Triazole and multi-fungicide resistance in agricultural pathogens

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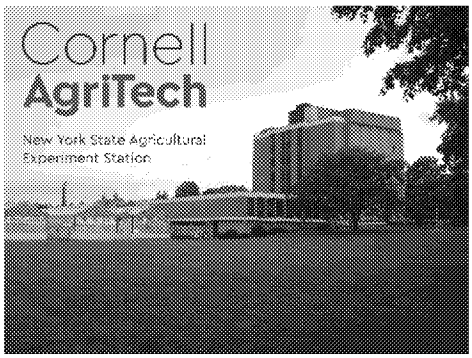
Tree Fruit & Small Fruit Pathology
Research/Extension/Teaching 50/15/35

Cornell AgriTech

- Stakeholder-Driven Specialty Crop Research
- Field, Digital, and Molecular Laboratories > Achieve Transition to Practice

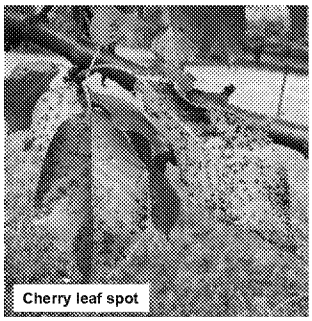
Antibiotic Resistance



Fungicide Resistance

Perennial fruit crops as model system for fungicide resistance

- Long-lived (> 5 years) & management periods for exceptionally long (> 6-7 months)
- Fruit pathogens have numerous secondary infection cycles > repeat treatments
- Localized populations w/ little influx of new members



Apple Scab (*Venturia inaequalis*) & Practical fungicide resistance

- Perennial problem & susceptible cultivars: favored by consumer and producer
- High input system (10+ fungicide applications/year) & resistance reported in most fungicide classes
- Practical Resistance: pathogen population is sufficiently resistant > results in management failure even under appropriate-use practices

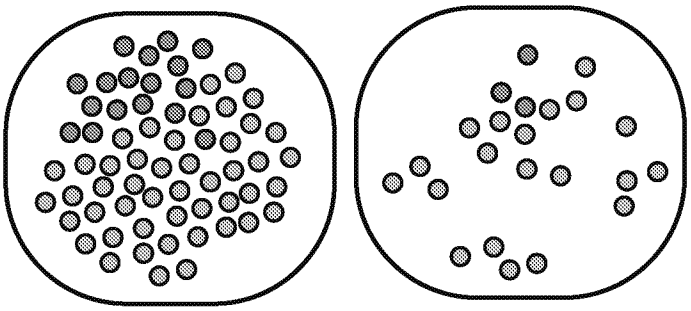
Phases of fungicide resistance development

1. Emergence*

2. Establishment

*Fungicides are not inherently mutagenic, mutations are **pre-existing**
*Advantageous mutations occur **infrequently**

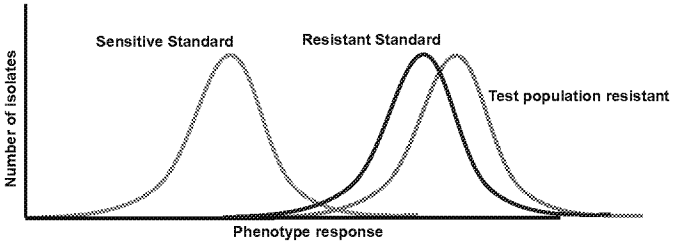
Application of a fungicide does not cause emergence, rather may select for establishment



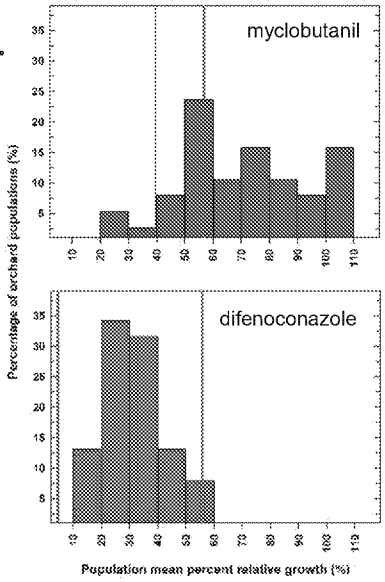
Pathogen Population
Sensitive Isolate
Resistant Isolate

Determining practical fungicide resistance

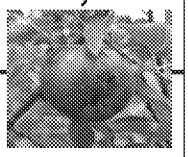
- Statistical test to compare the distribution of phenotype responses from the test population to that of a reference distribution
- Reference standards: Confirm proper application practice & level of disease incidence following product use

