Identifying and managing Fusarium species responsible for head scab #17-08-03-CS

Characterizing fungicide sensitivity of diverse *Fusarium* spp. and determination of value of fungicide applications through meta-analysis

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John Boyse, Research Technician

- Over 300 species of Fusarium
  - **Atleast 40 of those are known to infect cereal grains and cause mycotoxin contamination**
- Variety of mycotoxins produced, profiles depend on species
- Variability within species as well

Table 1. Mycotoxigenic *Fusarium* species associated with cereals and their mycotoxins

Fusarium species[a]	Mycotoxins[b]
F. acuminatum	**T2**, **MON**, HT2, DAS, MAS, NEO, BEA
F. anthophilum	BEA
F. avenaceum	**MON, BEA**
F. cerealis	**NIV**, **FUS**, **ZEN**, ZOH
F. chlamydosporum	**MON**
F. culmorum	**DON**, **ZEN**, **NIV**, FUS, ZOH, AcDON
F. equiseti	**ZEN**, **ZOH**, MAS, DAS, NIV, DAcNIV, FUS, FUC, BEA
F. graminearum	**DON**, **ZEN**, **NIV**, **FUS**, **AcDON**, DAcDON, DAcNIV
F. heterosporum	**ZEN**, ZOH
F. nygamai	BEA, FB_1, FB_2
F. oxysporum	**MON**, BEA
F. poae	**DAS**, **NIV**, **FUS**, MAS, T2, HT2, NEO, BEA
F. proliferatum	**FB_1**, **BEA**, **MON**, **FUP**, FB_2,
F. sambucinum	**DAS**, T2, NEO, ZEN, MAS, BEA
F. semitectum	**ZEN**, BEA
F. sporotrichioides	**T2**, **HT2**, **NEO**, MAS, DAS
F. subglutinans	**BEA**, **MON**, FUP
F. tricinctum	**MON**, BEA
F. verticillioides	**FB_1**, FB_2, FB_3

(Logrieco 2002)

Fusarium Characterization objectives

1) What species are present in Michigan wheat and corn?

- Identify if there are other species, so we can accurately manage the full spectrum
- Represent true population in experimental studies for breeding or management studies

2) What types of toxins can they produce?

- Chemotype the specific DON type, and possibility of presence of other toxins besides deoxynivalenol (DON) that could be a risk to food safety or important for virulence

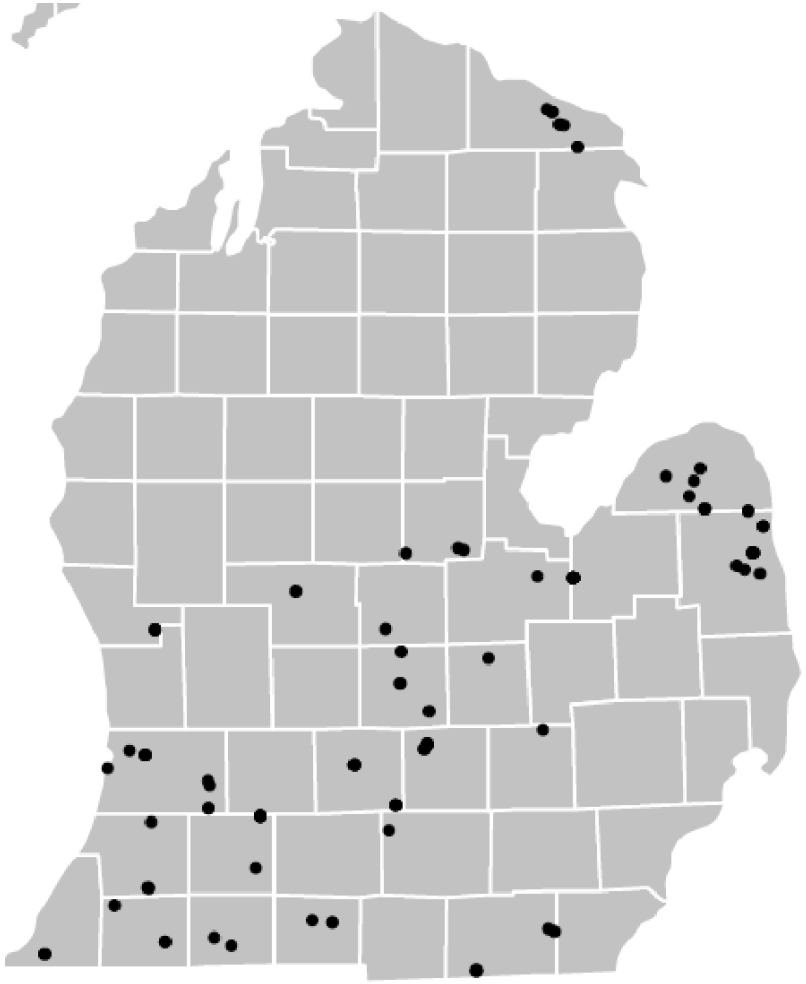
3) What levels of fungicide sensitivity are present in the population?

- Identify if any fungicide resistance currently exists
- Define current sensitivity to monitor changes in the future
- Investigate relationship of *in vitro* sensitivity with *in vivo* field efficacy

Isolate Collection

560 total isolates

- 170 from corn ears and grain
- 375 from wheat heads and grain
- Miscellaneous from wheat and corn roots
- Additional *F. graminearum* from soybean/dry bean
- Spanning 2014-2019
- All isolates were single spored

