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 goals of this project were to generate baseline sensitivities for pydiflumetofen in *F. graminearum* and populations across wheat and barley production regions in the US and examine for potential resistance development in triazole chemistries that have been the backbone of head scab management.

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.

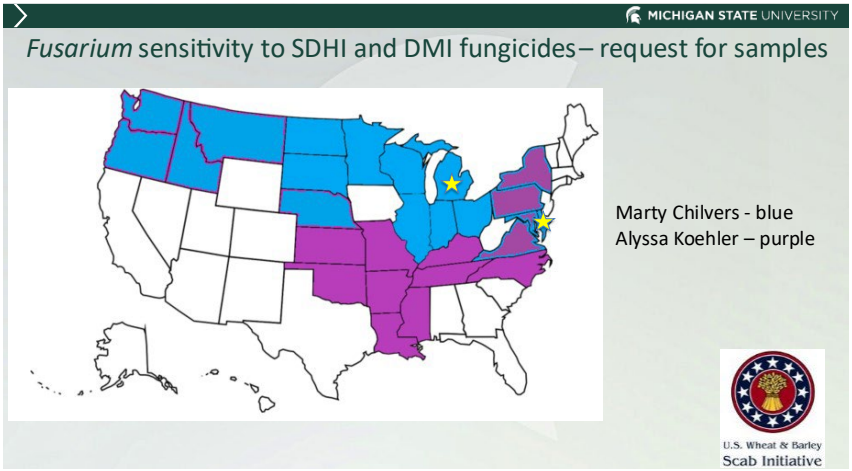

Figure: Centralized testing locations for the US, at Michigan State University (blue) and the University of Delaware (pink).

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 and 2025 seasons. 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	Isolate Count	Provided By
Michigan	2011-2019	200	Chilvers Lab
Michigan	2024	7	Keeley Satterfield and Christine Charles
Michigan	2025 (expected)	5	Keeley Satterfield
Illinois	2024	49	Briana Whitaker
Illinois	2025 (expected)	15	Briana Whitaker
Minnesota	2024	43	Beheshteh Zargaran
Nebraska	2024	12	Stephen Wegulo
Nebraska	2022	3	Chilvers Lab
North Dakota	2024	41	Briana Whitaker
North Dakota	2022	2	Chilvers Lab
Indiana	2024	27	Darcy Telenko
Indiana	2022	8	Chilvers Lab
Idaho	2022	6	Rawnaq Chowdhury
Idaho	2021	7	Rawnaq Chowdhury
Ohio	2023	26	Alyssa K. Betts
Maryland	2024	5	Alyssa K. Betts
Pennsylvania	2024	47	Alyssa K. Betts
New York	2024	115	Alyssa K. Betts
Delaware	2024	25	Alyssa K. Betts
Kentucky	2025 (expected)	16	Briana Whitaker
Kentucky	2023	26	Alyssa K. Betts
Missouri	2024	21	Alyssa K. Betts
Total	**2011-2025**	**706**	

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

62 strains of *F. graminearum* were tested for fungicide sensitivity. The suitability of half-strength PDA and YBA media was pre-evaluated for image analysis, and half-strength PDA provided better results due to clearer mycelial growth. Pydiflumetofen (SDHI) and tebuconazole (DMI) were each added to the half-strength PDA at concentrations of 0.008, 0.04, 0.2, 1, and 5 mg/L. Based on the growth rate at each concentration, EC50 calculations and discriminatory dose experiments were performed. Using a three-parameter logistic model, the average EC50 values were measured to be 0.165±0.048 mg/L for pydiflumetofen and 0.658±0.135 mg/L for tebuconazole. When analyzing the correlation between LogEC50 values and relative growth rates at various concentrations, the highest correlation was observed at 0.04 mg/L for pydiflumetofen (R^2=0.86, MAE=0.119, RMSE=0.172) and 0.2 mg/L for tebuconazole (R^2=0.792, MAE=0.09, RMSE=0.128)*

* R^2 : Coefficient of Determination, MAE : Mean Absolute Error, RMSE : Root Mean Squared Error

