The Environment and Azole Resistance in *Aspergillus*

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Sally A. Miller
Department of Plant Pathology

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*)

Aspergillus fumigatus

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


A. flavus


A. niger

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 B_1 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 37C, pH 3.7-7.6;
    - Growth range 12-65C, pH 2.1-8.8
- Cleistothecia and ascospores resistant to environmental stressors

Mycology Online Univ. Adelaide

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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

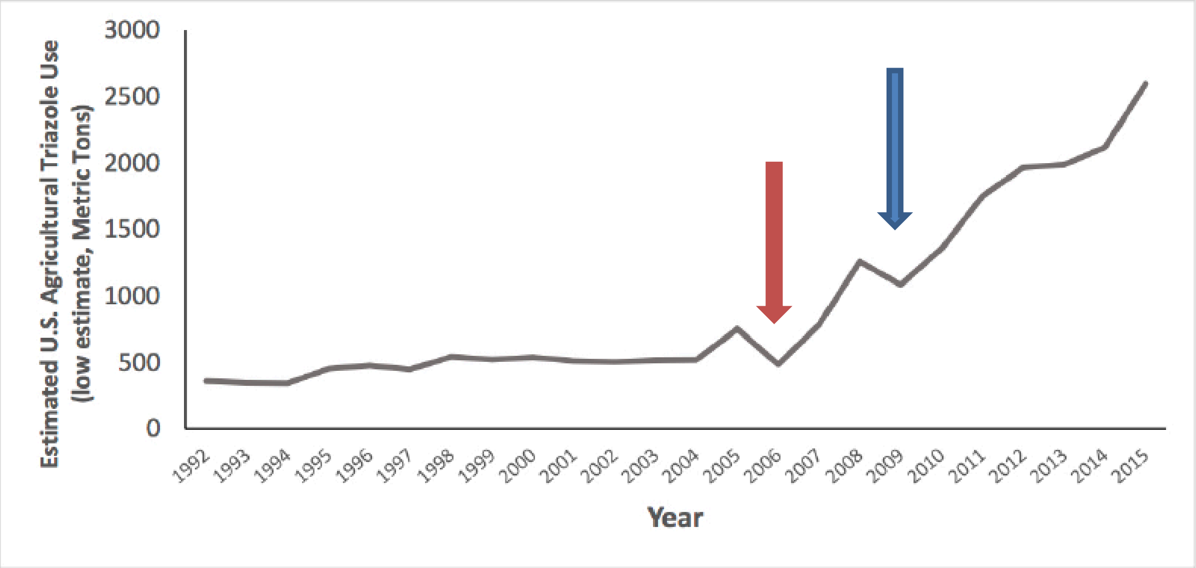
Epidemiology of Aspergillosis (*A. fumigatus*)

- 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

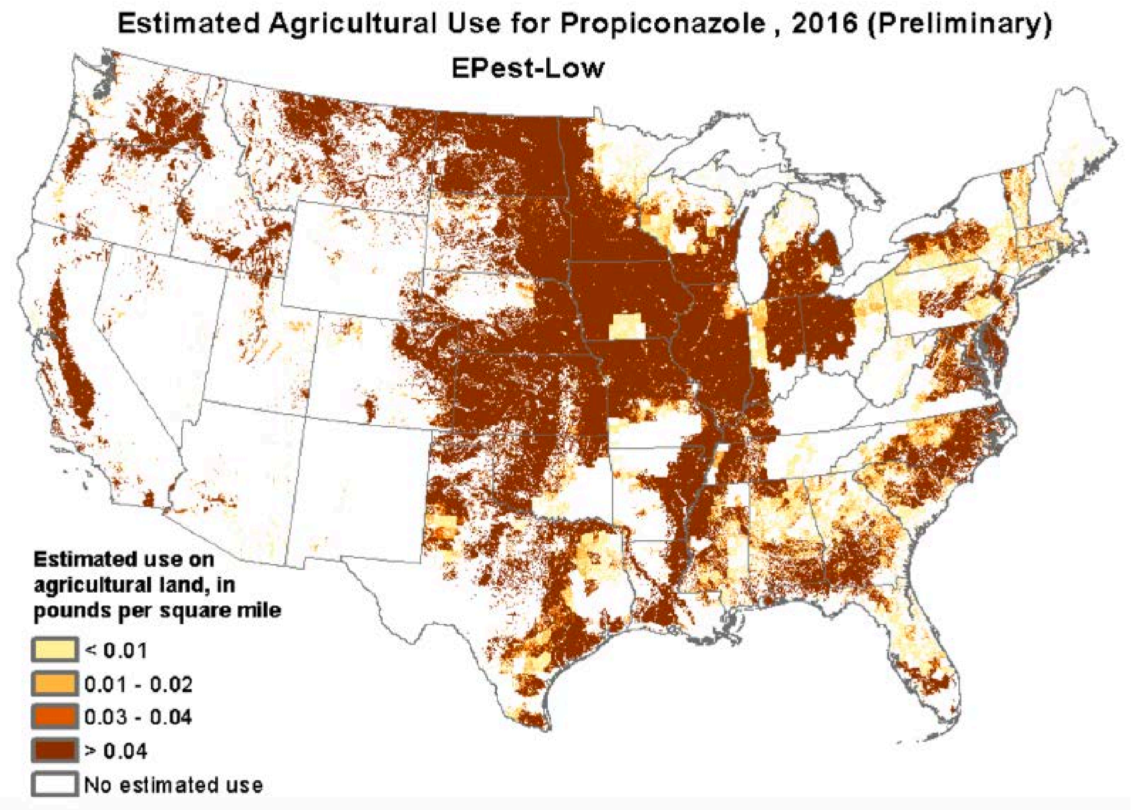
Agricultural Triazole Use in US



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

UNH Extension

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

Pierce Paul, OSU Plant Pathology

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

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Thank you