Project FY22-MG-009: Assessment of Fungicide Sensitivity in Field Populations of Fusarium Causing FHB

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1. What are the major goals and objectives of the research project?

Fungicides are commonly used as part of an integrated management plan to reduce Fusarium head blight caused predominantly by the fungus, *F. graminearum*. In 2019, Syngenta released Miravis Ace, a premix fungicide that contains an active ingredient in the "second generation" succinate dehydrogenase fungicide class (adepidyn (pydiflumetofen); SDHI, FRAC group 7) and a triazole (propiconazole), for use in suppressing FHB. This is the first fungicide containing an active ingredient other than a DMI labelled for use in suppressing FHB; however, additional second-generation SDHI active ingredients have been used for several years to suppress numerous fungal diseases in wheat and cropping systems such as corn and soybeans. SDHI resistance has been observed in other pathosystems, including diseases in wheat and barley; however, no studies have examined populations of *F. graminearum*. The goal of this project was to generate baseline sensitivities for pydiflumetofen in *F. graminearum* and populations across wheat and barley production regions in the US. Objectives include:

Objective 1. Establish centralized testing locations and protocols for fungicide sensitivity testing for Fusarium isolates as part of the USWBSI
Objective 2. Develop baseline sensitivity and associated virulence of current and historic isolates of Fusarium to SDHI and DMI fungicides collected from FHB symptomatic wheat in US wheat production areas
Objective 3. Place unique and/or valuable isolates into a national storage facility to facilitate collaboration between MGMT and PBG RACS

2. What was accomplished under these goals or objectives? *(For each major goal/objective, address these three items below.)*

What were the major activities?

<u>Objective 1. Establish centralized testing locations and protocols for fungicide sensitivity testing for Fusarium isolates as part of the USWBSI</u>

In project year 1 (2022-2023), Dr. Alyssa Koehler and I established our laboratories as centralized testing locations for *Fusarium* isolate fungicide sensitivity. We have had video calls and meetings to coordinate sample solicitation, isolate collection, and fungicide sensitivity testing strategies. We have solicited head scab sample submissions from our fellow extension pathologists across the country. APHIS permits were renewed through the new e-permit system to allow import of isolates and infected wheat and barley heads from across the country.

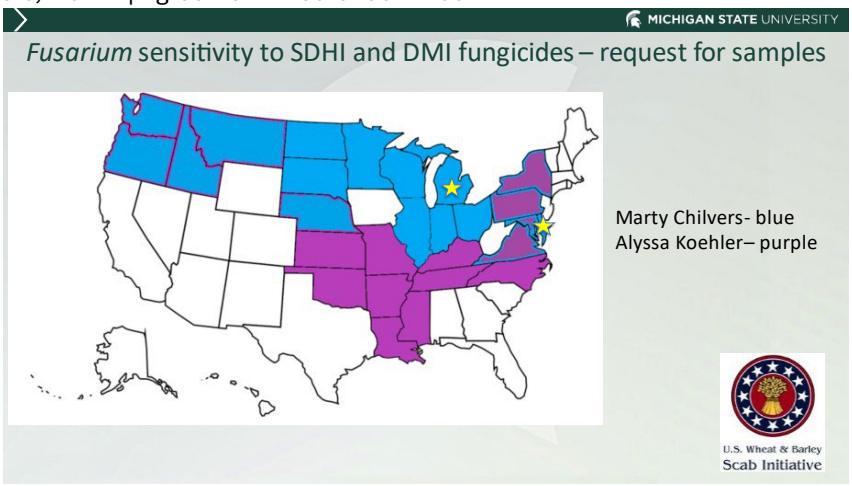

Figure: Centralized testing locations for the US, at Michigan State University (blue) and the University of Delaware (pink).

The collection of isolates in 2022 and 2023 was slow due to the low head scab disease years. However, we have been receiving a good number of samples for the 2024 season. We have also been in discussions with several groups to secure access to existing collections including historical and contemporary isolates to be used in this project.

Table: Summary of samples or isolates submitted for fungicide sensitivity testing. In addition, to these isolates listed there are historical collections available in various labs around the country that we plan to include to allow for contrast of fungicide sensitivity values between contemporary and historical isolates.