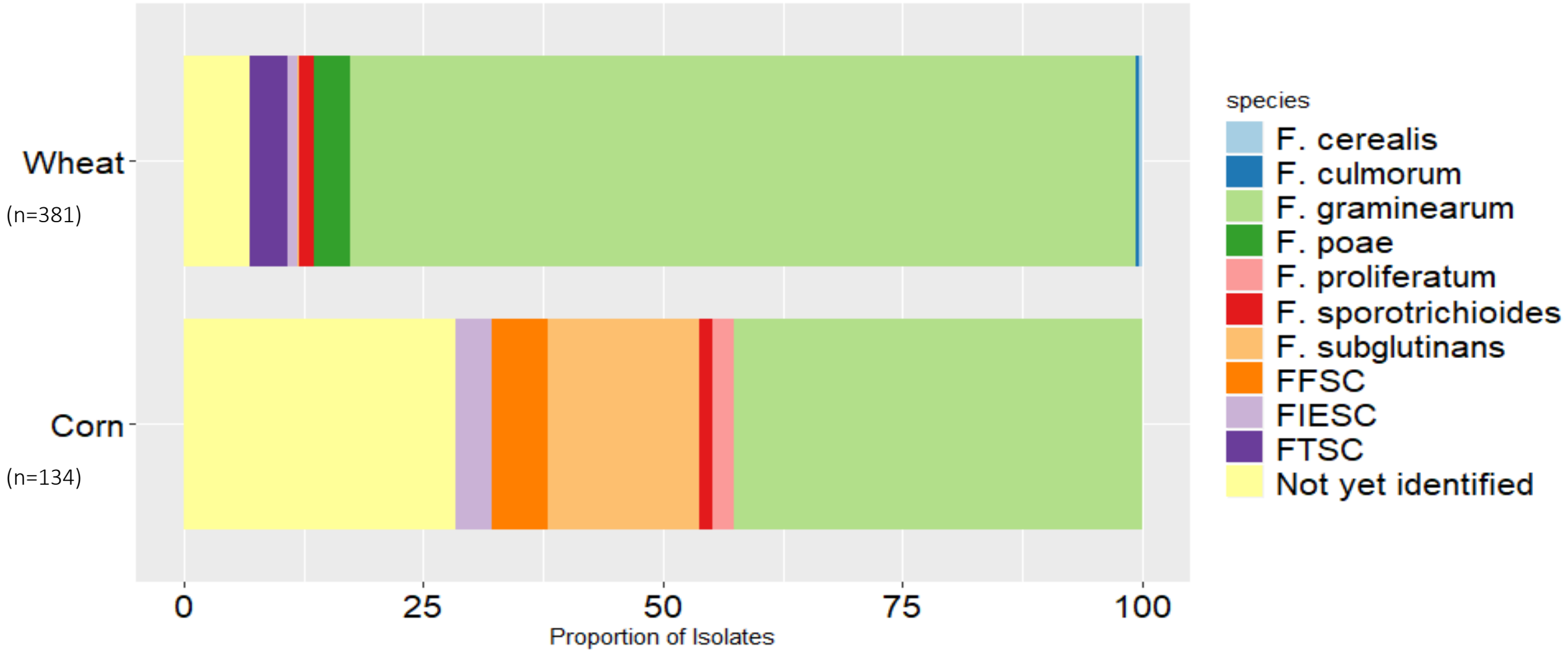


Species and chemotype characterization Using Multi-Locus Genotyping Assay (MLGT)

Species ID and chemotype in collaboration with Dr. Todd Ward at USDA-ARS Mycotoxin Prevention Unit

Sequencing of Translation Elongation Factor 1-α, for isolates not identified by the assay

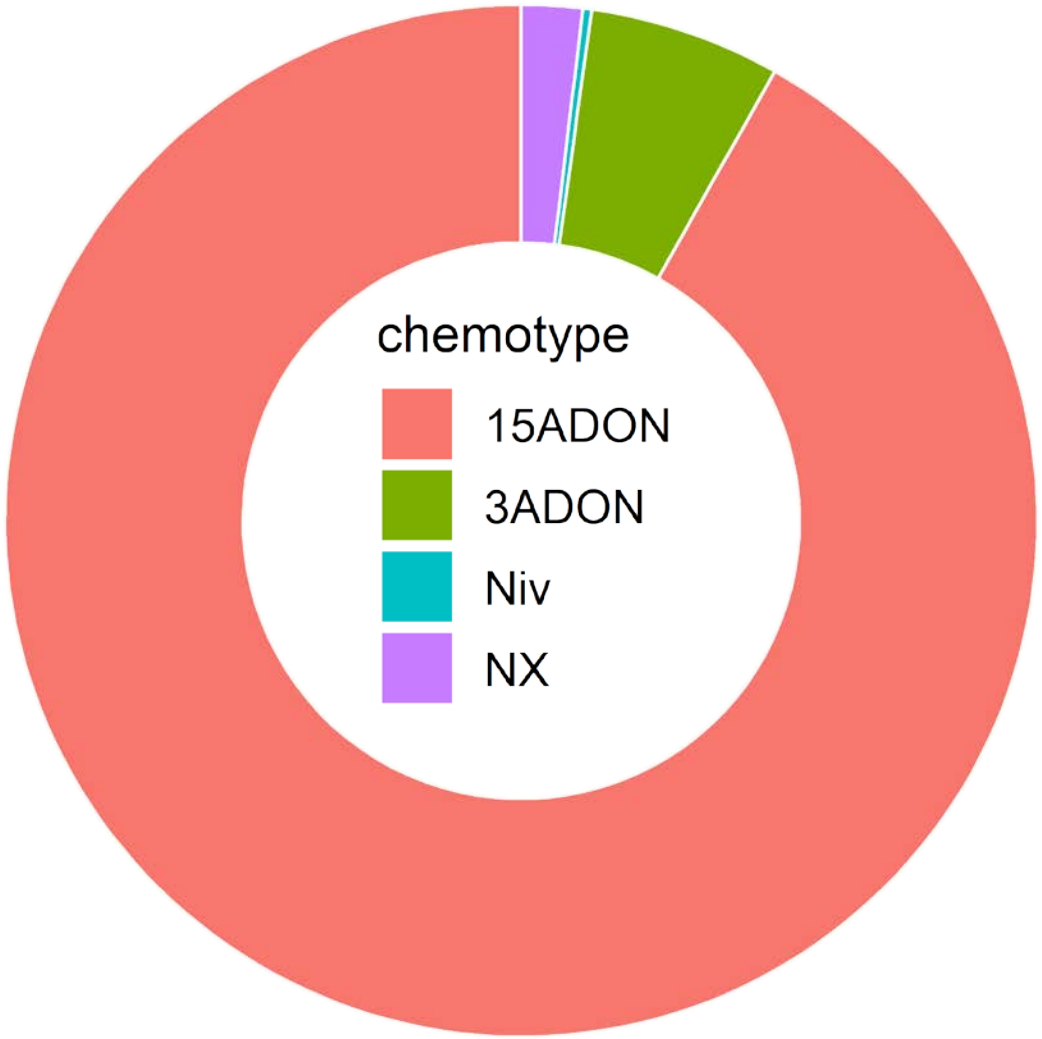
Identification of *Fusarium* isolates



Colors indicating the percentage of isolates identified in each species or species complex

F. graminearum chemotype identification

- Chemotype refers to the genotype of toxin production, that determines what "version" of toxin the isolate produces
- Most *F. graminearum* isolates were of the 15ADON chemotype, however **22** isolates were 3ADON and **7** were of the recently elucidated NX chemotype
- One *F. cerealis* isolate was of the nivalenol (NIV) chemotype

