Field Crop Pathogen Resistance and Link to Human Health

Indiana CCA
December 14, 2022

Marin Talbot Brewer
Fungal Biology Group and Department of Plant Pathology
University of Georgia, Athens, USA
@marintalbrew

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Questions that guide my talk

- Why are *A. fumigatus* and aspergillosis a concern?
- What evidence is there that antifungal resistance develops in agricultural environments?
- Are triazole-resistant (DMI) *A. fumigatus* in agricultural environments and retail plant products in the US?
- What are the hotspots of resistant *A. fumigatus*?
- What can we do to better understand and mitigate this issue?

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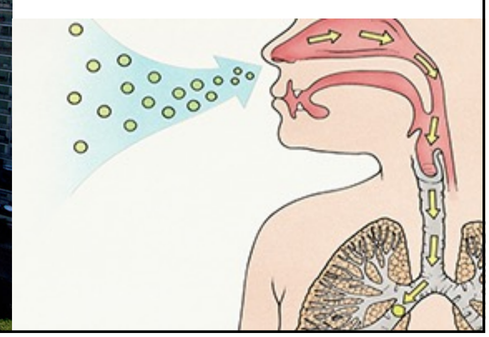
Aspergillus fumigatus is a plant-associated human pathogen

- thermotolerant saprotroph
- abundant in soil, compost, and plant debris
- most common cause of aspergillosis

- environmentally acquired
- the immunocompromised and patients with lung disease are most susceptible



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Aspergillosis

- Respiratory infections caused by *Aspergillus* spp.
- Commonly acquired by inhaling *Aspergillus fumigatus* spores
- Affects humans and other animals
- Treated using triazole drugs

Healthy Patients	Patients with Prior Lung Disease	Immunocompromised Patients
Asthma Alveolitis	Aspergilloma Chronic Pulmonary Aspergillosis	Invasive Aspergillosis (IA)

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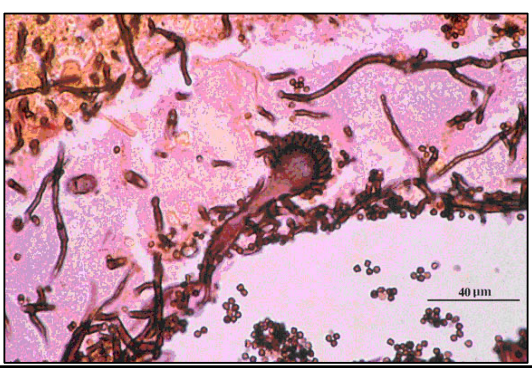
Azole-resistant *Aspergillus fumigatus*

- Azole antifungals first line of defense for aspergillosis
- Azoles (DMIs) used to combat plant-pathogenic fungi
- Azoles inhibit an enzyme (Cyp51A) the fungus needs to grow properly
- Resistance develops by mutations in target enzyme Cyp51A
- Resistance found worldwide in patients and the environment



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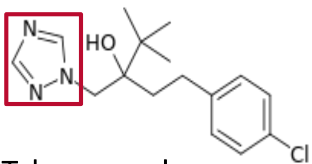
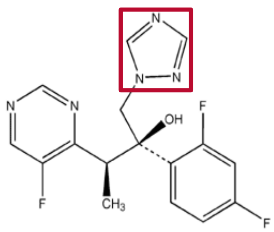
Azole-resistant *Aspergillus fumigatus* is deadly

