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Genomic Surveillance and Epidemiology

Forum on Microbial Threats Workshop

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Evidence *ARAF* infections in humans come from the environment

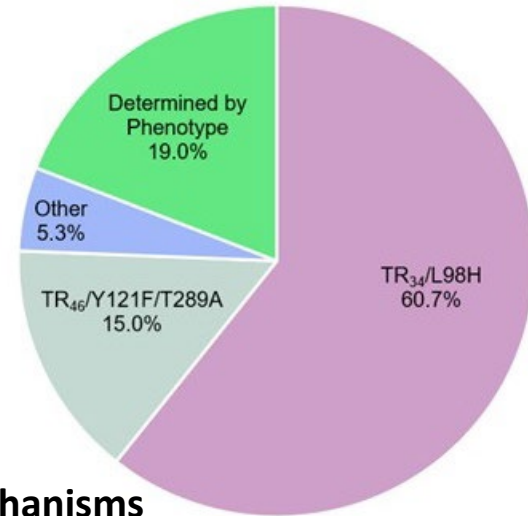
1. azole naïve patients have pan-azole resistant infections
2. pan-*ARAF* with same TR resistance detected worldwide in humans and the environment
3. human and environmental *ARAF* isolates with TR alleles share multilocus genotypes and nearly identical WGS genotypes

Which direction are *ARAF* moving?

Contribution of azoles in wood preservation and topical residues?



Caroline Burks



**Resistance mechanisms
for environmental *ARAF***

Is *ARAf* abundant in agricultural environments in the southeastern US?

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



**UNIVERSITY OF
GEORGIA**



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

Minimum inhibitory concentrations (MIC) based on broth microdilution assay

Tebuconazole-resistant <i>A. fumigatus</i> strains ($n = 172$) isolated from agricultural environments in the southeastern U.S. where azole fungicides were applied. Resistance is defined as final drug concentration $\geq 2 \mu\text{g/mL}$ or $\geq 0.5 \mu\text{g/mL}$.									
Azole	Final Drug Concentration ($\mu\text{g/mL}$)								
	>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

What is the mechanism of resistance?

12 pan-ARAf with TR₄₆/Y121F/T289A from compost pile and pecan debris.

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

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

Qols (strobilurins) are not used to treat humans

Qol resistant = G143A in *CytB*

Scan genomes of environmental and human ARAf isolates

benzimidazole resistant = mutations in *BenA* (tubulin)

SDHI resistant = mutations in *Sdh* genes



Earl Kang

Pan-ARAF are multifungicide resistant!