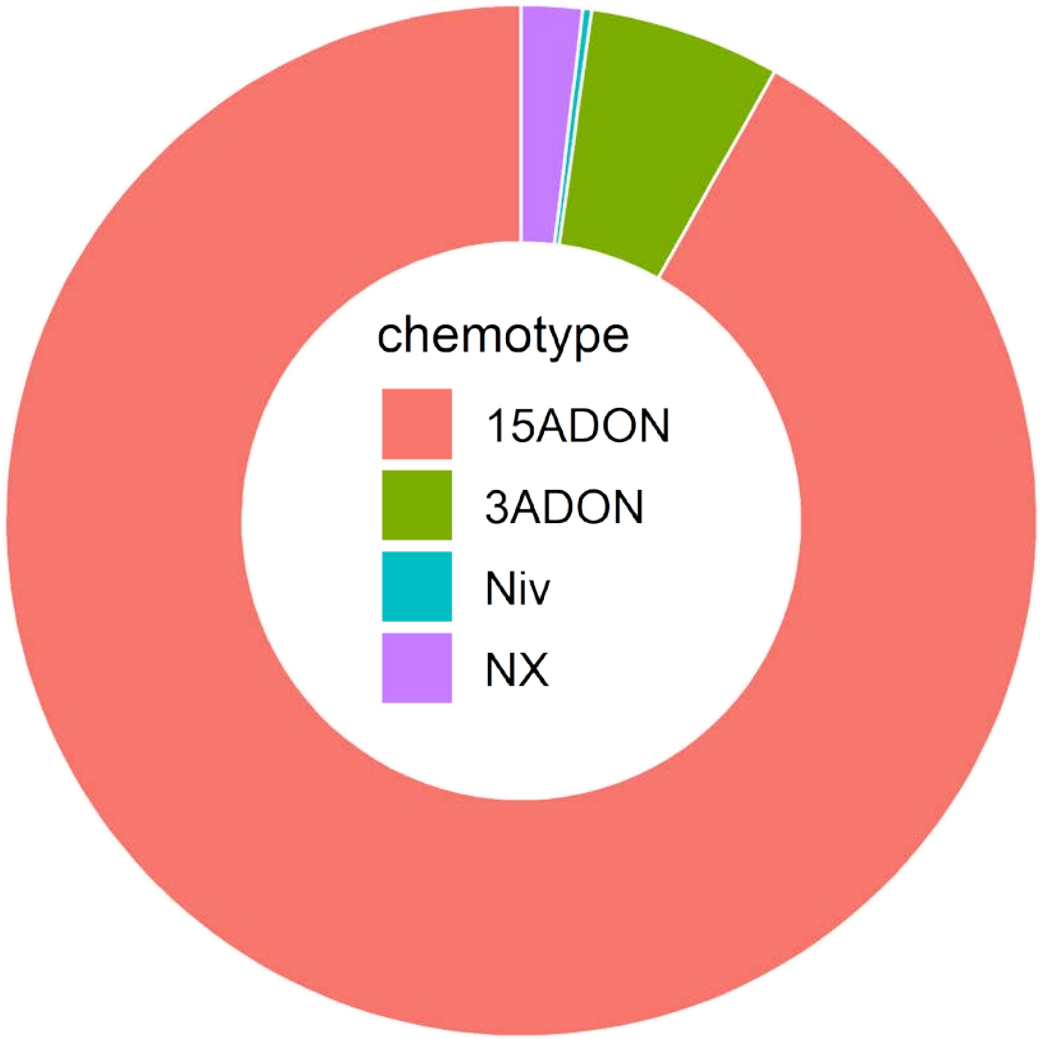


F. graminearum chemotype identification

- 3A DON chemotype known to produce more DON and be more aggressive

- Interesting because many other places in the United States have seen a shift towards the 3A DON chemotype
  - Most of Canada, North Eastern U.S, Dakotas

- However Ontario, Wisconsin, and Nebraska majority 15-ADON



What levels of fungicide sensitivity are present in the population?

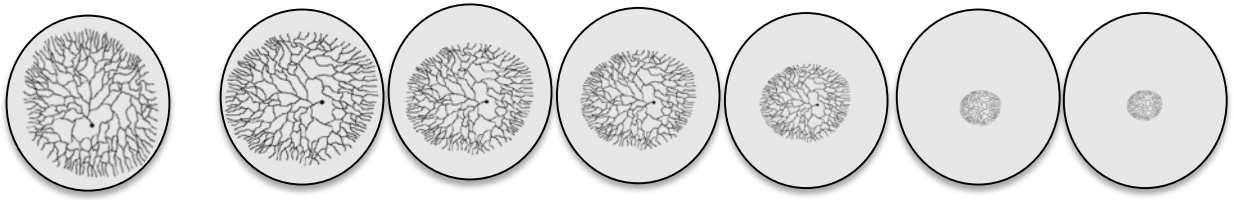
- Identify if any fungicide resistance currently exists
- Define current sensitivity to monitor changes in the future
- Investigate relationship of *in vitro* sensitivity with *in vivo* field efficacy

Class	Active ingredient	Product name	Head scab Rating	Approximate Release year
Triazole	Metconazole ★	Caramba	G	2010
	Propiconazole	Tilt	P	1997
	Prothioconazole ★	Proline	G	2006
	Tebuconazole ★	Folicur	F	2006
	Prothioconazole Tebuconazole	Prosaro	G	2008
SDHI	Pydiflumetofen Propiconazole ★	Miravis Ace	G	2018

Approach for Triazole Fungicide Sensitivity Testing:

- Determine EC50 Values for a subset of 50 isolates for three most commonly used chemistries
  - EC_{50} = Effective Concentration of fungicide that reduces the growth by 50%, when compared to growth with no fungicide (control)
- Screen remaining isolates at a single concentration of 1ppm
  - Chose dose based on correlation of relative growth with initial EC50 values
  - Find any isolates suspect of resistance
  - Follow-up by determine additional EC50 values