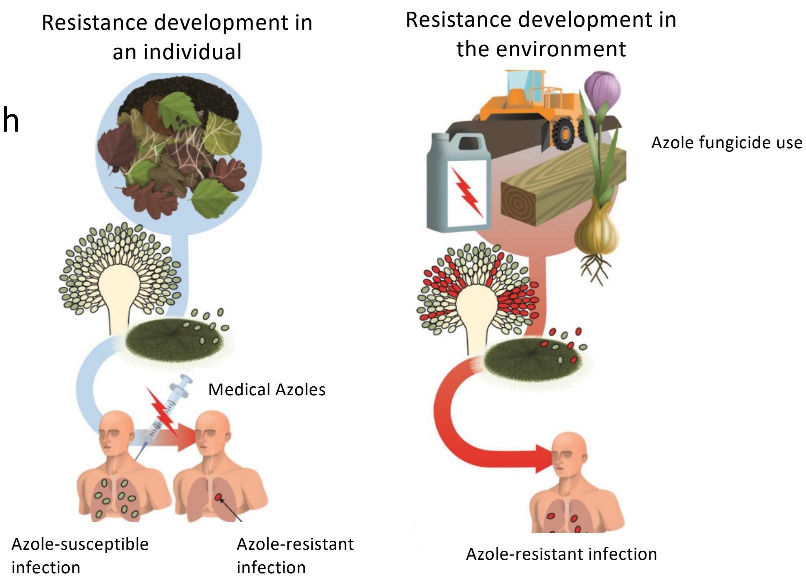
- cross resistance to azole antifungals and fungicides
- mortality high in patients with resistant infections



Tebuconazole



Voriconazole



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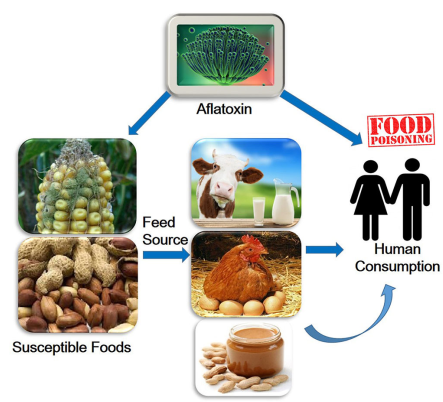
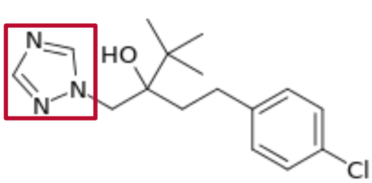
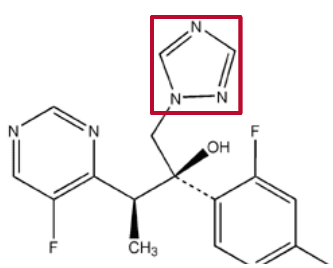
Why are azoles used in agriculture?

- Food security
- Mycotoxin reduction
- Very effective & inexpensive broad spectrum activity against plant-pathogenic fungi
- Resistance development slow compared to other single-site fungicides (MBC, QoI, SDHI)



Tebuconazole





Voriconazole

Kumar *et al.* 2017. *Frontiers in Microbiology*

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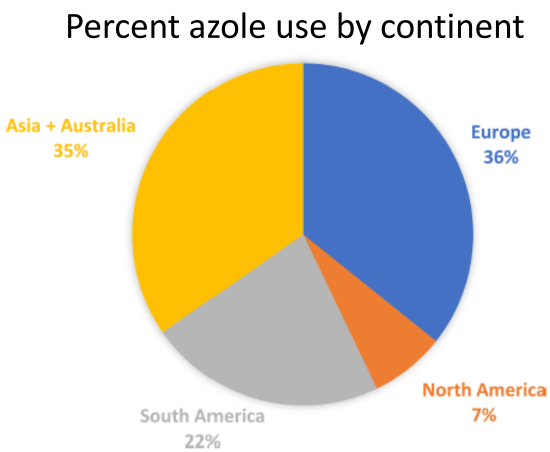
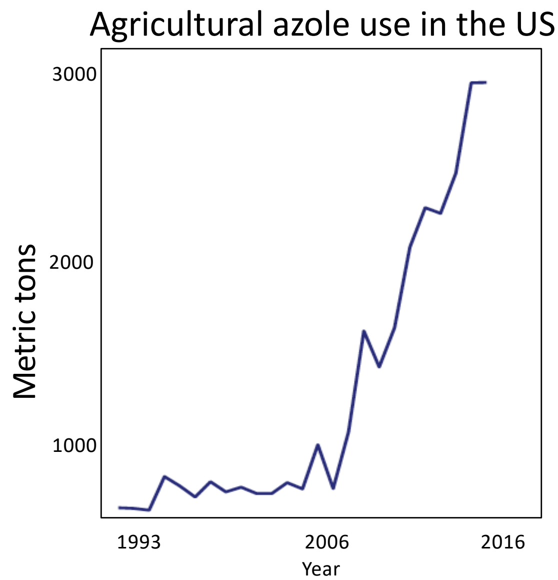
Azole use in agriculture is increasing