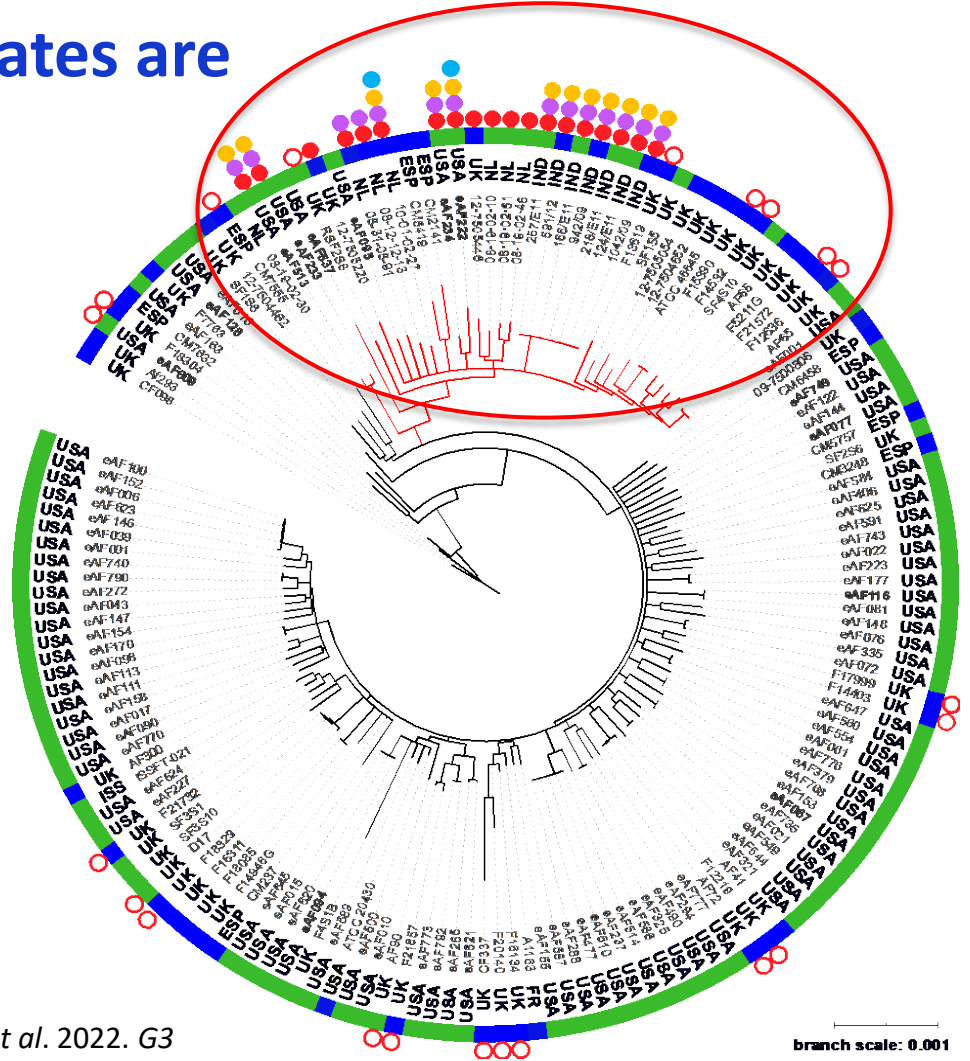
Isolates	Source	Pan-ARAF <i>Cyp51</i>	QoI resistance <i>CytB</i>	benzimidazole resistance <i>BenA</i>	SDHI resistance <i>SdhB</i>
A1163	Human	WT	WT	WT	WT

All azole-sensitive environmental and clinical isolates were WT for *CytB*, *BenA*, *SdhB*

Environmental and clinical isolates are multifungicide-resistant

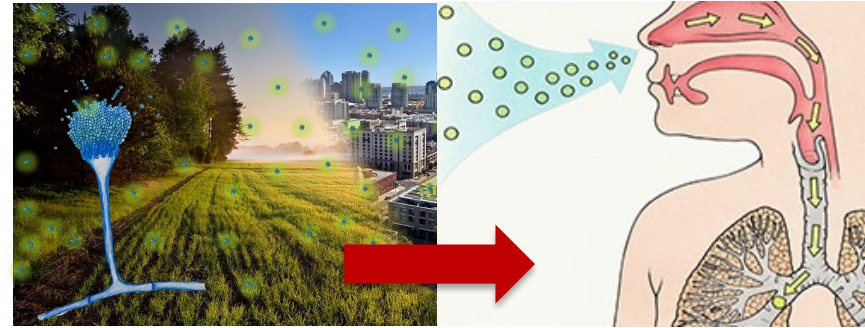
- environmental
- clinical
- azole resistant (non-TR)
- pan-azole resistant (TR-based)
- benzimidazole resistant
- QoI resistant
- SDHI resistant