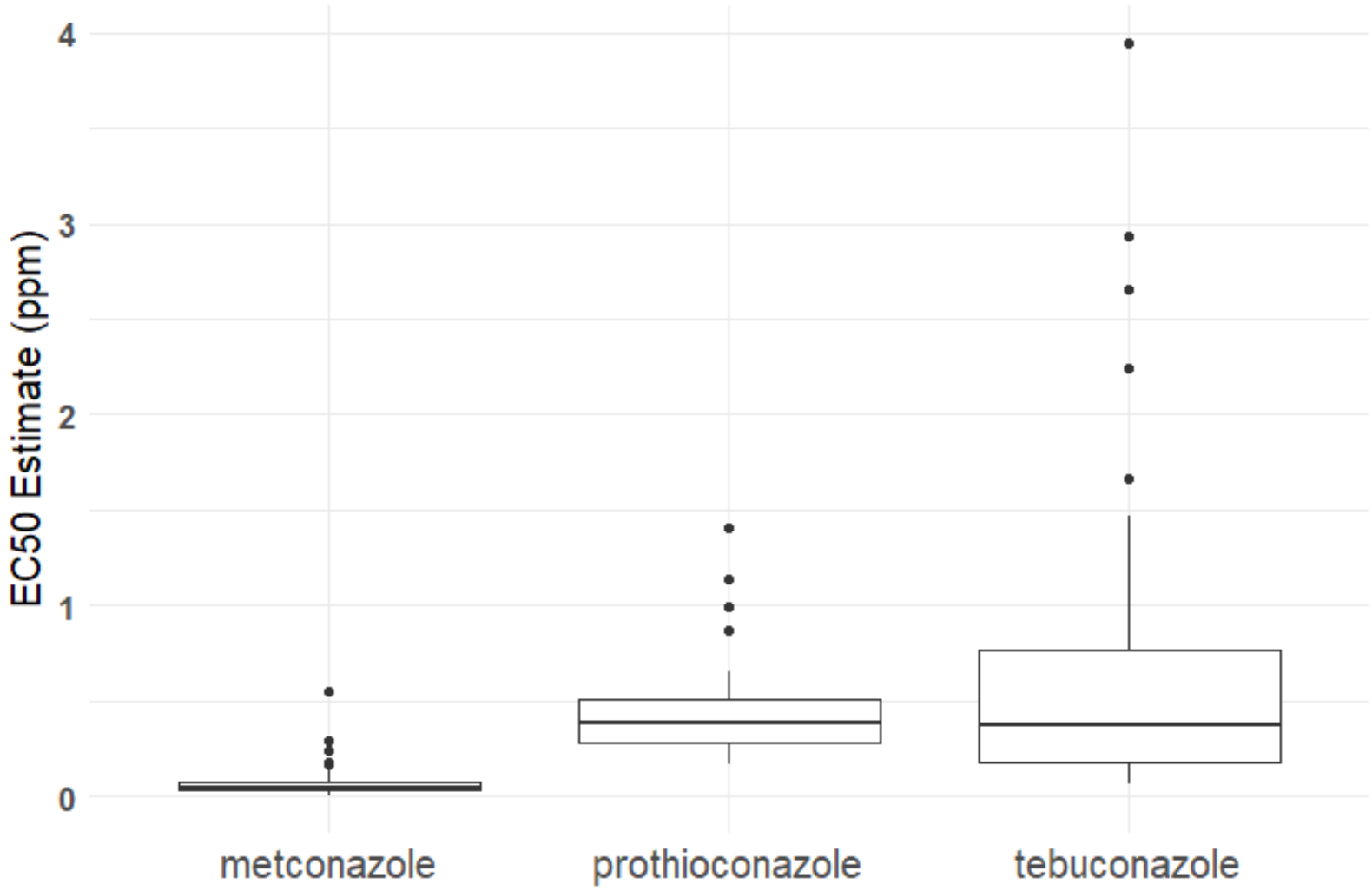
Mycelial Growth Assay



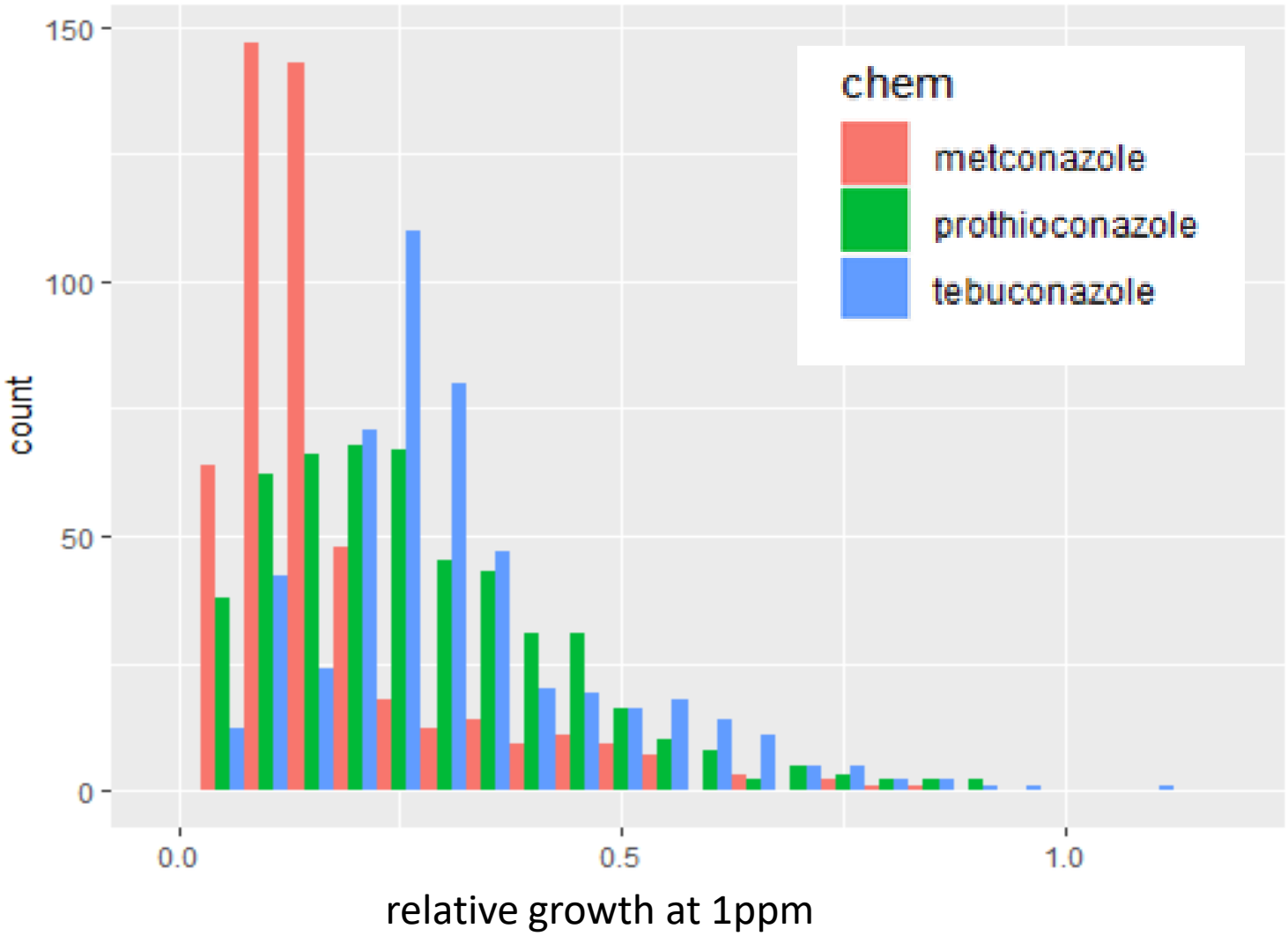
0 ug/mL 0.003 0.03 0.3 1 3 30

Agar plugs were placed on ½ strength PDA plates amended with commercial formulations of fungicides and grown for 5 days