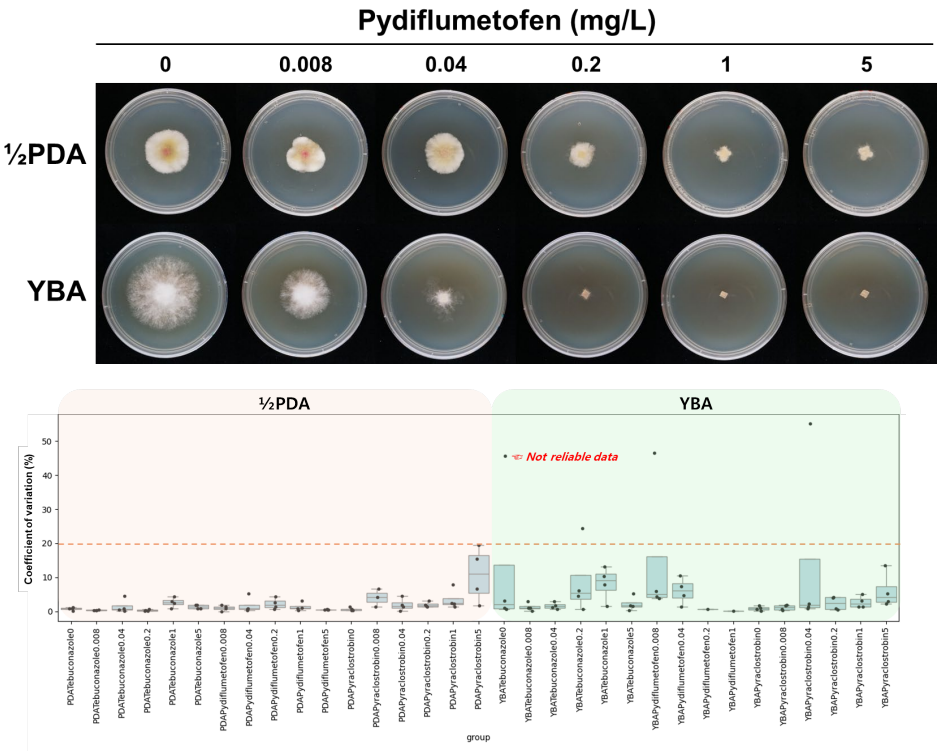

Figure: Sensitivity testing of pydiflumetofen on half-strength PDA and YBA media, and evaluation of the coefficient of variation (CV). Demonstrating reduced variation with half-strength PDA vs YBA media.