This is occurring in a single clade of *Aspergillus fumigatus*



Environmental and clinical isolates of pan-ARAF are multifungicide-resistant

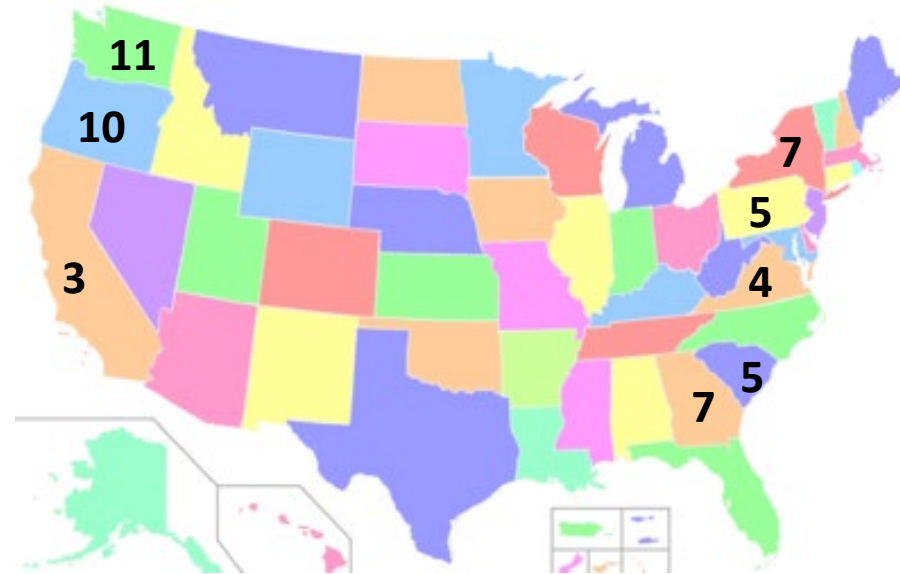
- Many pan-ARAF resistant to benzimidazoles
- Most pan-ARAF resistant to Qols
- Few pan-ARAF resistant to SDHI
- Multifungicide-resistant *A. fumigatus* in environment and clinic
- Multifungicide-resistant isolates geographically widespread and in single clade
- **Exclusively agricultural fungicide-resistance markers (*cytB* G143A and *sdhB* H270Y) in pan-ARAF isolates from patients further supports an agricultural origin of pan-azole resistance**



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 broth microdilution assay



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

Minimum inhibitory concentrations (MIC) based on broth microdilution assay

Tebuconazole- and Itraconazole-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 $\geq 2 \mu\text{g/mL}$ or $\geq 0.5 \mu\text{g/mL}$.

Azole	Final Drug Concentration ($\mu\text{g/mL}$)								
	>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

What is the mechanism of resistance?

~20 pan-ARAf with TR₄₆/Y121F/T289A or TR₃₄/L98H from grape, wheat, herbs, peach, tulips, compost; WGS of 135 strains

Are ARAf in our food supply or plant-based retail products?

- Samples gathered from retail stores



Caroline Burks



Product	Location of Origin
Grape	California, USA
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