EC50 Estimations from 45 *Fusarium* graminearum isolates

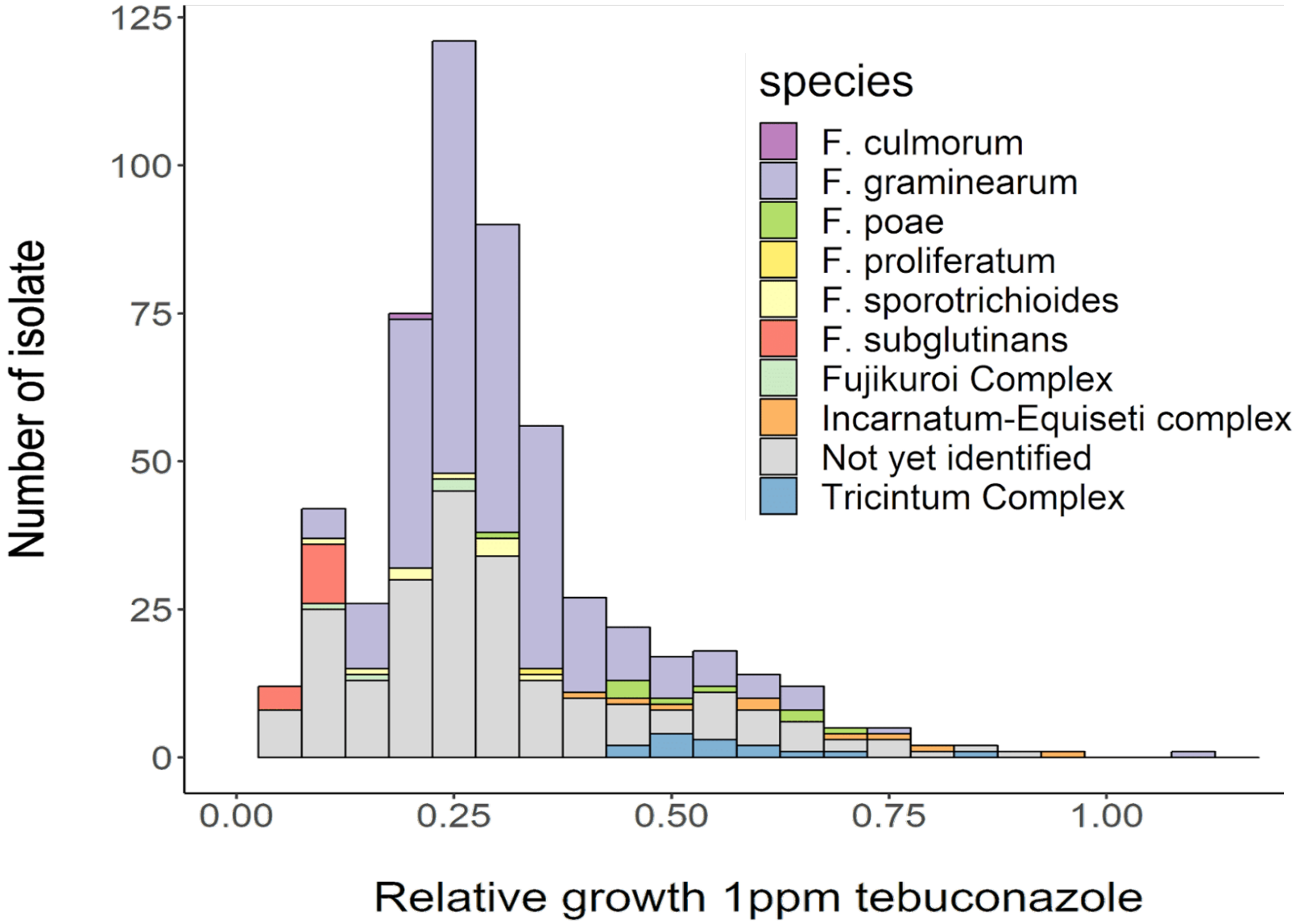


Relative Growth Distribution- Screen at 1ppm



- 1ppm chosen as relative growth at this concentration correlated best with EC50s in initial set of 50
- n=**501 isolates**
- Shows the same trends as initial EC50 estimates
- 81 isolates greater than 50% relative growth at 1ppm, could be resistant

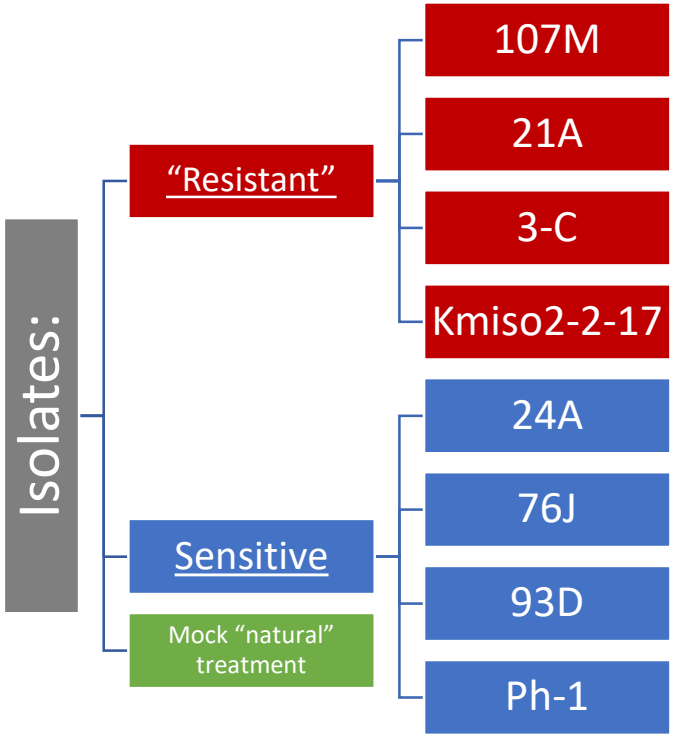
Species differences in fungicide sensitivity



- Some species seem to be inherently more sensitive or resistant
- Can further test this hypothesis with EC50 assays and *in vivo* work

Characterizing Fungicide Sensitivity
Validating *In Vitro* EC50 values of *F. graminearum*:

- Each plot was sprayed with a unique combination of an isolate and a fungicide treatment
- Four of the most sensitive isolates *in vitro* were compared to four of the least sensitive
- Random Complete Block Design with 4 replicates



X

Untreated

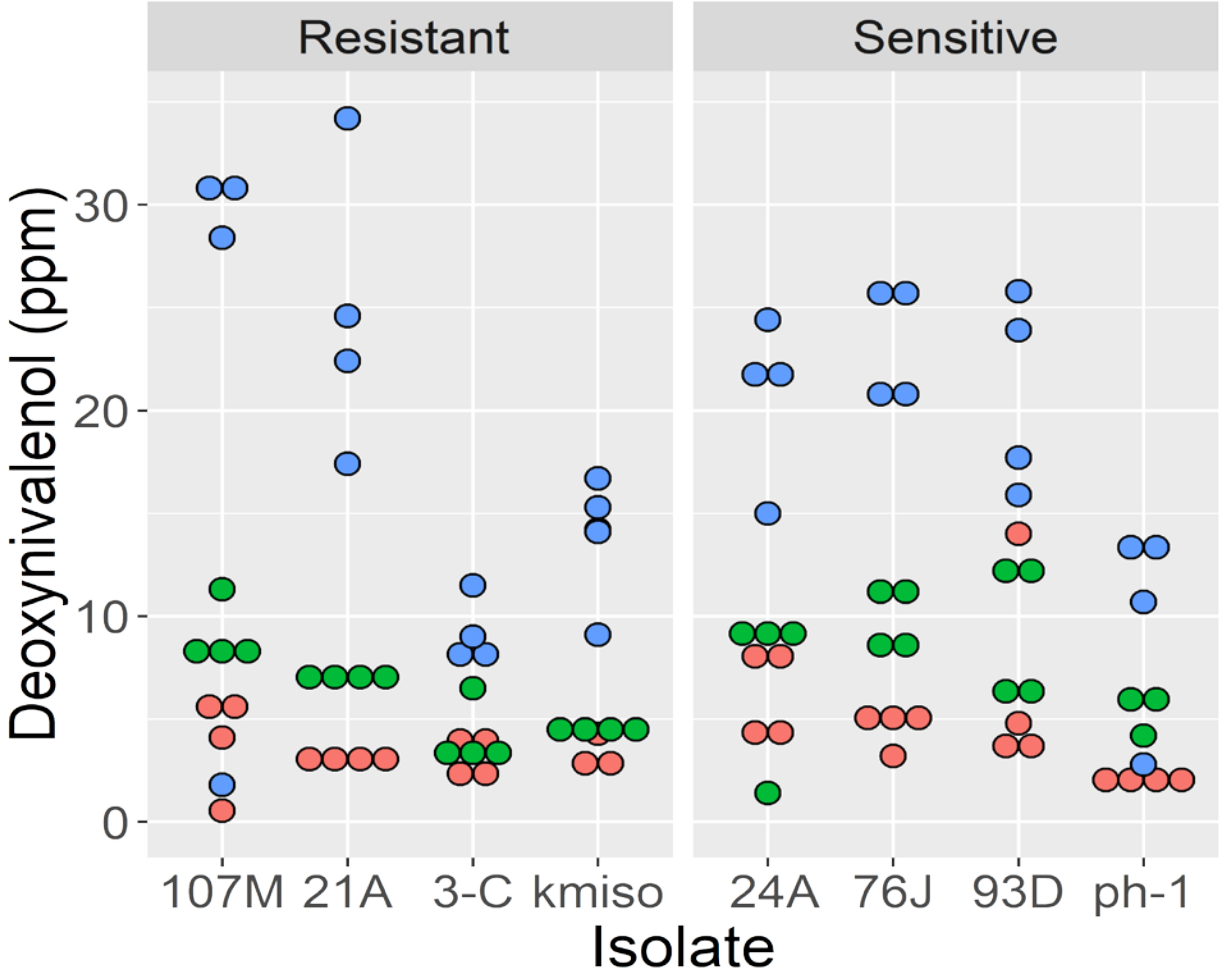
Tebuconazole (Folicur)