Jørgensen and Heick. 2021. *Front Cell Infect Microbiol*



Toda *et al*. 2021. *Environmental Health Perspectives*

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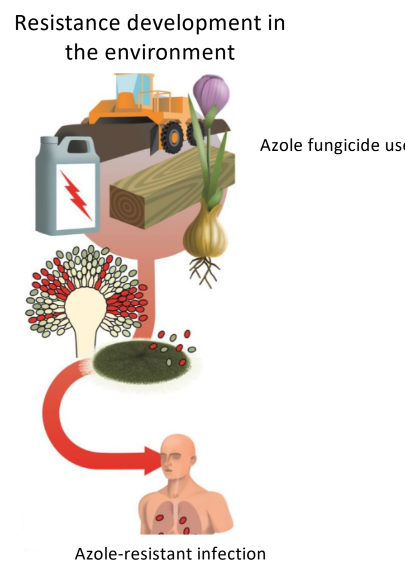
Evidence azole-resistant *A. fumigatus* infections in humans come from the environment

1. azole naïve patients have azole resistant infections
2. Resistant *A. fumigatus* with the same resistance mutations detected worldwide in humans and the environment
3. human and environmental strains with the resistance mutations share nearly identical DNA

Which direction are resistant strains moving?

Contribution of azoles in wood preservation and topical residues?



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Is azole resistant *A. fumigatus* abundant in agricultural environments in the Southeast?

Collected over 700 *A. fumigatus* from 50 agricultural sites in Georgia and Florida, USA

Soil and plant debris from fields and orchards with peanuts, grapes, pecan, apples, strawberries, tomatoes, and oranges; compost; and debris from pecan processing



Michelle Momany

CDC
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Cross resistance of tebuconazole-resistant *Aspergillus fumigatus* to antifungal drugs

Minimum inhibitory concentrations (MIC) based on broth microdilution assay

Tebuconazole-resistant *A. fumigatus* strains (*n* = 172) isolated from agricultural environments in the southeastern U.S. where azole fungicides were applied. Resistance is defined as final drug concentration ≥ 2 µg/mL or ≥ 0.5 µg/mL.

	Final Drug Concentration (µg/mL)								
Azole	**>16**	**16**	**8**	**4**	**2**	**1**	**0.5**	**0.25**	**<0.25**
Tebuconazole	**11**	**1**		1	85	68	6		
Itraconazole					11	140	21		
Voriconazole	**12**					1	81	72	6
Posaconazole						15	93	58	6

We detected 12 highly resistant strains with known resistance mutations from a compost pile and pecan debris.

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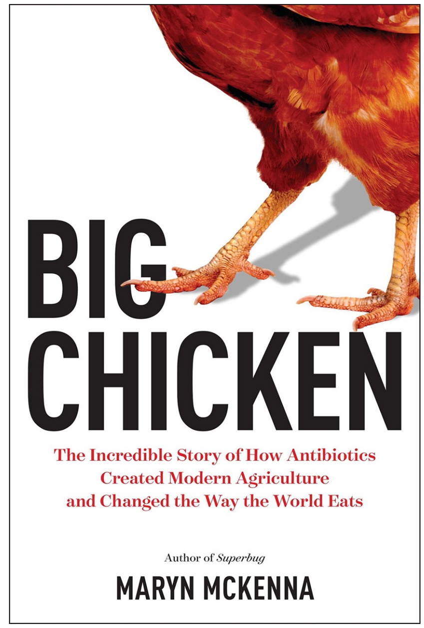
Do azole-resistant *Aspergillus fumigatus* show signatures of agricultural origins?

Are azole-resistant human and agricultural isolates resistant to other fungicide classes?