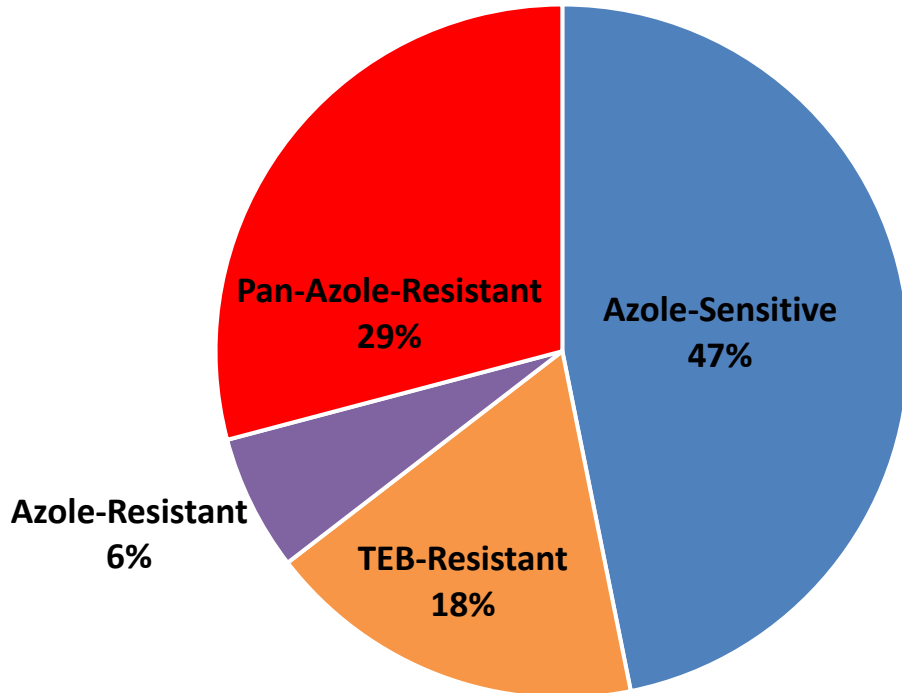


Over 500 *A. fumigatus* isolates screened

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



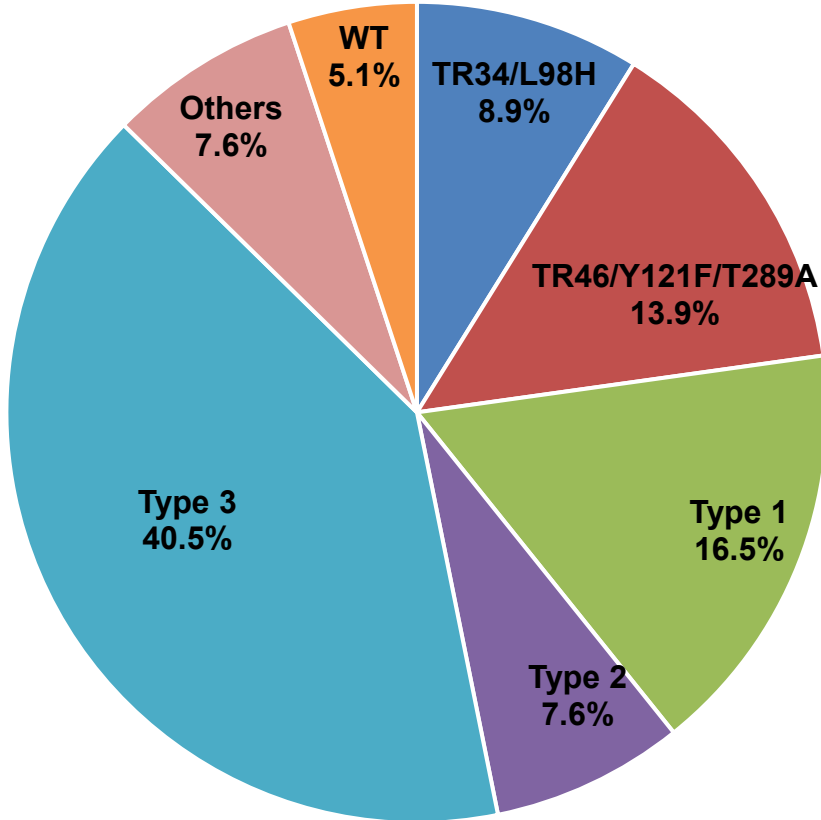
Diverse azole-resistance phenotypes



Resistance classifications:

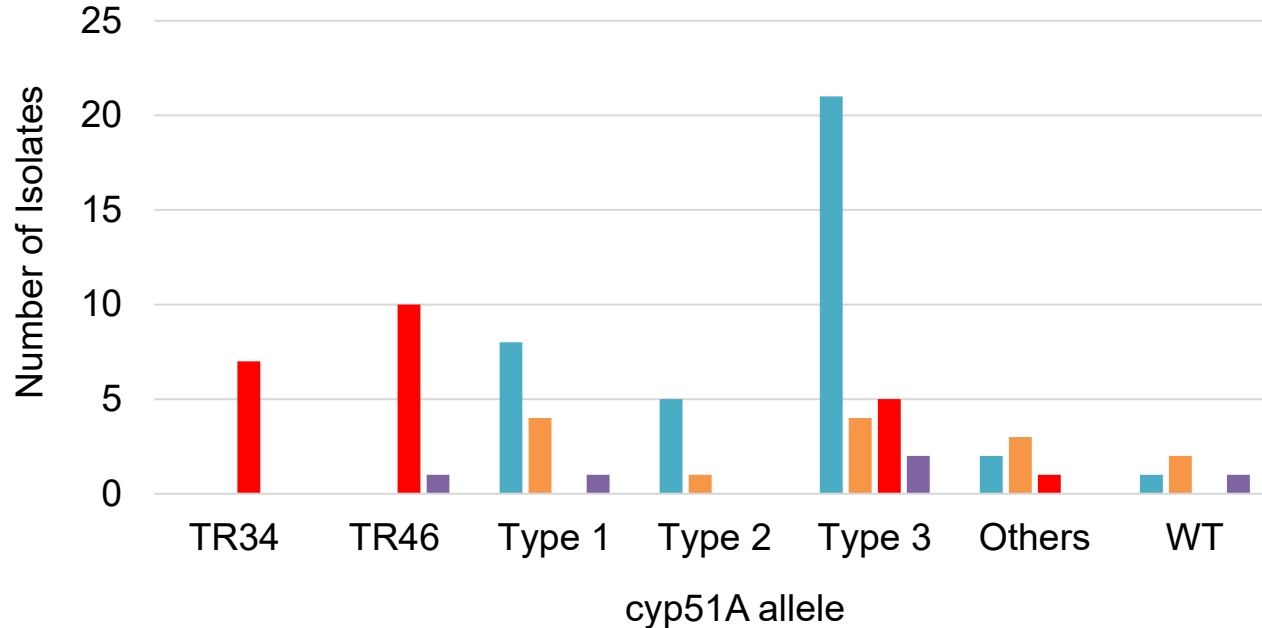
- **Azole-sensitive:** not resistant to any azole tested
- **TEB-resistant:** resistant to only TEB
- **Azole-resistant:** resistant to only 1 medical azole; may or may not be resistant to TEB
- **Pan-azole-resistant:** resistant to more than 1 medical azole and TEB

Diverse *cyp51A* genotypes



TR ₃₄	TR ₃₄ /L98H
TR ₄₆	TR ₄₆ /Y121F/T289A