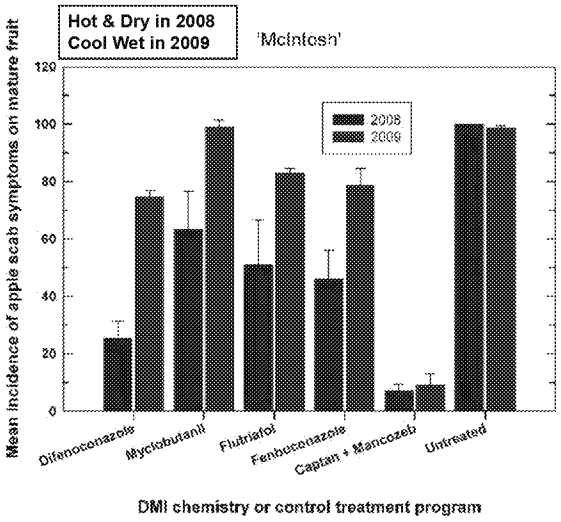


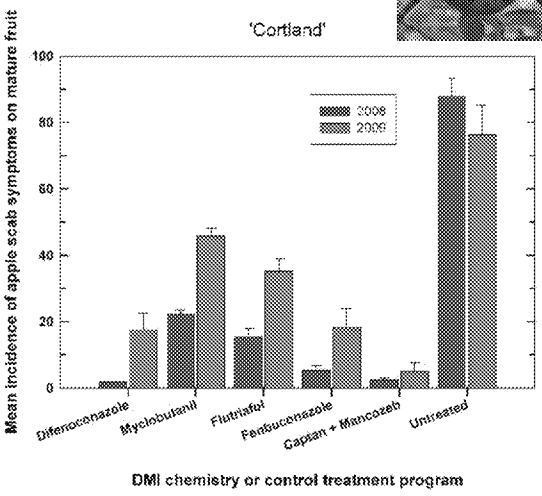
121 apple orchard populations



Practical triazole resistance > Environment, Host, & Chemistry

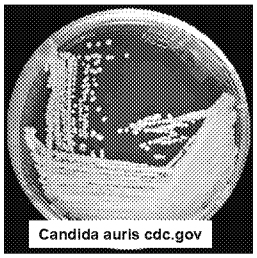




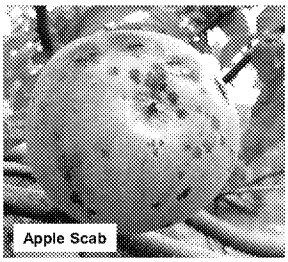


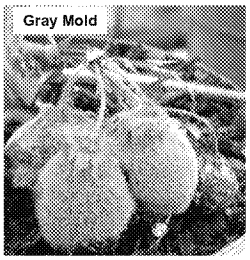
Cross & Multiple Fungicide Resistance

- **Cross-resistance** – resistance to multiple fungicides that share the same biochemical mode of action or target site
- **Multiple resistance** –resistance development to two or more unrelated fungicide classes resulting from sequential selection or multi-drug resistant mechanism


Candida auris cdc.gov

Black Sigatoka APSnet.org


Apple Scab

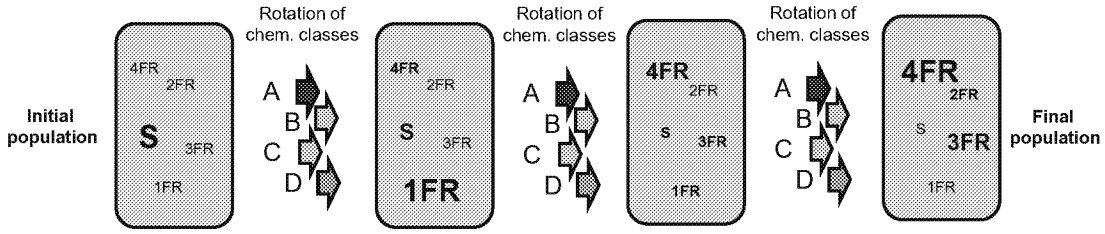

Gray Mold

Fungicide Rotation w/ single-resistance > Multiple Resistance

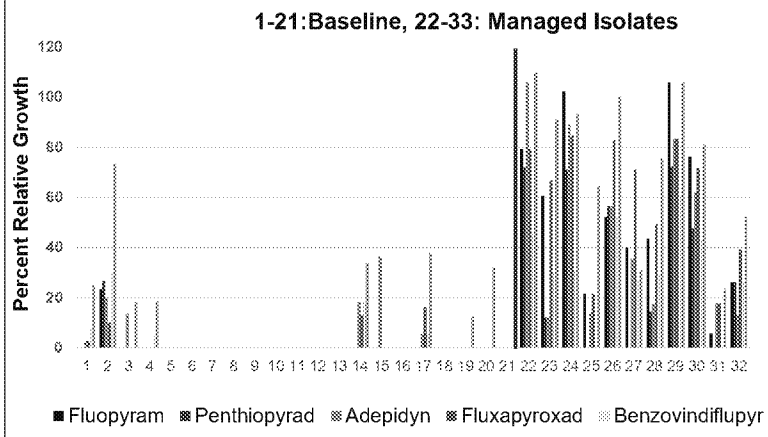
	Thiophanate-methyl	Pyraclostrobin	Cyprodinil	Fenhexamid	Iprodione	Boscalid	Fludioxonil
Thiophanate-methyl							
Pyraclostrobin	>50%						
Cyprodinil							
Fenhexamid			20-50%				
Iprodione							
Boscalid					5-20%		
Fludioxonil						<5%	