QoIs (strobilurins) are not used to treat humans



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Do azole-resistant *Aspergillus fumigatus* (AR*Af*) show signatures of agricultural origins?

Are azole-resistant clinical and agricultural isolates resistant to other classes of fungicides?

QoIs (strobilurins) are not used to treat humans

Are there known QoI resistance mutations in azole-resistant strains (G143A in cytochrome B)

Look at the DNA sequence of environmental and human strains in publicly available databases

benzimidazole (MBC) resistant = mutations in tubulin

SDHI resistant = mutations in succinate dehydrogenase

Earl Kang

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Azole resistant *A. fumigatus* strains are multifungicide resistant!

Isolates	Source	Azole resistance *Cyp51*	QoI resistance *CytB*	benzimidazole resistance *BenA*	SDHI resistance *SdhB*
A1163	Human	no (WT)	no (WT)	no (WT)	no (WT)

All azole-sensitive environmental and human isolates did not have mutations

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Environmental and human isolates are multifungicide-resistant

environmental

human

azole resistant

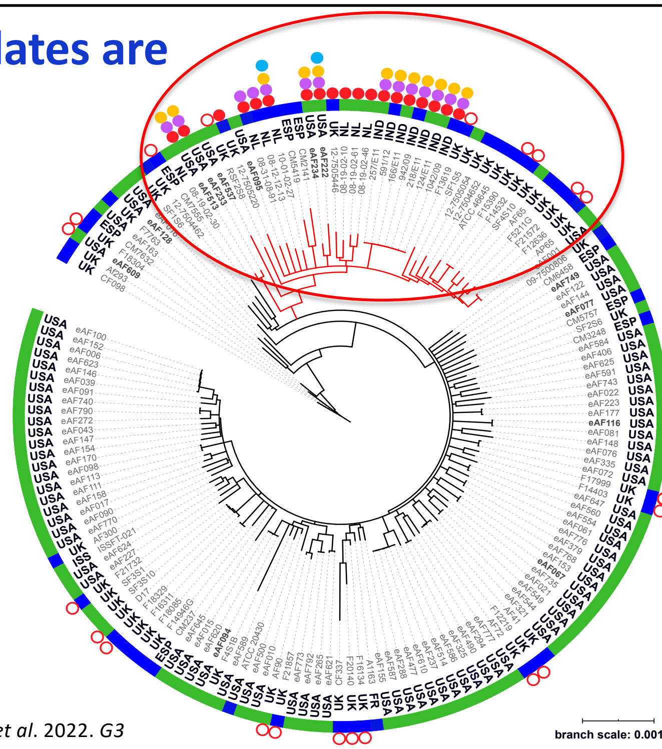
high azole resistant

benzimidazole resistant

QoI resistant

SDHI resistant

This is occurring in a single branch of *Aspergillus fumigatus* tree



Kang *et al.* 2022. *G3*

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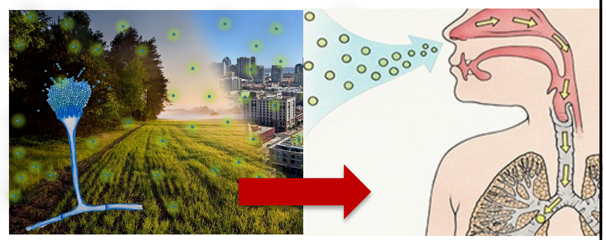
Environmental and human isolates of azole-resistant *A. fumigatus* are multifungicide-resistant

- Many resistant to benzimidazoles
- Most resistant to QoIs
- Few resistant to SDHI



- Multifungicide-resistant *A. fumigatus* in environment and humans
- Multifungicide-resistant isolates geographically widespread and in single branch of tree
- **Exclusively agricultural fungicide-resistance markers in azole-resistant strains from humans supports an agricultural origin of azole resistance**