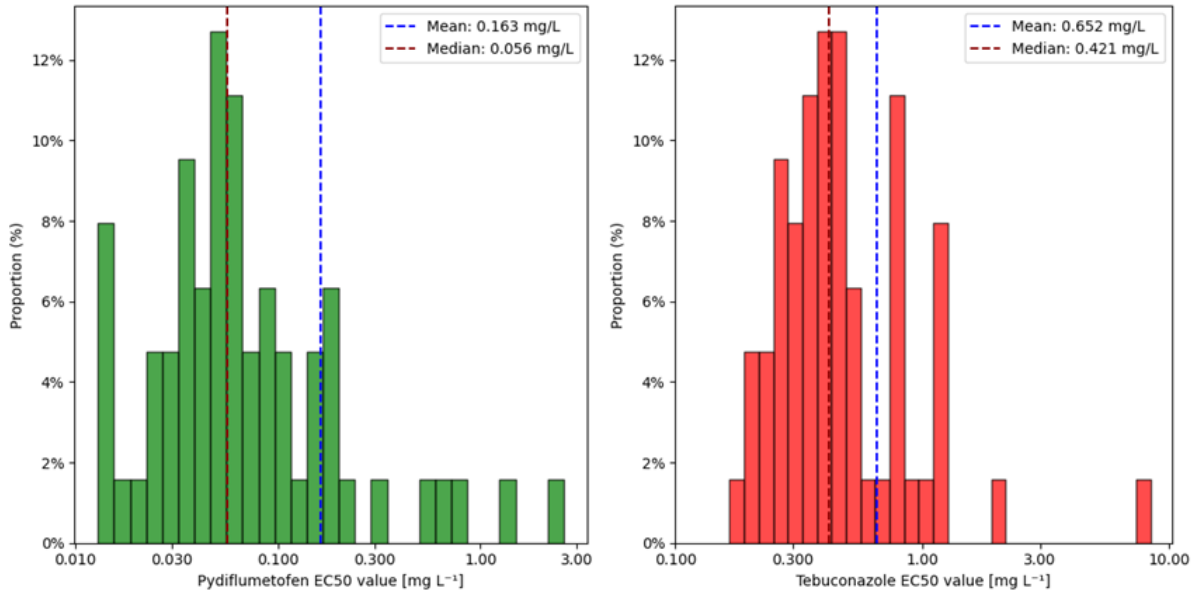

Figure: EC50 values of 62 *F. graminearum* strains for pydiflumetofen and tebuconazole. Note isolates on the right tale of the distribution.

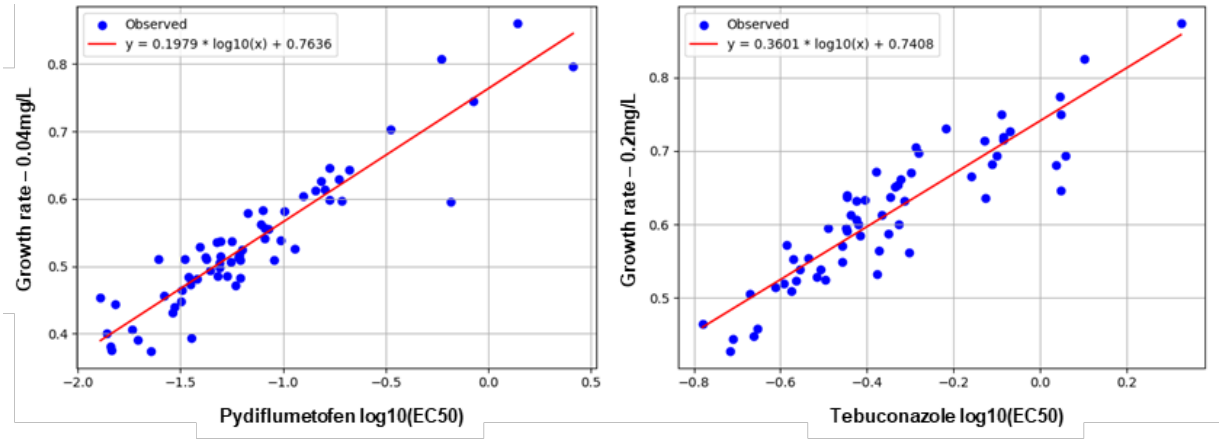

Figure: Correlation analysis between logEC50 and mycelial growth rate in fungicides.

These identified discriminatory concentrations will be used to screen the remaining ~400-500 Fusarium isolates. Approximately 250 isolates have been collected by the Chilvers lab from the Midwest and West, and 250 isolates collected by the Betts lab from the East and Southern United States.

In addition to the above activities, we have been active in submitting work for publication:

We assisted Dr. Alyssa Betts' lab in submitting a manuscript describing initial pydiflumetofen baseline fungicide sensitivity from across the United States.

Cinderella, J., Emanuel, I.B., McCoy, A.G., Chilvers M.I., Anderson, K., Bergstrom, G.C., Bockus, W.W., Bradley, C.A., Breunig, M., Byamukama, E., Cowger, C., Faske, T.R., Friskop, A.J., Kelly, J., Kleczewski, N.M., Mideros, S., Noller, J., Paul, P.A., Price, T., Rawat, N., Shim, S., Stevens, J., Telenko, D., Betts, A.K Submitted Sep 17, 2024. Resubmitted May 30, 2025. Establishment of

baseline sensitivity levels of Fusarium graminearum from wheat grown in the United States to pydiflumetofen fungicide. Plant Disease

We also submitted a manuscript to Phytopathology describing in detail the *Fusarium* species composition, chemotype and fungicide sensitivity in Michigan from both wheat and corn.