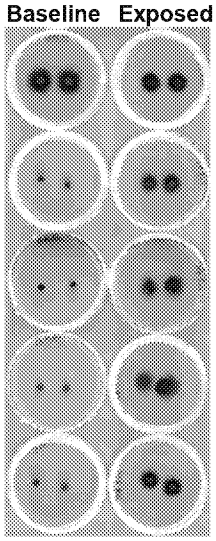
Logistic Regression Analysis of 2130 *Botrytis* isolates from Eastern US

Hu, Cox, and Schnabel, Phytopathology 106:1513-1520



Isolates from managed populations > super isolates w/ multiple-resistance





No Fungicide Control

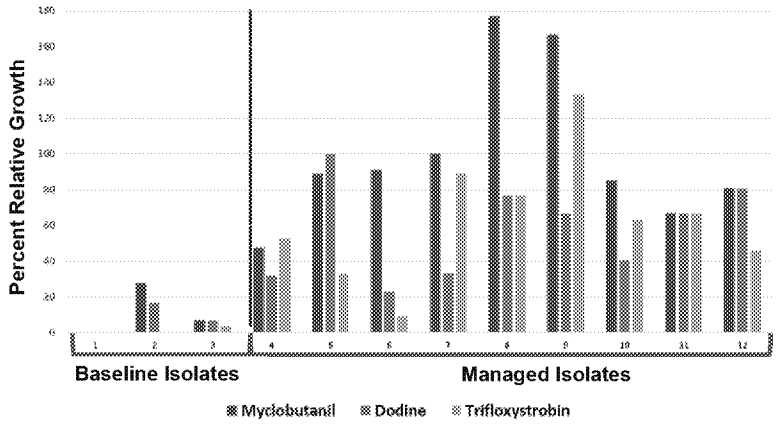
Fluopyram

Penthiopyrad

Pydiflumetofen

Fluxapyroxad

Isolates from managed populations > super isolates w/ multiple-resistance



Resistance extended to multiple classes of fungicides

No mutations in target genes detected

Investigating multi-drug resistance mechanisms

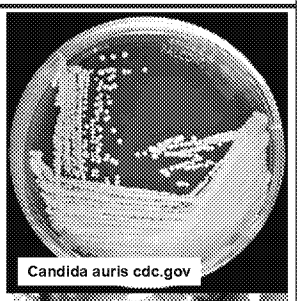
Genomics of isolates with multiple resistance

Functional annotation of *Venturia inaequalis* genomes.				
Isolate Name	**ID: 10.3.14**	**ID: 6.31.13**	**ID: 12.2.13**	**ID: 2.42.14**
Fungicide Phenotype	**Baseline sensitive**	**DMI resistant**	**DMI/dodine Resistant**	**DMI/dodine/QoI resistant**
Genome Size (Mb)	**39**	**48**	**61**	**44**
Scaffolds	3303	7555	6920	619
N50	26689	35520	36116	221483
UniProt	601	576	581	774
CAZyme	346	350	346	396
BUSCO	**95.74%**	**96.34%**	**96.73%**	**97.49%**
Pfam	11441	11477	11562	14903
Transposable elements	**48**	**71**	**71**	**153**

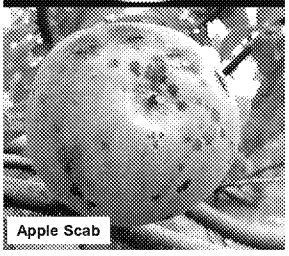
- Dynamic genome size [39-61Mb] > from non-coding regions
- Transposable element numbers in coding sequences increase with fungicide resistance phenotype

Summary & Takeaways

- **Tree fruit: long-lived, receive multiple treatments, exposed to pathogen in comprising environments**
  - **C. auris: not found as an epiphyte in apple & stone fruit**
- **Fungicide resistance: population size affects risk of emergence & reduced time to selection > practical resistance**
- **Development of Multi-fungicide Resistance:**
  - **Using a fungicide on a population with resistance**
    - **May drag isolates w/ resistance to other chemistries**
  - **Continual fungicide exposure (regardless of the class)**

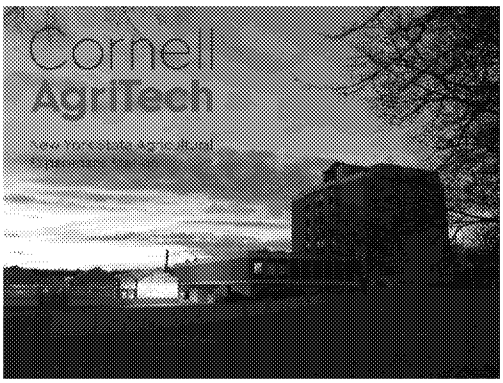


Candida auris cdc.gov



Apple Scab

Acknowledgements & funding sources



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