Metconazole (Caramba)

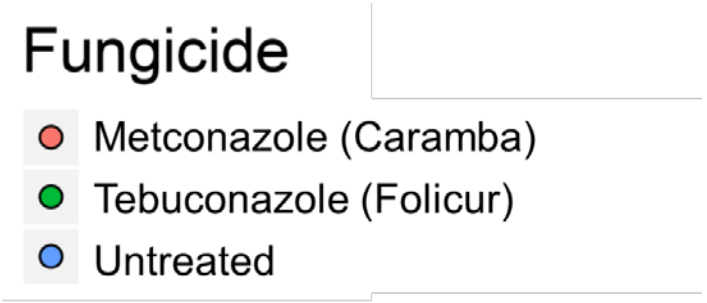
= 27 treatments

Validating *In Vitro* EC50 values: Results

DON production



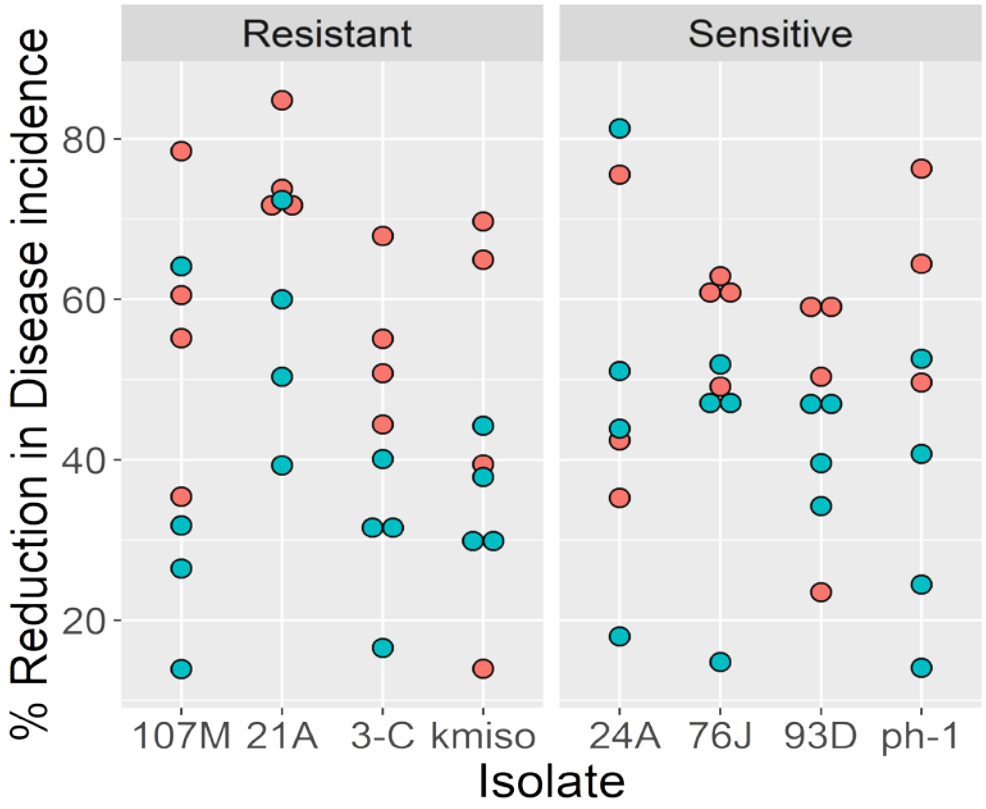
- Resistance was not a significant factor in DON production
- Large isolate differences
- Ph-1, model isolates one of the lowest producers



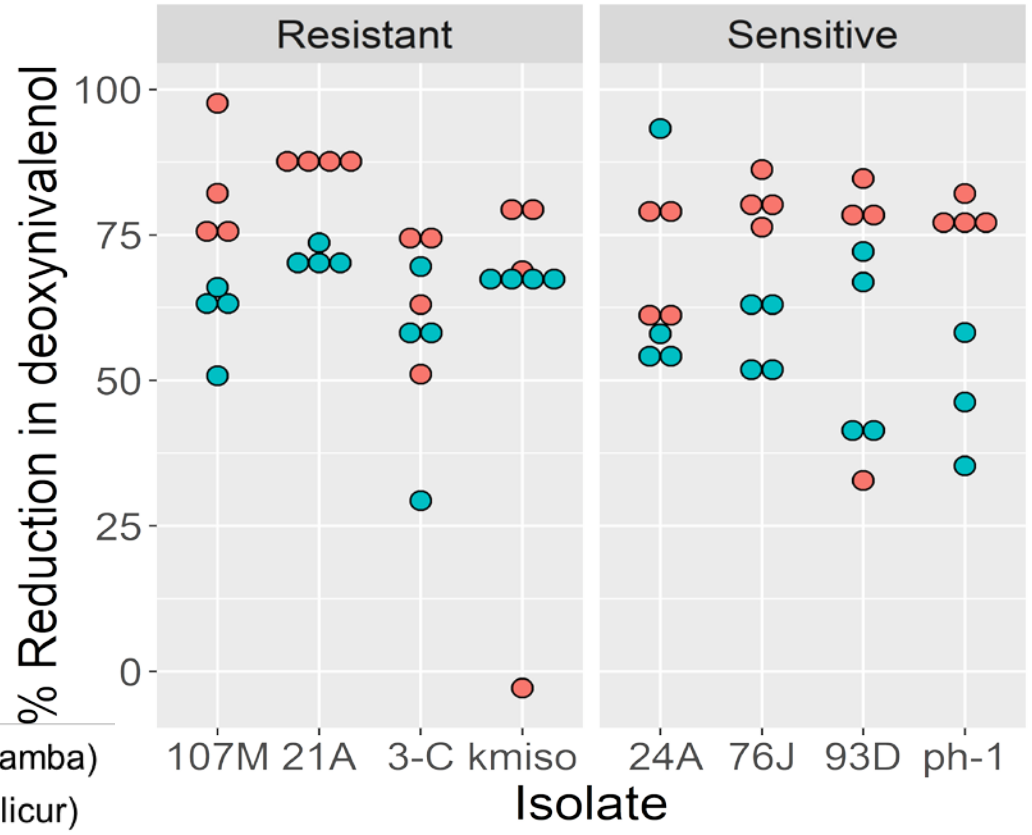
Validating *In Vitro* EC50 values: Results

Resistance was not a significant factor in the percentage disease reduction or deoxynivalenol reduction from the fungicide

Disease reduction from fungicide



DON reduction from fungicide



Fungicide

- Metconazole (Caramba)
- Tebuconazole (Folicur)

Good News!

- We have a majority of 15-ADON populations, known to be less aggressive compared to 3-ADON
- We do not seem to <u>currently</u> have any triazole fungicide resistance at field rates of chemistry

Interesting News!