Type 1	T248N/E255D
Type 2	Y46F/V172M/I242V/T248N/E255D/K427E
Type 3	Y46F/V172M/T248N/E255D/K427E
WT	WT
Other	A9T/Y46F/V172M/T248N/E255D/K427E V172M/T248N/E255D V172M/T248N/E255D/K427E W415G Y46F/H147Y/V172M/T248N/E255D/K427E Y46F/V172I/T248N/E255D/K427E

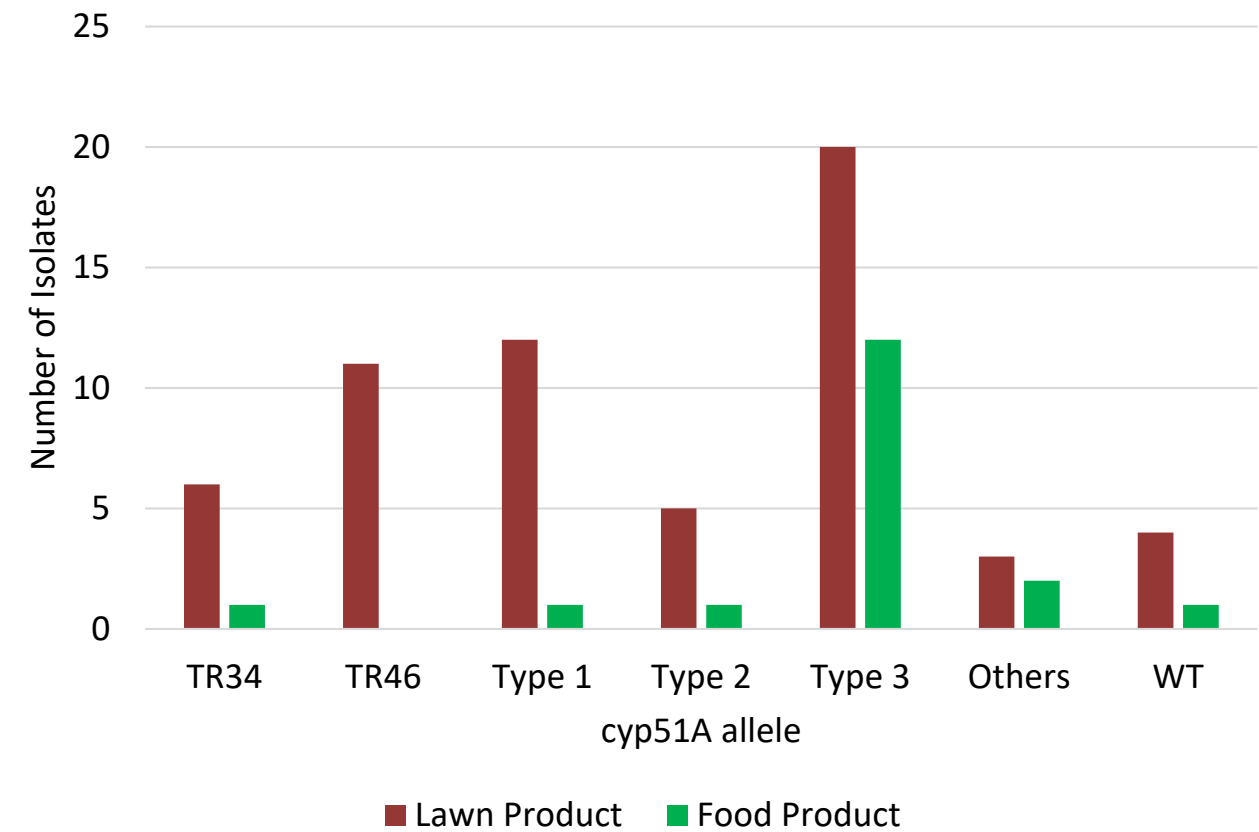
Association of resistance phenotypes and *Cyp51A* genotype