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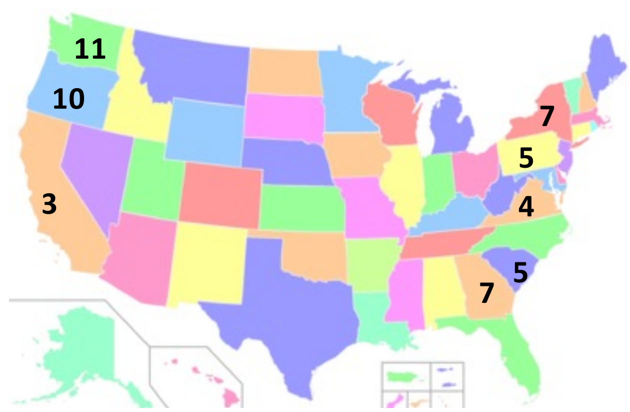
Surveillance of *Aspergillus fumigatus* in East and West Coast agricultural environments

Sampled soil and debris from 52 sites from the East and West Coasts in 2018 and 2019

- Tulip, hemp, wheat, apple, grape, herbs, flowers, brassica, cucurbit, peanut, peach, corn, and soybean
- Organic farm soil and compost from GA used for comparison
- 727 isolates, screened on TEB- and ITC-amended media followed by by broth microdilution assay



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Cross resistance of tebuconazole-resistant *Aspergillus fumigatus* to antifungal drugs

Minimum inhibitory concentrations (MIC) based on broth microdilution assay

Teb- and Itra-resistant *A. fumigatus* strains (*n* = 160) isolated from agricultural environments on the East and West Coasts where azole fungicides were applied. Resistance is defined as final drug concentration ≥ 2 µg/mL or ≥ 0.5 µg/mL.

	Final Drug Concentration (µg/mL)								
Azole	**>16**	**16**	**8**	**4**	**2**	**1**	**0.5**	**0.25**	**<0.25**
Tebuconazole	15	13	11	26	78	17			
Itraconazole	11	5	3	7	44	71	19		
Voriconazole	8	1		18	6	23	89	15	
Posaconazole					2	41	68	24	25

~20 highly resistant strains from grape, wheat, herbs, peach, tulips, compost; currently sequencing their DNA and adding them to the tree

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Are azole-resistant *A. fumigatus* in our food supply or plant-based retail products?

- Samples gathered from retail stores

Caroline Burks

Product	Location of Origin
Grape	California
Almond	California, USA
Peanut	North Carolina, USA
Pecan	Texas, Georgia, Unknown
Apple	Washington, New York, USA
Compost	Unknown, USA
Soil	Unknown, USA, Canada
Flower bulbs	The Netherlands, USA, Unknown, Costa Rica, China

USDA

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Over 500 *A. fumigatus* strains screened

Products	Total Isolates	Growth on selective medium		Selected for MIC
		Tebuconazole	Itraconazole	
Peanut	147	0	33	33
Compost	133	35	76	44
Flower Bulb	109	42	51	20
Soil	85	7	26	17
Grape	35	0	0	8
Pecan	12	1	0	6
Almond	2	0	0	2
Apple	2	0	0	0
Total	525	85	186	130

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Azole-resistant strains associated with lawn products

Products	Isolates Tested	Resistant Isolates (% Product)			
		TEB	ITC	VOR	POS
Compost	44	25 (56.8%)	10 (22.7%)	9 (20.5%)	9 (20.5%)
Peanut	33	4 (12.1%)	2 (6.1%)	1 (3.0%)	1 (3.0%)
Bulb	20	15 (75.0%)	9 (45.0%)	15 (75.0%)	13 (65.0%)
Soil	17	5 (29.4%)	1 (5.9%)	1 (5.9%)	1 (5.9%)
Pecan	6	4 (66.7%)	1 (17.7%)	0	0
Almond	2	2 (100.0%)	1 (50.0%)	0	0
Grape	8	0	0	0	0
Apple	0	0	0	0	0
Total	130	55 (42.3%)	24 (18.5%)	26 (20.0%)	24 (18.5%)