- Have NX producers of *F. graminearum* in northern Michigan– unique landscape, crops?
- We do have variation *in vitro* fungicide response, so should continue to monitor resistance build-up in the future
- Variation in species, specifically *F. tricinctum complex* species

Proposed Future Work

1. Characterize effects of diverse *Fusarium* species, and determine their sensitivity to triazole fungicides and new SDHI - Miravis Ace
2. Meta-analysis of Michigan fungicide efficacy data to determine response of different fungicide timings
3. Continued field trials examining best disease management practices with emphasis on fungicide efficacy, and fungicide timing. Monitoring emerging diseases and communicating disease management in support of Michigan wheat producers.

Characterize effects of diverse *Fusarium* species

- Interested in Fusarium tricinctum complex species
- Pathogenicity
  - Preliminary work shows they are pathogenic
  - Determine if there is a range of virulence
- Determine their sensitivity to triazole fungicides and new SDHI - Miravis Ace
  - Determine EC50 values for these species
  - See if it is significantly different from sensitivity of *F. graminearum*
  - Follow up with greenhouse assays to see if they are sensitive in vivo and can be controlled with currently used commercial products

Meta-analysis of Michigan fungicide efficacy data to determine response of different fungicide timings

- Pooling data into a “Meta-analysis” allows us to better answer big picture questions- especially ones that are variable every year
- Martin Nagelkirk has fungicide efficacy trials dating back to 2008
- Chilvers lab has several years now as well

Meta-analysis goals

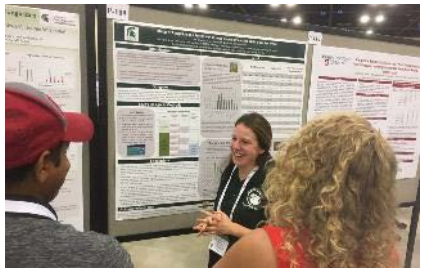
- What ranges of **yield** response can a grower expect from applications at different timings?
- What advantage does two fungicide applications have over a single flowering application?
- How often would an early foliar application (feekes 6, jointing) help control an epidemic of foliar disease? Impact yield?

Monitoring emerging diseases and communicating disease management in support of Michigan wheat producers

- Possible Bacterial disease
  - Assisted wheat breeders in determining cause of speckling
- Stinking Smut
  - Determining causal agent in conjunction with Diagnostic Services
  - Preparing extension material

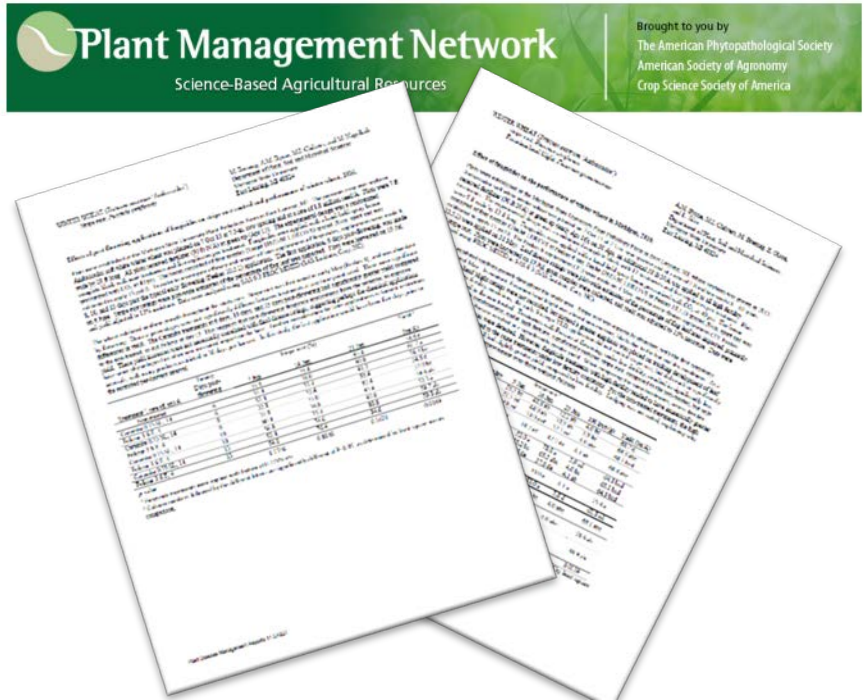
Outreach & Outputs

Find us on Facebook



@MikaelaBreunig
@MartinChilvers1

1,032 Impressions



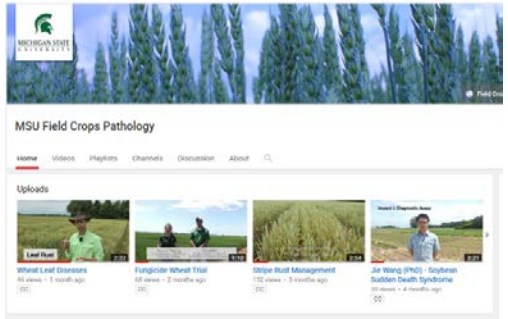
Using fungicides to suppress Fusarium head scab in wheat

Wheat fields are beginning to show signs of head emergence. This marks the time to consider applying fungicides to reduce the risk of head scab and leaf diseases.

June 5, 2019 - Author: Martin Nagelkirk, Michigan State University Extension, and Martin Chilvers, MSU Department of Plant, Soil and Microbial Sciences

The wet weather this spring has greatly elevated the risk of Fusarium head scab. At this point, the use of a fungicide at flowering is the best response

YouTube



Budget request:

Project Budget				
A. Personnel Wages	FY 20-21	FY 21-22	Non-MWP Funds**	Source**
Research associates/post-docs/on-call/technical			$ 12,000.00	USWBSI
Fringe associated*				
Graduate students	$ 23,311.43			
Fringe associated* (includes tuition)	$ 16,645.00			
Undergraduate students				
Fringe associated (summer work)*				
B. Non-expendable equipment (attach explanation)				
C. Materials, supplies & publications				
D. Travel				
E. Other direct costs (attach explanation, list of items and individual costs).				
Grand Total	$ 39,956.43	$ -	$ 12,000.00	

Other projects (non-funded unless noted)

- Fungicide efficacy trials for products and timings, conducted on campus (Industry supported)
- Multistate coordinated head scab management trials (USWBHSI)
- Novel SDHI testing in collaboration with University of Michigan Chemistry (published later this year)
- Seed treatment trials with UW-Madison and Dennis

Leveraged dollars:

- USWBHSI w/ Martin Nagelkirk $16,000. Integrated Management of FHB and DON in soft winter wheat in Michigan
- Project GREEEN w/ Kurt Steinke $25,000. Demonstration of Varietal Response to Fungicide and Nitrogen Applications on Wheat Yield, Disease Incidence and Profitability
- BASF/FMC/Syngenta/Verdesian $20,000. Fungicide efficacy testing for wheat diseases
- Corn Marketing Program of Michigan $40,000. Gibberella ear mold, seedling damping off and root rot, and responding to emerging diseases
- USDA-NIFA $499,279. Impact of production system, plant species and stress on whole plant microbiome and productivity. Greg Bonito, Martin Chilvers, Frances Trail

Contributions:

- $5,000 for venting of drier, also used by Eric's crew for drying Fusarium inoculum
- $10,000 to $12,000 towards combine wheat head and auger to facilitate wheat harvest for all programs
- $1,000 from Dick Chase movin on endowment to Pennington's drone purchase

Thank you!