TR ₃₄	TR ₃₄ /L98H
TR ₄₆	TR ₄₆ /Y121F/T289A
Type 1	T248N/E255D
Type 2	Y46F/V172M/I242V/T248N/E255D/K427E
Type 3	Y46F/V172M/T248N/E255D/K427E
WT	WT
Other	A9T/Y46F/V172M/T248N/E255D/K427E V172M/T248N/E255D V172M/T248N/E255D/K427E W415G Y46F/H147Y/V172M/T248N/E255D/K427E Y46F/V172I/T248N/E255D/K427E

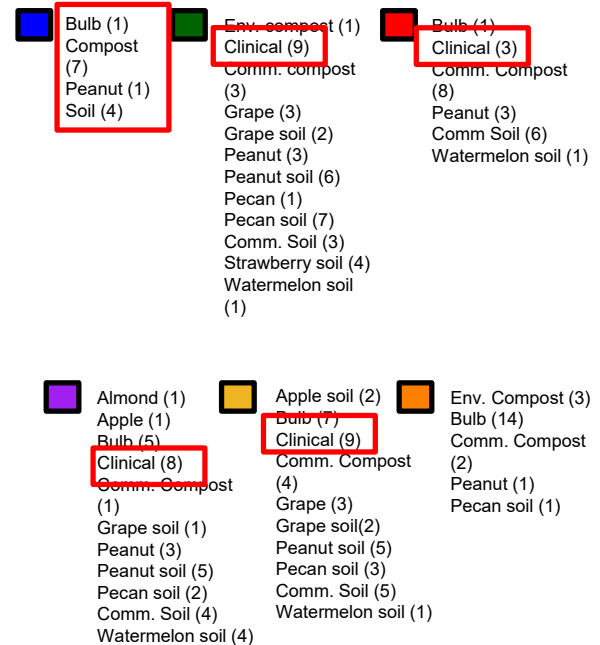
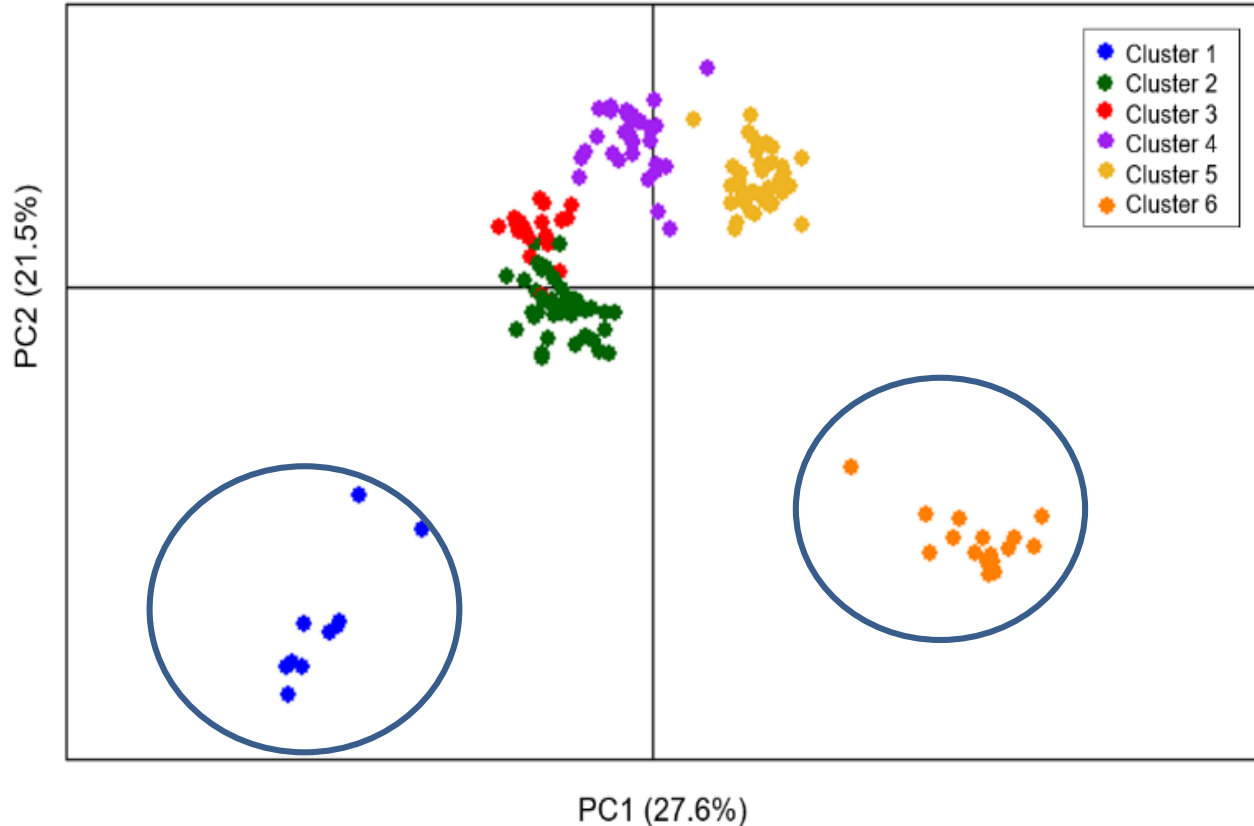
■ Azole-Sensitive
 ■ TEB-Resistant
 ■ Pan-Azole-Resistant
 ■ Azole-Resistant