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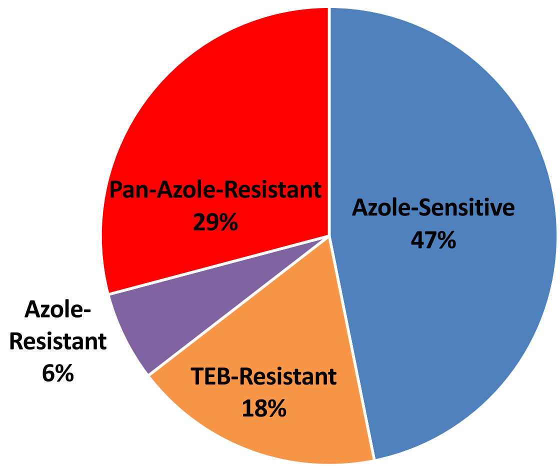
Diverse azole-resistant strains of *A. fumigatus*



Resistance classifications:

- **Azole-sensitive**: not resistant to any azole tested
- **TEB-resistant**: resistant to only TEB
- **Azole-resistant**: resistant to only 1 medical azole; may or may not be resistant to TEB
- **Pan-azole-resistant**: resistant to more than 1 medical azole and TEB

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Conclusions: azole-resistant *A. fumigatus* in our food supply and plant-based retail products

- Pan-azole-resistance found in commercial compost, soil, peanut, and flower bulbs
- Lawn and garden products contain by far the most pan-azole-resistant isolates and present the most danger to immunocompromised people

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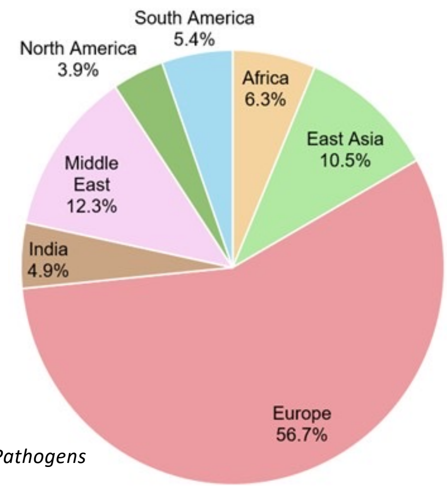
What are the hotspot of azole-resistant *A. fumigatus* in the environment?

Caroline Burks

- Collected data from all published reports of AR*Af* in the environment
- None from human patients or other animal infections, assumed saprophytic
- 1292 AR*Af* isolates in the environment based on synthesis of the available literature (52 papers)

- Sampling bias



Burks *et al*. 2021. *PLOS Pathogens*

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Azole resistance across environments

Caroline Burks

- Developed environments
  - parks, hospitals, homes
- Agricultural environments
  - crop and horticultural production
- Commercial products
  - items sold in retail stores
- Other environments
  - forests, sawmills

Burks *et al*. 2021. *PLOS Pathogens*

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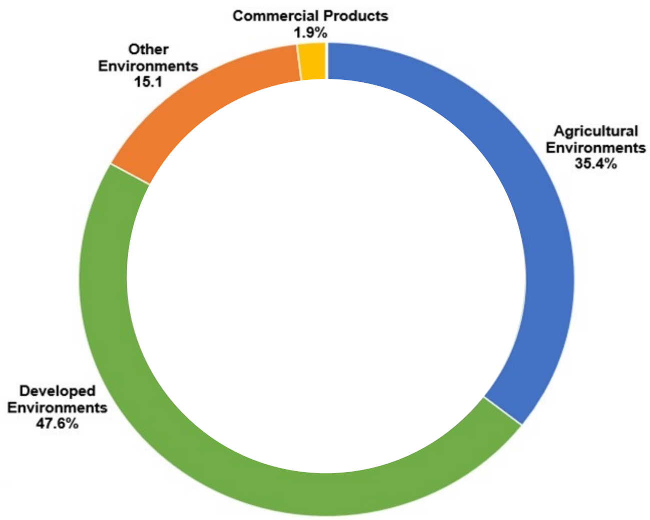
Hotspots of azole-resistant *A. fumigatus* in the environment



- Developed environments
  - 47.6% of resistant isolates
  - 28/52 studies
  - most from hospitals or flower gardens
- Agricultural environments
  - 35.4% of resistant isolates
  - 31/52 studies
  - most from flower farms
- Commercial products
  - 1.9% of resistant isolates
  - 4/52 studies
  - flower bulbs, soil, seeds