Breunig, M., Byrne, A.M., Jacobs, J.L., Ward, T.J., McCoy, A.G., and Chilvers, M.I. Submitted June 12, 2024, resubmitted June 16, 2025. Characterization of Fusarium species composition, chemotype, in planta and in vitro fungicide sensitivity of isolates from wheat and corn in Michigan, USA. Phytopathology

The abstract is as follows:

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.

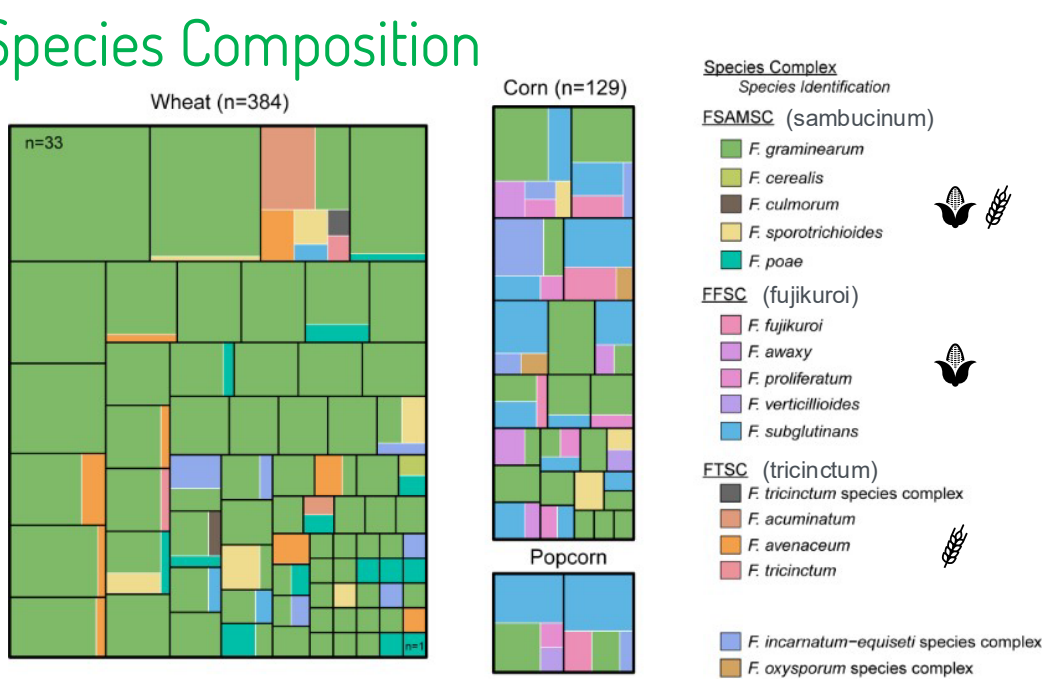

Figure: *Fusarium* species composition as collected from wheat and corn between 2011 and 2019 from across Michigan.