State	Year	# of Fields	Minimum # Isolates Expected	# Isolated	Notes	Provided By
ND	2024	10	20		**Heads currently being collected**	Andrew Friskop
MI	2024	4	12	0	PLP and KBS. Heads received, in progress	Christine Charles (KBS)
IN	2024	11	33	0	Heads received, in progress	Darcy Telenko
NE	2024	4	12	0	Heads received, in progress	Stephen Wegulo
IL	2024	33	66	60	Minimum 2 isolates (2 heads) per field (close to complete)	Briana Whitaker
ID	2023	1	1	1	Isolated by Rawnaq	Rawnaq Chowdhury
IN	2022	8	8	8	From Nik, isolated	Chilvers lab
NE	2022	1	3	3	From Nik, isolated	Stephen Wegulo
ND	2022	1	2	2	From Nik, isolated (Expecting 2024 from Andrew Friskop?)	Andrew Friskop
ID	2022	6	6	6	Isolated by Rawnaq	Rawnaq Chowdhury
ID	2021	7	7	7	Isolated by Rawnaq	Rawnaq Chowdhury

MI	2011-2019		200	200		Chilvers lab
Total			370			

In addition to the isolation of *Fusarium* from head scab samples described above. We have also been able to collect fungal and oomycete isolates from roots of wheat seedlings. The isolations were performed across eight site years in Michigan from two varieties of non-treated wheat seed. Of relevance to this USWBSI project, approximately half of the fungal isolates recovered from the roots of wheat seedling were Fusaria. Including, isolates of *F. graminearum, F. sporotrichioides*, members of the *Fusarium tricinctum* species complex, and members of the *F. incarnatum-equiseti* species complex. Seedling pathogenicity and fungicide tests with these isolates are currently being conducted. These experiments will provide valuable data on the role of *Fusarium* spp. not only as they affect head scab but also their role in seedling infection and root rot. This data will improve our understanding of *Fusarium* in the wheat system.

Objective 2. Develop baseline sensitivity and associated virulence of current and historic isolates of Fusarium to SDHI and DMI fungicides collected from FHB symptomatic wheat in US wheat production areas

As described above we have a good collection of Fusarium isolates for fungicide sensitivity screening. A visiting PhD student, Mr. Sungyu Choi visiting my lab from Dr. Hyunkyu Sang's lab in Chonnam National University in Korea worked on medium for fungicide sensitivity testing. Although we had previously used half strength potato dextrose agar, we seemed to get better uniformity growth and fungicide inhibition data from YBA medium (10g/L yeast extract, 10g/L peptone and 20glL sodium acetate in distilled water). We are testing YBA in comparison to half strength PDA in a few more trials until we are confident that YBA will be a better medium for the fungicide sensitivity testing. Dr. Koehler's lab have also been conducting similar tests.

A graduate student was recruited in Fall of 2023 and is currently conducting fungicide sensitivity assays. We look forward to presenting results at the 2024 USWBSI forum.

We have submitted a manuscript to Phytopathology describing in detail the *Fusarium* species composition, chemotype and fungicide sensitivity in Michigan from both wheat and corn. The abstract of the submission is as follows:

Abstract:

Mycotoxin contamination of wheat and corn grain from Fusaria is a major agricultural concern. To characterize the population of *Fusarium* in Michigan, 569 isolates were collected and species composition (see figure below), chemotype, *in vitro* and *in planta* fungicide sensitivity were determined. In wheat, the *Fusarium sambucinum* species complex comprised 90% of isolates of which 82.5% were *F. graminearum*. In corn, the *Fusarium sambucinum* species complex comprised 40% with 37% identified as *F. graminearum,* while species from *Fusarium fujikuroi* species complex comprised 50%. Within this complex *F. awaxy* (4.6%) was present and is a first report in the United States. Across *F. graminearum* isolates, chemotypes were found at the following proportions 92% 15-ADON, 6% 3-ADON, 1.6% NX and no NIV. *In vitro* mycelial growth sensitivity assays to triazole fungicides demonstrated *Fusaria* were most sensitive to metconazole. Species-specific differences in sensitivity

were identified, with *Fusarium tricinctum* species complex members significantly less sensitive than *F. graminearum* isolates, and the *Fusarium fujikuroi* species complex was significantly more sensitive (see figure below). Within *F. graminearum,* 10 isolates had EC_{50} values 10-fold greater than sensitive isolates. A subset of these *F. graminearum* isolates were chosen to investigate if reduced sensitivity *in vitro* would lead to practical resistance *in vivo*. Field plots were inoculated with spore suspensions however,no differences in the relative fungicide efficacy were found, signaling no practical resistance currently exists despite differences *in vitro*. While currently there may not be practical resistance, monitoring should continue as there is variation in *in vitro* sensitivities present within and among species.