Burks *et al*. 2021. *PLOS Pathogens*

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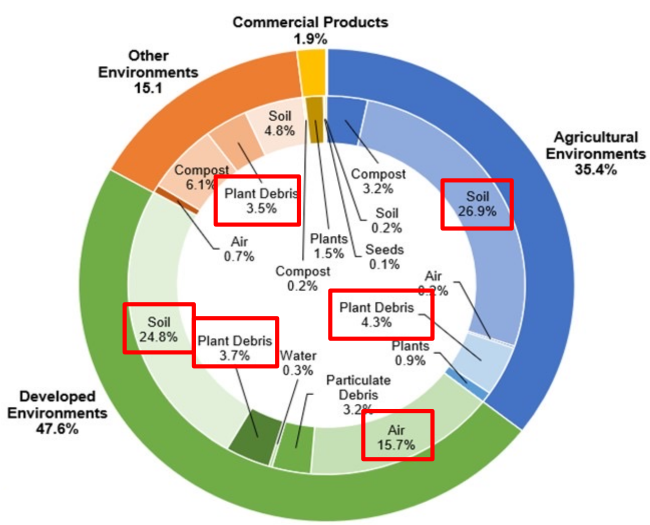
Hotspots of azole-resistant *A. fumigatus* in the environment



- Soil has the greatest proportion of resistant isolates
  - Mainly from agriculture/developed environments
- Air samples had the second greatest proportion
  - Almost all from developed environments
- Plant debris had the third greatest proportion
  - Equally from agriculture/ developed/other

Burks *et al*. 2021. *PLOS Pathogens*

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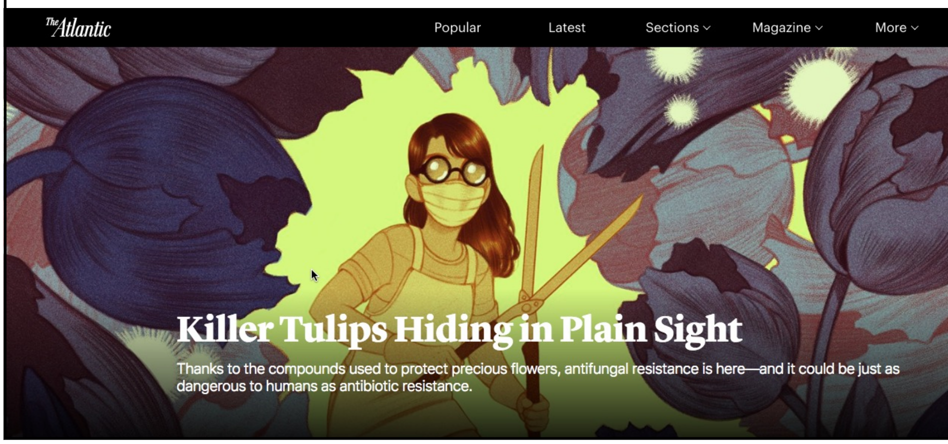
Hotspots of azole-resistant *A. fumigatus* in the environment

- flower gardens
- flower farms
- flower bulb waste
- green waste (pre-compost)
- wood chippings (pre-compost)
- compost piles
- retail compost
- retail flower bulbs

Burks *et al.* 2021; Schoustra *et al.* 2019; Kang *et al.* 2020; others and ongoing studies



vineyards, wheat fields, peach and apple orchards, peanut and pecan debris, retail raw peanuts may also play a role

by Maryn McKenna

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What can we do to minimize the risk?