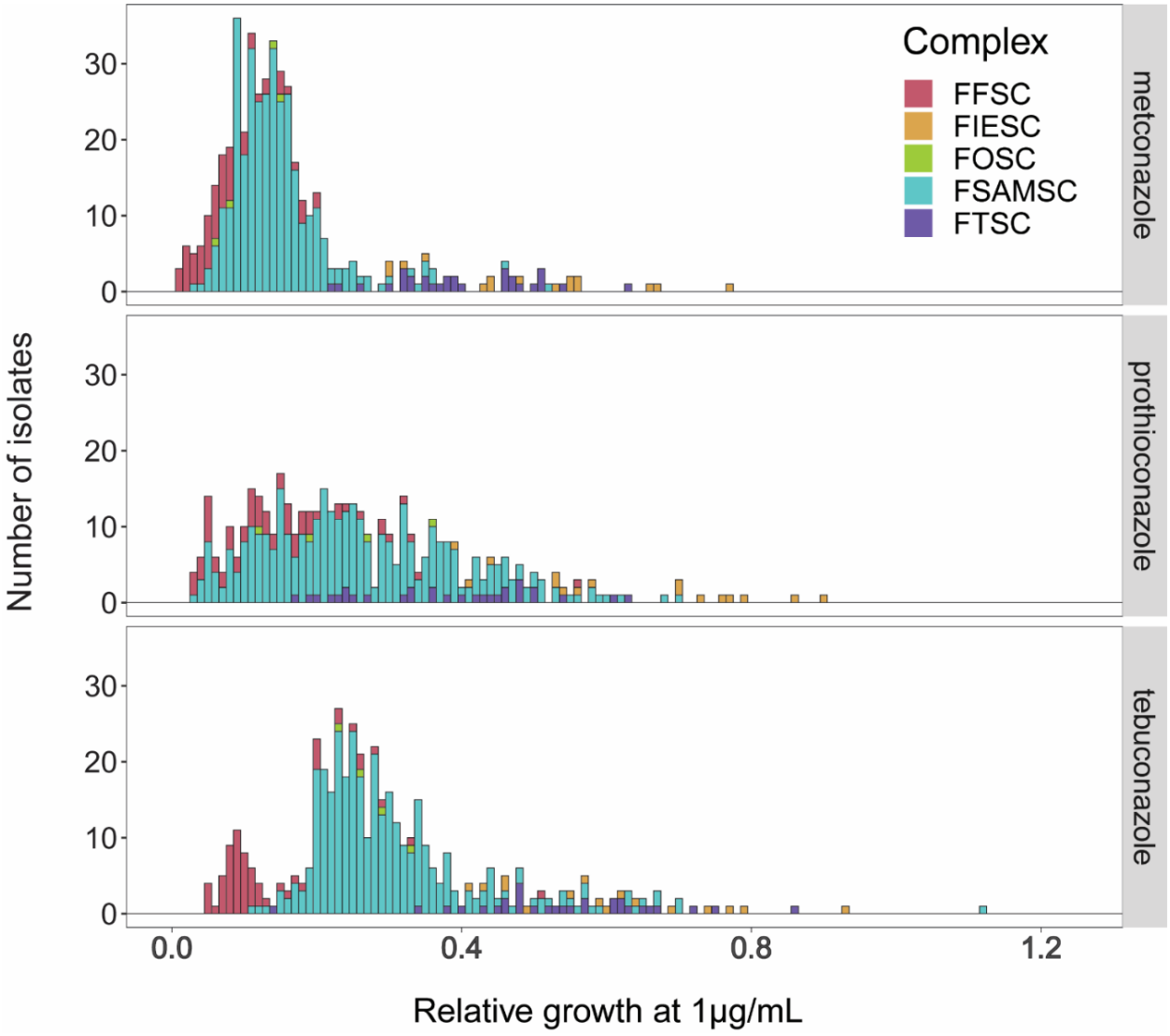

Figure: Distribution of mean relative growth values from two replicates of 445 *Fusarium* isolates tested in mycelial growth assays in ½ strength PDA plates amended with 1µg/mL commercial formulation of metconazole (Caramba), prothioconazole (Proline), and tebuconazole (Folicur). Different colors represent 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. Betts 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.

The Chilvers lab have also provided 26 Michigan isolates of 3ADON *F. graminearum* isolates to Dr. Chris Toomajian from the University of Kansas.

What were the significant results?

A collection of *F. graminearum* isolates, acquired from the Midwestern United States between 2022 and 2024, was used to conduct fungicide sensitivity tests. The majority of the initial 62 isolates screened displayed susceptibility to the two fungicides, pydiflumetofen and tebuconazole. However, for pydiflumetofen, six isolates showed a different growth curve from the baseline and had EC50 values greater than 0.33 mg/L. Additionally, for tebuconazole, one isolate showed a high relative growth rate (88.5±0.01%) at the maximum experimental concentration of 5 mg/L, suggesting it is a resistant strain to this fungicide. Screening of the remaining 400 isolates collected from across the United States will provide additional information as to how widespread any insensitivity/resistance is in wheat and barley cropping systems.

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 2025 season.