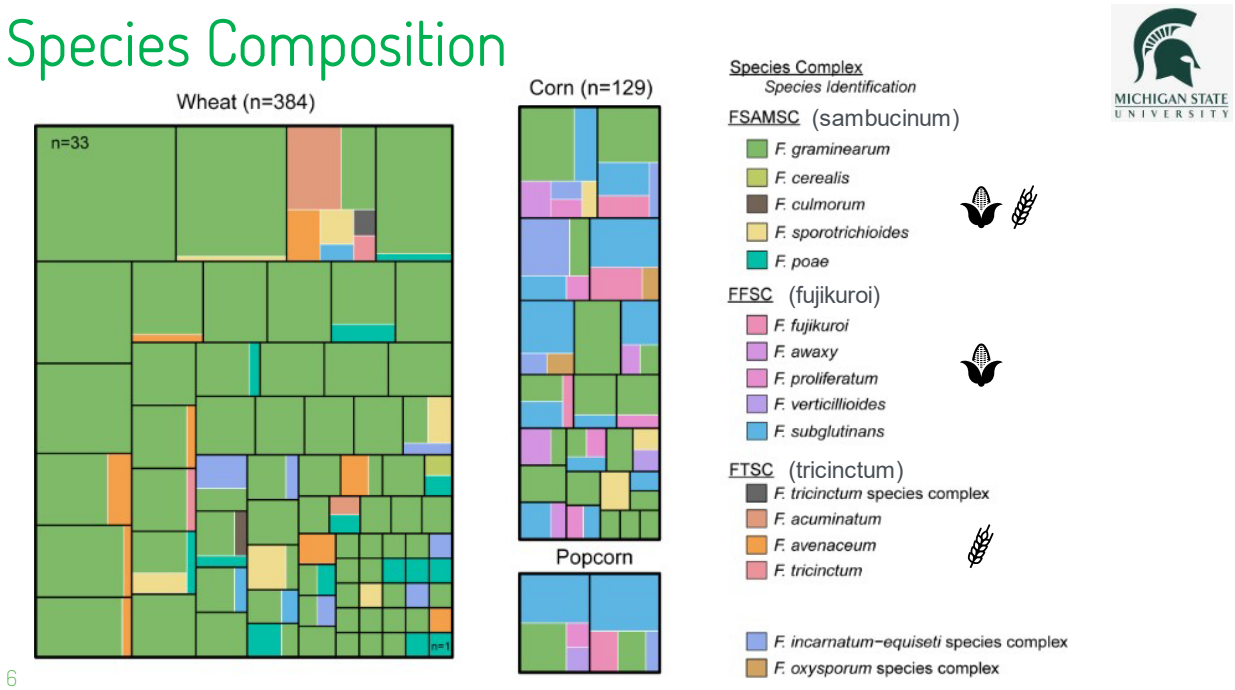

Figure: *Fusarium* species composition as collected from wheat and corn between 2011 and 2019 from across Michigan.