- Continued surveys and monitoring
- Act as stewards of the environment
- Use single-site fungicides prudently for disease control
- Improve debris pile, waste pile, and compost management
- Improve and recommend Integrated Pest Management (IPM) strategies

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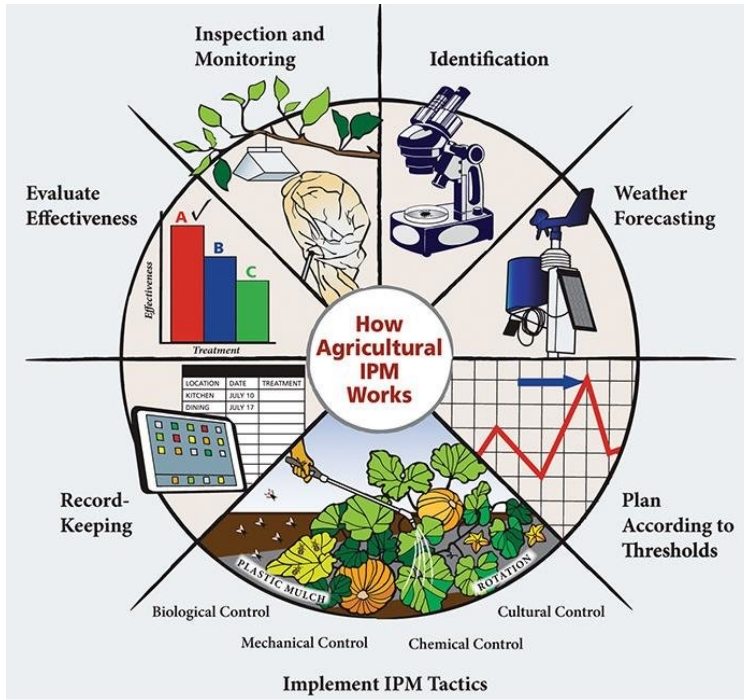
Integrated Pest Management (IPM) in agriculture

Plant disease resistant varieties
Monitor for disease and resistance
Use alternative control strategies
Minimize fungicide applications
Use precision spray technology



New York State Integrated Pest Management Program

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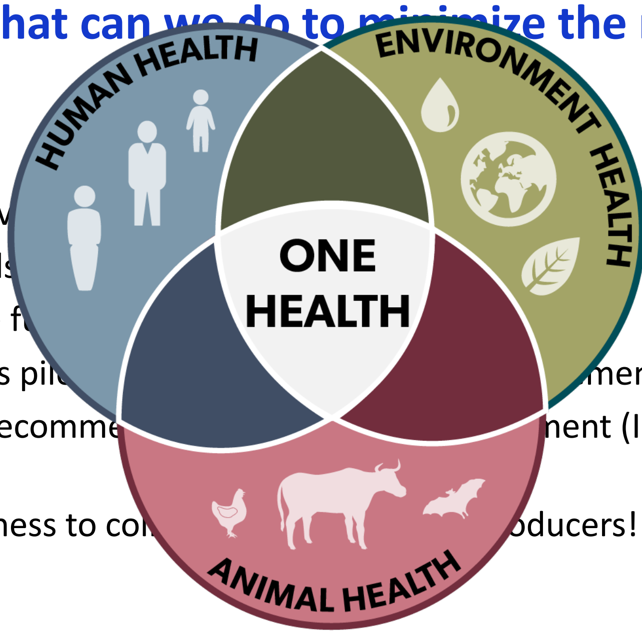
What can we do to minimize the risk?

- Continued surv
- Act as stewards
- Use single-site f
- Improve debris pil ment
- Improve and recomme nent (IPM) strategies
- Spread awareness to co oducers!!!



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Acknowledgements

Current & Past Lab Members

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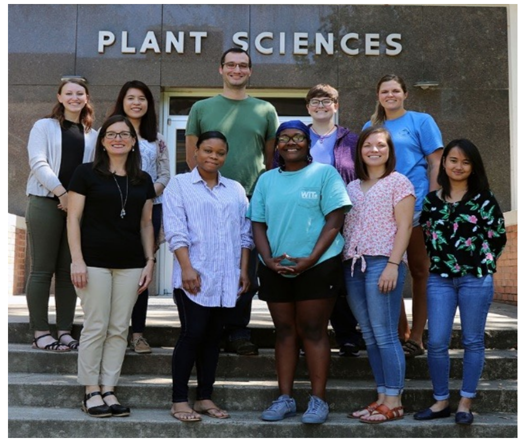
Justina Stanislaw

And to so many who helped us sample field sites or who sent us soil and plant debris samples from around the US



Brewer Mycology Lab

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