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

Conversations have been conducted 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?**
Graduate student Gloria Baker was recruited in Fall of 2023 and postdoc Dr. Sunkyu Choi was recruited in April of 2025 and together they are conducting fungicide sensitivity assays. Dr. Austin McCoy who holds a research assistant position in the Chilvers lab has participated in the project, in particular assisting with analysis and writing of papers in conjunction with the Betts lab. Additionally, several undergraduates have been involved in assisting with the project. We look forward to presenting results at the 2025 USWBSI forum.

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. Peer reviewed publications have 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 for the 2025 season. We have completed EC50 determination for the three primary fungicide modes of action that are used within field crops, including FRAC3 triazole, FRAC7 succinate dehydrogenase inhibitors, and FRAC11 quinone outside inhibitors. This work has established discriminatory doses that will be used to screen the remaining ~500 isolates from across the US.

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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
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?

Objective 1

In project year 1 (2022-2023), Dr. Martin Chilvers and I established centralized testing at each of our respective locations (Figure 3). Video calls and virtual meetings have been continued to coordinate email correspondence for sample request, isolate collections, and approaches for fungicide sensitivity screening. Samples from extension specialists across the US have been solicited via email and through awareness at presentations during the spring 2025 NCERA184 meeting.

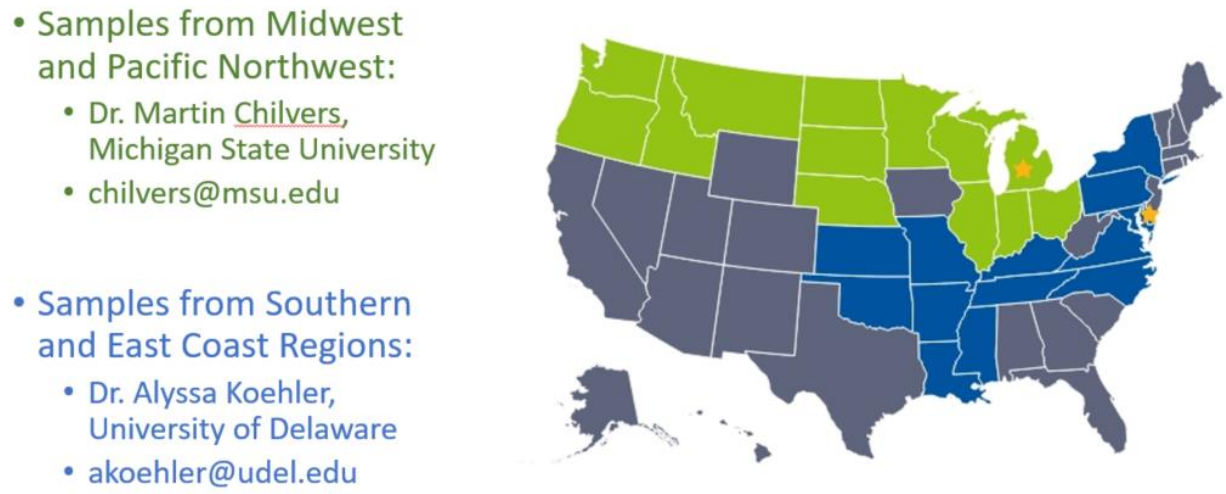


Figure 3: Centralized testing locations at the University of Delaware (blue) and Michigan State (green)