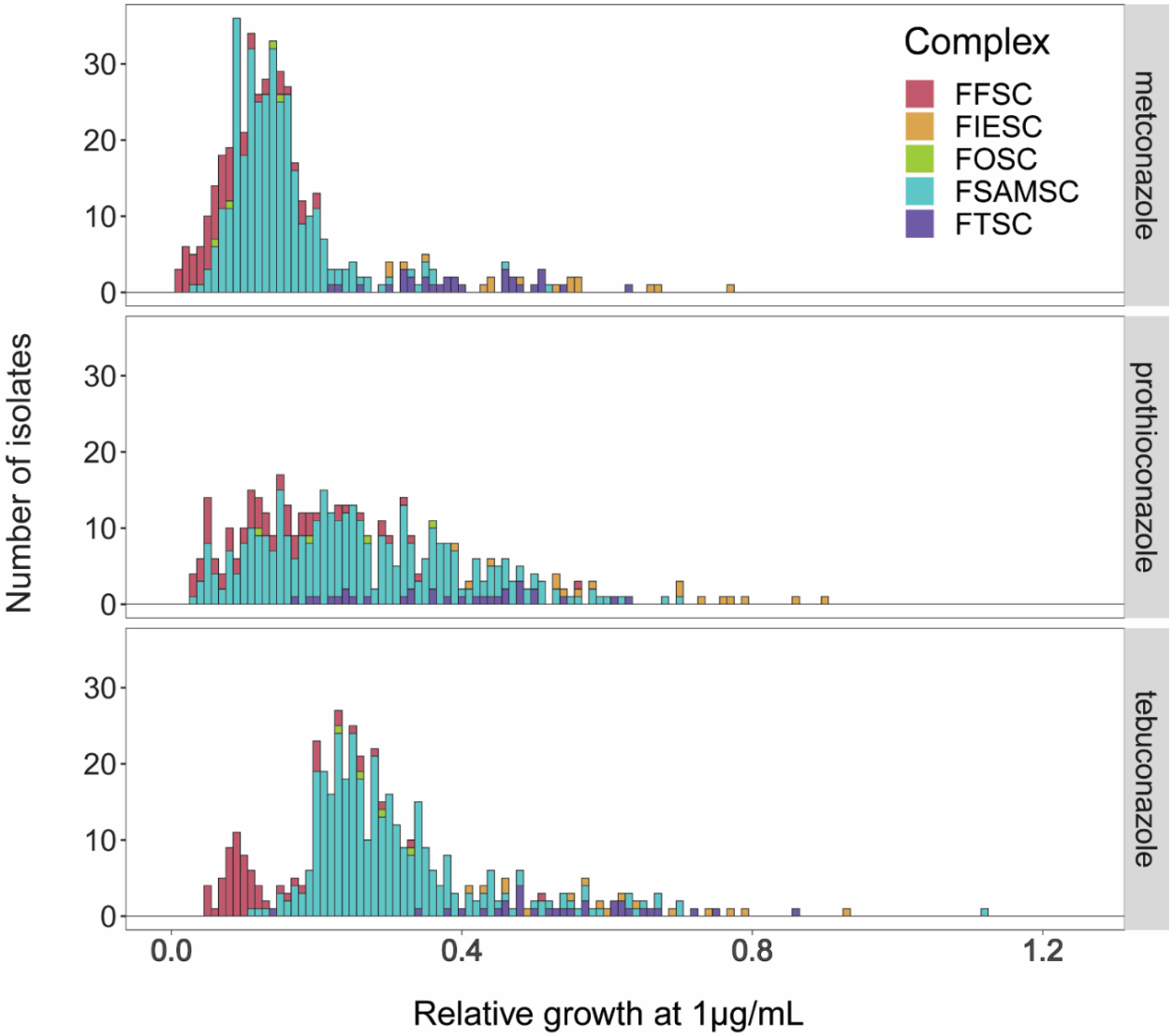

Figure: Distribution of mean relative growth values from two replicates of 445 *Fusarium* isolates tested in mycelial growth assays in ½ strength PDA plates amended with 1µg/mL commercial formulation of metconazole (Caramba), prothioconazole (Proline), and tebuconazole (Folicur). Different colors represent different species complexes FFSC (*Fusarium fujikuroi*), FIESC (*Fusarium incarnatum-equiseti*), FOSC (*Fusarium oxysporum*), FSAMSC (*Fusarium sambucinum*), and FTSC (*Fusarium tricinctum*).

Objective 3. Place unique and/or valuable isolates into a national storage facility to facilitate collaboration between MGMT and PBG RACS

Dr. Koehler and I have participated in conversations with our USDA colleagues with respect to which isolates might be valuable and how many and how these might be plated into a national storage facility to facilitate collaboration between MGMT and PBG RACS.
We have also provided 26 isolates of 3ADON F. graminearum isolates to Dr. Chris Toomajian from the University of Kansas.

What were the significant results?

Although relatively low levels of head scab have been experienced for the last two seasons. At MSU we have been provided with 23 unique sample locations from across 4 states and a total of 234 samples. We are working through these head samples and performing Fusarium isolations. In discussions with colleagues, we have also been granted access to historical collections from a number of states.

List key outcomes or other achievements.

Two centralized testing locations are in operation and we have continued to improve the protocols for fungicide sensitivity testing. We are currently collecting isolates from the 2024 season.

Baseline efficacy within wheat and malting barley isolates have begun to be established across multiple states and in comparison to historic isolates.

Conversations have been initiated to discover the process and resources available to secure high value isolates in national storage facilities and to work across MGMT and PGB projects to maximize utility of isolate collections.

3. What opportunities for training and professional development has the project provided?

A postdoc has been heavily involved in the project, and a graduate student has been recruited to work on this project. The lab also has 4 undergraduate students that are involved in assisting with the project.

4. How have the results been disseminated to communities of interest?

A presentation was given at the 2024 USWBSI Scabinar, which provided an overview of the project, what has been found to date and a discussion on the importance of fungicide sensitivity screening work. A peer reviewed publication has been submitted and additional papers are in progress. The work has also been used to communicate to farmers and industry about the status of fungicide sensitivity within Fusarium and the potential risk fungicide resistance poses.

5. **What do you plan to do during the next reporting period to accomplish the goals and objectives?** We will continue to solicit and collect isolates from across our testing region. The past two seasons have been quite low FHB pressure, but it seems that pressure was elevated in many states, so we anticipate higher sample numbers for 2024. Once isolates have been completed for the 2024 season we will assess the collection that we have, which will inform the number of historical isolates that we pull in for comparative analysis.