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517-353-9967

U.S. Wheat & Barley Scab Initiative

Michigan Wheat Program

Project GREEEN 10 YEARS & GROWING

Michigan State University AgBioResearch

Corn Marketing Program of Michigan CMPM

GAPS

DuPont

National Sclerotinia Initiative

NCSRP North Central Soybean Research Program

USDA NIFA

Asgrow

Pioneer A DuPont Company

Valent

Bayer

United Soybean Board Making Your Checkoff Pay Off.

Michigan Soybean Promotion Committee www.michigansoybean.org

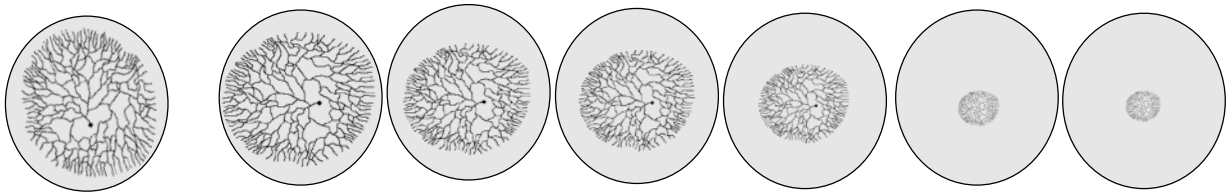
Foliar Timing Trial

Treatment	Timings	Product
1	Untreated	Prosaro
2	T1 (Fks 5/6)	Prosaro
3	T2 (Fks 9)	Prosaro
4	T3 (Fks 10.5.1)	Prosaro
5	T3.5 (one week after T3 – Milk/grain fill)	Prosaro
6	T1 + T3	Prosaro
7	T2 + T3	Prosaro
8	T1 + T2 + T3	Prosaro
9	T1 (Stratego YLD 4 fl oz) T3 (Prosaro 6.5 floz) "Industry Standard"	Stratego Prosaro

- On campus - PLP Farm, un-misted, non-inoculated
- Variety: Ambassador soft white winter wheat
- Randomized Complete Block Design- 5 replications

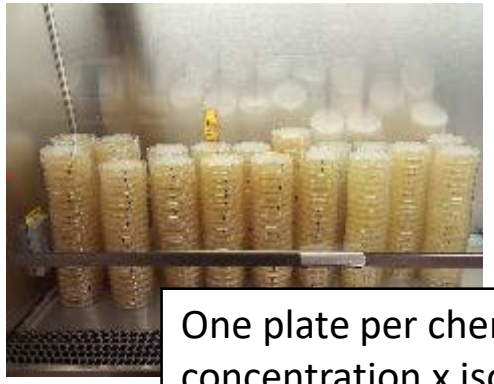
Characterizing Fungicide Sensitivity: Methods

Mycelial Growth Assay

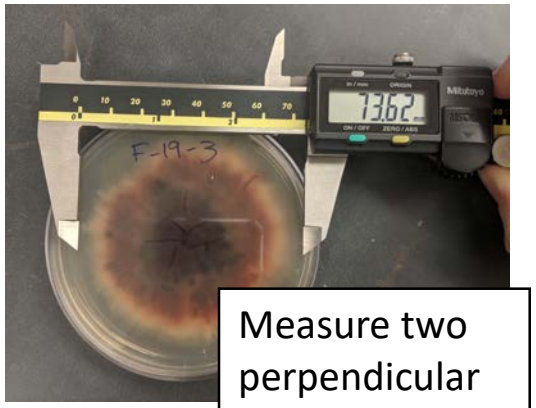


0 ug/mL 0.003 0.03 0.3 1 3 30

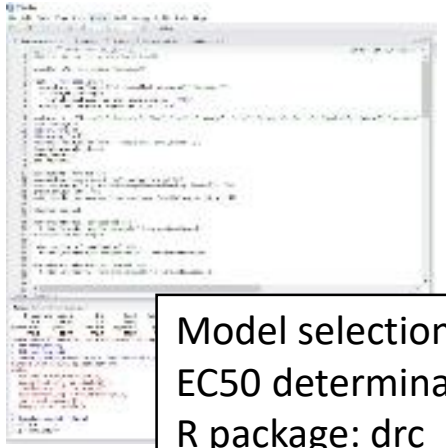
Agar plugs were placed on ½ strength PDA plates amended with commercial formulations of fungicides and grown for 5 days



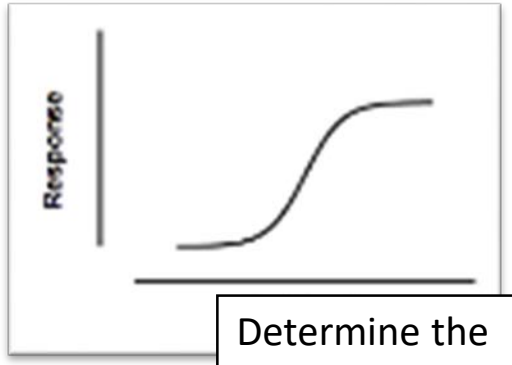
One plate per chemistry x concentration x isolate , repeated three times



Measure two perpendicular directions



Model selection and EC50 determination
R package: drc



Determine the absolute EC50

EC_{50} = Effective Concentration of fungicide that reduces the growth by 50%, when compared to growth with no fungicide (control)

Conclusions- Characterization of Fungicide Sensitivity

- No practical resistance currently exists in Michigan populations of *F. graminearum* despite widespread use of triazoles in wheat (and corn) throughout Michigan for the last 10 years
  - No differences in the relative fungicide efficacy between invitro sensitive and resistant isolates
- Diverse cropping system and pathogen lifestyle reducing selection pressure?
  - Fitness penalty reducing selection pressure? “sensitive” may be more pathogenic?
- If isolates with EC_{50} values greater than 10ppm are found in the future, these would warrant further field testing
- Introduction of SDHI chemistry (Adepidyn) will be valuable rotation partner if there would be problems
  - Current population very sensitive, we tested 100 isolates for a baseline sensitivity
- Other species however could have a different relationship and would need to be validated separately

Outputs

- Chilvers M.I. and Nagelkirk M. May 31, 2017. Using fungicides to suppress head scab in wheat. Wheat wisdom email listserv and website. Michigan Wheat Program
- Nagelkirk M. and Chilvers M.I. May 26, 2017. Using fungicides to suppress head scab in wheat. MSUE News for Ag
- Stripe rust management YouTube video https://www.youtube.com/watch?v=dLo1s1futP0&t=1s
- Fungicide wheat trial YouTube video
- https://www.youtube.com/watch?v=Fzs3nHhqqWE
- Wheat leaf disease identification YouTube video
- https://www.youtube.com/watch?v=cEUsg-Jv_5Q
- Byrne, A.M., Chilvers, M.I. M. Breunig, Olson, E., Siler, L. 2017. Effect of fungicides on performance of winter wheat in Michigan, 2016. Plant Disease Management Reports 11:FC029
- Breunig, M., Byrne, A.M., Chilvers, M.I., Nagelkirk, M. 2017 Effects of post-flowering applications of fungicides on stripe rust control and performance of winter wheat, 2016. Plant Disease Management Reports 11:CF027