Objective 2

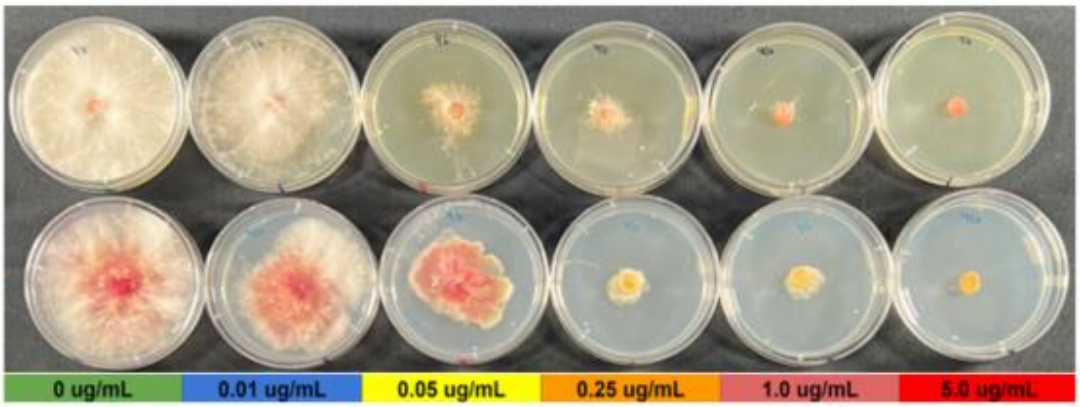
Base line EC50 values from 2019-2020 have been established. From the original 16 states, mycelial growth EC50 values averaged by state and year(s) ranged from 0.16 to 0.56 µg/mL, cumulatively averaging 0.37 µg/mL and 0.31 µg/mL in 2020 and 2021, respectively. The average EC50 value of historic isolates was 0.27 µg/mL. These values have been presented in a manuscript that was submitted this reporting year. We now continue to solicit isolates and improve screening methods for more high throughput screening with a discriminatory dose assay.

Objective 3

Dr. Chilvers and I have been in touch with USDA colleagues and there have been multiple zoom meetings to connect across projects and determine how to best approach isolate storage. Due to space limitation, we will need to prioritize valuable isolates and we will work across the MGMT and PBG RACS to characterize isolates and make these decisions regarding long term storage if/when isolates with reduced sensitivity are identified.

What were the significant results?

In 2022, our testing location received 279 isolates from six states (KS, KY, LA, MD, NC, PA). In 2023 we received 123 isolates from four states. In 2023, 40 representative isolates were screened using a new YBA media. From this set EC50 values ranged from 0.04 µg/mL to 3.3 µg/mL. Since this range was wider than what had previously been observed, we began a series of experiments to further compare YBA and PDA media. The main advantage of YBA is that *Fusarium* growth is more symmetric, but there are tradeoffs in visualization as the lighter hyphae benefit from backlighting that is not needed when rating bright pink plants (Figure 4). Media type did not change the overall results and YBA was selected for continued testing in 2024. In the 2023 trial, EC50 values on PDA trended higher than 2020-2022 trials, which indicated that the 2023 YBA screen may have had isolates with higher EC50 values rather than being an artifact of media selection. Due to this, we wanted to run more isolates in a full EC50 panel in 2024/25 prior to setting the first discriminatory dose.



Fusarium graminearum isolate growing on Yeast Bacto Agar (top) and Potato Dextrose Agar (bottom).

Figure 4: Comparison of growth on YBA versus PDA

In 2024 a total of 254 isolates were added to the DE location collection (Table 3). A set of 54 *F. graminearum* isolates were screened during 2024/25 in a full EC50 panel with doses 0, 0.01, 0.1, 1, 10 and 100 µg/mL pydiflumetofen on YBA media with two replications. Data was best analyzed by four-parameter logistic model. The results were unexpected with multiple isolates showing EC50 values higher than previous testing. From the original 16 states, mycelial growth EC50 values averaged by state and year(s) ranged from 0.16 to 0.56 µg/mL, cumulatively averaging 0.37 µg/mL and 0.31 µg/mL in 2020 and 2021, respectively. The average EC50 value of historic isolates was 0.27 µg/mL. In 2023 testing of isolates from 2022, EC50 values ranged from 0.04 µg/mL to 3.3 µg/mL. In this new set containing isolates from 2023 and 2024, individual EC50 values ranged from 0.24 to 17.25 µg/mL, with an average of 2.7 µg/mL (Table 4). While many isolates had EC50 values consistent with previous trials, 4there were multiple isolates with higher EC50 values than previously observed.

