *A. flavus*: widespread in grains, oilseeds, peanuts, spices, others; aflatoxin producer
  - *A. niger*: grains, vegetables, others
Although A. fumigatus grows optimally at 37C and a pH 3.7 to 7.6, it can be isolated wherever decaying vegetation and soil reach temperatures range between 12C and 65C and the pH ranges between 2.1–8.8 (Kwon-Chung & Sugui 2013)

Aflatoxin B₁ is a potent carcinogen and also causes acute liver damage, stunting in children, malnutrition, etc.

Top row photos: *Aspergillus flavus* on corn ear (left) and kernel (right).
Bottom row photos: *Aspergillus niger* on corn ear (left) and onion (right).
Biology of *Aspergillus*

- Conidia highly resilient
- Resistant to heat shock, freezing/thawing, osmotic stress, low pH, alkalinity, desiccation/rehydration, low oxygen, ionizing radiation, UV, C/N starvation
  - *A. fumigatus*
    - Optimum growth 37°C, pH 3.7-7.6;
    - Growth range 12-65°C, pH 2.1-8.8
- Cleistothecia and ascospores resistant to environmental stressors
Although A. fumigatus grows optimally at 37°C and a pH 3.7 to 7.6, it can be isolated wherever decaying vegetation and soil reach temperatures range between 12°C and 65°C and the pH ranges between 2.1–8.8 (Kwon-Chung & Sugui 2013)

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Top row photos: *Aspergillus flavus* on corn ear (left) and kernel (right).

Bottom row photos: *Aspergillus niger* on corn ear (left) and onion (right).
Ecology of *Aspergillus*

- *Aspergillus* spp. are ubiquitous in the environment
  - Predominant fungi in decomposing vegetation
- Widely varying habitats
  - Plants, soil, compost, water, aerosols, animal systems, indoor environments
    - Decomposition, biodeterioration, food spoilage
- Extremely flexible nutrient utilization pathways: digest, then consume
  - Glycosylhydrolases, extracellular proteinases break down plant cell polysaccharide and protein substrates
Epidemiology of Aspergillosis (A. fumigatus)

• Community-acquired vs. hospital acquired

• Airborne route
  – Small (2-3 um), hydrophobic airborne conidia; aerial mycelia
  – Conidia may undergo cycles of settling and becoming airborne for months
  – Conidia can travel deep into lungs on inhalation

• Water route
  – High conidial loads in surface water but not ground water
  – Detected in water in hospitals; biofilms in water-handling systems
  – Conidia can be transported in aerosols
  – Strains may differ in adaptability to wet environments
Azole Resistance in *Aspergillus* – Triazole Use on Crops

- Use in U.S. agriculture began increasing in 2006
  - 11-14 of 76 million acres in corn belt sprayed with a fungicide in 2007
  - High commodity prices
  - “Plant health” marketing of QoI (strobilurin) and DMI (triazole) fungicides for corn, soybeans
    - EPA registered plant health use – 2009
  - Fusarium head scab emergence in wheat – triazole fungicides most effective

Agricultural Triazole Use in US

Source: Mother Jones April 11, 2019
Azole Resistance in *Aspergillus* – Triazole Use on Crops

- Triazoles use in the MW US
  - Corn
    - 1 application at tasseling
    - Triazole alone or paired with another fungicide, e.g. strobilurin
  - Wheat
    - 1-2 applications of triazoles for head scab
  - Soybeans
    - 1 application
  - Vegetables, fruits
    - Multiple applications but many are combination products
    - Lower acreage in MW than field crops

Source: Mother Jones April 11, 2019
Azole Resistance in *Aspergillus* – Potential Contributing Environmental Factors in the Midwest

- **Conservation Agriculture/reduced tillage**
  - Crop residues remain on soil surface; corn after corn common
  - Known to increase fungal diseases of corn (e.g. grey leaf spot) – reservoir of inoculum
  - Greater exposure of fungal decomposers to fungicides?

- **Climate change**
  - More intense rainfall events in Midwest; fungi generally favored by high moisture, high humidity
  - Greater use of fungicides to manage crop diseases
  - Increased reproduction by *Aspergillus* spp.?
At least 30% of the surface covered with residue.
Aspergillus flavus favored by high moisture.
Possible Mitigating Factors

• Physical factors
  – Corn and soybean crop canopies closed when triazoles are applied
  – Fungicides do not penetrate canopy
    • In corn, rarely reach the ear – poor fungicidal control of ear rots
    • Unlikely to come in contact with residue on soil surface possibly harboring *Aspergillus* spp.
  – Reduced tillage systems uncommon in vegetable crops
Possible Mitigating Factors

• Economic factors
  – Meta-analysis of fungicide tests in corn belt: low probability of recovery of cost of fungicide application in absence of disease (Paul et al. Phytopathology 2011)
  – Low prices for corn and soybeans contraindicate fungicide use

• Chemical factors
  – Combination products: triazole with fungicide with different mode of action
  – Reduced rates of active ingredients
  – Possible resistance to partner fungicide
Reducing Risk of Environmental Azole Resistance

- Integrated Pest Management
  - Utilize crop varieties with high levels of disease resistance
  - Utilize cultural practices to reduce disease risk
  - Apply fungicides only when disease risk is high enough to threaten significant yield and economic loss (economic threshold)
  - “Plant health” applications not consistent with IPM
  - Follow FRAC guidelines to reduce fungicide resistance development
  - Substitute biologicals when possible
Thank you