Pan-azole-resistant isolates associated with lawn products

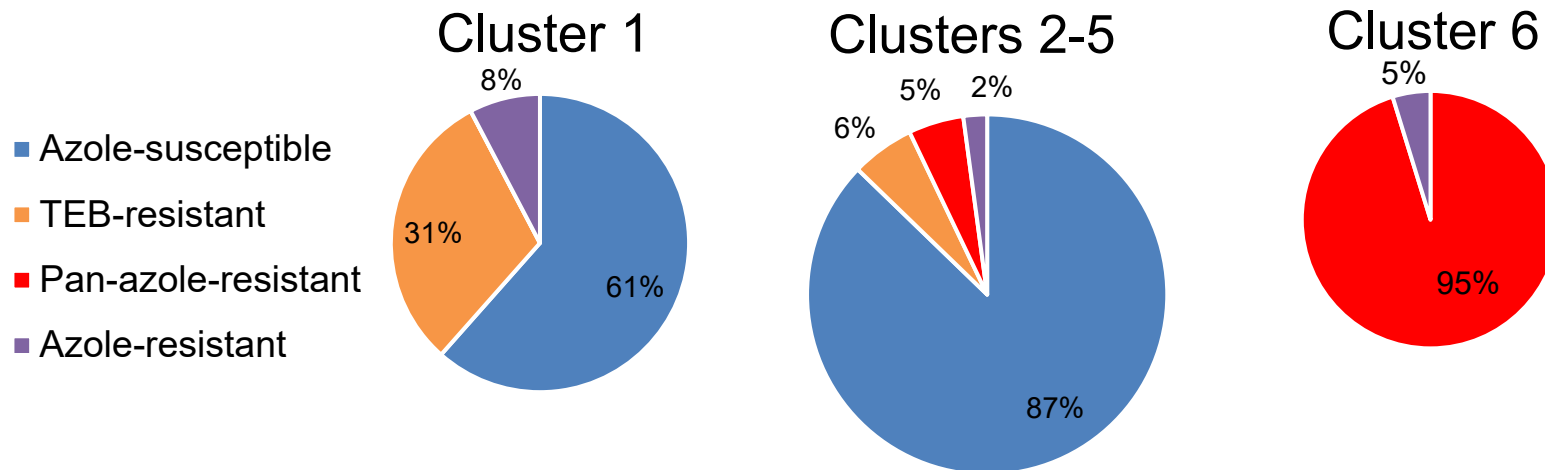


TR ₃₄	TR ₃₄ /L98H
TR ₄₆	TR ₄₆ /Y121F/T289A
Type 1	T248N/E255D
Type 2	Y46F/V172M/I242V/T248N/E255D/K427E
Type 3	Y46F/V172M/T248N/E255D/K427E
WT	WT
Other	A9T/Y46F/V172M/T248N/E255D/K427E V172M/T248N/E255D V172M/T248N/E255D/K427E W415G Y46F/H147Y/V172M/T248N/E255D/K427E Y46F/V172I/T248N/E255D/K427E

How are these isolates related to *A. fumigatus* from agricultural and patient environments?



Cluster 6 is pan-azole-resistant with TR alleles



Conclusions: ARAf in our food supply and plant-based retail products

- Pan-azole-resistance in commercial compost, soil, peanut, and flower bulbs
- Isolates with TR₃₄/L98H, TR₄₆/Y121F/T289A, Y46F/V172M/T248N/E255D/K427E, and Y46F/H147Y/V172M/T248N/E255D/K427E alleles are pan-azole resistant
 - non-*cyp51A*-based resistance mechanism?
- *Afum* populations in US are genetically structured
- Lawn and garden products contain the most pan-azole-resistant isolates
- Azole residue profiling of substrates with high levels of ARAf
- Compiling environmental genomes with publicly available ARAf genomes from patients in USA and elsewhere to find shared genotypes

Acknowledgements

Current & Past Lab Members

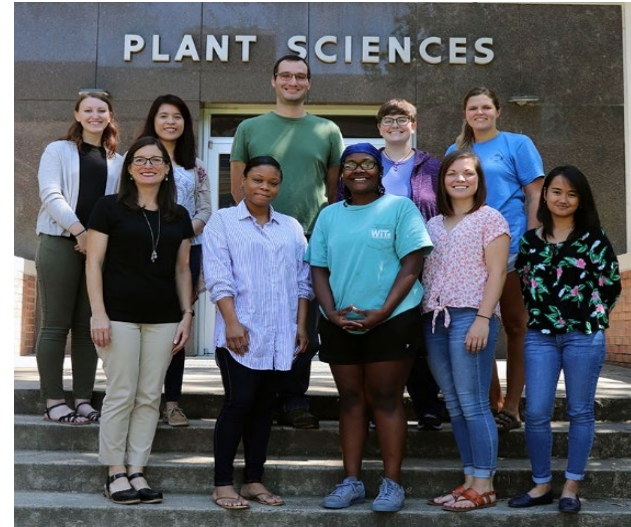
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Brewer Mycology Lab