Table 3: Location and number of *Fusarium* isolates collected from wheat and barley during the 2024 growing season for fungicide sensitivity screening

State	Number of Isolates
DE	25 (from Barley and wheat)
KY	15 (from wheat)
LA	11 (from wheat)
3MD	9 (from wheat)
MO	21 (from wheat)
NY	132 (from Barley)
MO	21 (from wheat)

Table 4: Minimum and Maximum EC50 values from *Fusarium* isolates collected in 2023 and 2024

Collection State	Year	Number Screened	Minimum EC50	Maximum EC50
DE	2023	7	1.29	2.83
DE	2024	6	0.47	3.88
KY	2023	5	0.75	2.22
LA	2024	6	0.47	3.08
MD	2024	4	1.91	17.25
MO	2024	6	0.43	2.03
OH	2023	7	0.24	6.32
PA	2024	6	0.74	7.16
NY	2023	7	0.40	16.20

While DE trials were ongoing, the Chilvers lab also compared YBA and half-strength PDA. We have made the group consensus to return to half strength PDA for clearer visualization of mycelial growth. We had anticipated selecting a discriminatory dose for DE testing from the 2024/25 trials, but the range of EC50 values greatly expanded from initial testing. There are six isolates in particular that will be shared with the Chilvers lab and included in future tests within the Betts lab (Table 5). The Chilvers lab has selected 0.04 mg/L as their discriminatory dose for pydiflumetofen. In 2025, we will continue to screen newly received isolates along with representative isolates with higher EC50 values in 2024/25 screening and isolates from the previous 2020-2022 screening. Our 2025/26 screening will include doses 0, 0.04, 0.4 , 4, and 40 µg/mL. In addition to year 4 EC50 panel screening, we will place priority on preparing isolates for shipment to transfer isolates collected from 2023-2025 to the Chilvers lab for discriminatory dose screening for tebuconazole and to compare consistency of pydiflumetofen results. If particular isolates continue to show elevated EC50 values, these will be further explored by the Chilvers lab.

Table 5: Isolates and locations with the highest EC50 values observed in 2024/25 screening

Isolate Name	Isolate Location	EC50 (µg/mL)
MD1-5	Maryland	17.25
MD2-5	Maryland	6.29
NY4	New York	6.32
OH60A	Ohio	7.16
PA2-2	Pennsylvania	16.20
PA4-3	Pennsylvania	12.23

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 have collected isolates from the 2024 season and are currently collecting from 2025. We will continue EC50 screening to determine optimal discriminatory dose values and share isolates with the Chilvers lab for discriminatory dose screening of tebuconazole and to confirm isolate responses to pydiflumetofen.

Baseline efficacy within wheat and malting barley isolates have begun to be established across multiple states and in comparison to historic isolates. One publication has been submitted and a second focused on malting barley will be submitted in 2025.

Conversations have been held 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. If isolates with unique response to fungicide screening are identified we will work with USDA colleagues to place these in national storage.

3. **What opportunities for training and professional development has the project provided?**
This project is currently supporting a research technician gaining new skills in fungicide efficacy screening. Our lab has collaborated with the post doc in Dr. Chilvers lab to standardize EC50 data analysis and modeling. The standardize model fitting protocols and analysis methods have been used to prepare two manuscripts. Dr. Chilvers was able to have A new graduate student joined the lab of Dr. Chilvers and our lab is facilitating collection of data that will be passed to this student for future publications that result from data collected in 2024-2026.

4. **How have the results been disseminated to communities of interest?**
Updates on fungicide sensitivity were disseminated through articles in the University of Delaware's Weekly Crop Update, which reaches over 700 growers, consultants, and stakeholders and provides a platform to discuss disease concerns and other production issues. Updates on project results were also highlighted at Delaware Ag Week held in January 2025 and at the NCERA184 meeting in February 2025. Once manuscripts are published, research summaries will be prepared for publication with the Crop Protection Network to further broaden the audience receiving project results.

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. In 2022 and 2023 FHB pressure was generally low across the country, but there was elevated pressure in 2024. It appears 2025 will also have moderate to high pressure and we anticipate similar collection sizes as 2024. Now that we have begun to identify discriminatory dose ranges, this will allow for larger numbers of isolates to be screened and the ability to expand to other modes of action. As we enter the final year of this project, we will work on preparing isolates to ship to MI for inclusion in screening with the work of graduate student Gloria Baker.