Evaluating fungicide resistance in leaf spot pathogens of Michigan sugarbeet, 2025-26

Emily Jordyn Weedon[1], Alexandra P. Hernandez[1], Sarah Ruth[1], Linda E. Hanson[1,2], and Jaime F. Willbur[1]; [1]Michigan State University, [2]USDA-ARS

Background: Leaf spot management in Michigan relies heavily on the application of fungicides to control diseases such as Cercospora leaf spot (CLS) and Alternaria leaf spot (ALS) throughout the season. Fungicides registered to manage these two diseases include benzimidazoles (FRAC Group 1), demethylase inhibitors (DMI or triazoles; FRAC Group 3), quinone outside inhibitors (QoI; FRAC Group 7) and organotin fungicides (FRAC Group 30). Reports of reduced sensitivity and decreased fungicide efficacy have been documented in Michigan populations of *Cercospora beticola* (Rosenzweig et al. 2015; Rosenzweig et al. 2020), and *Alternaria* spp. (Rosenzweig et al. 2017). The work done in the current study summarizes the fungicide sensitivities of recent and current isolates of *C. beticola* and *Alternaria* spp. impacting Michigan sugarbeets.

Objective 1: Characterize fungicide sensitivity in *C. beticola* isolates. Isolates were collected from symptomatic sugarbeet tissue sourced from fields across nine Michigan countries from 2021-2025. *In vitro* fungicide sensitivity testing was conducted on plates amended with fungicide using the gradient spiral dilution method (Förster et al. 2004) and spore suspensions from pure isolate cultures were streaked onto the plates. Using a standardized template, the effective concentration of fungicide to inhibit mycelial growth by 50% (EC_{50}) was calculated for seven active ingredients registered for leaf spot management in Michigan including: difenoconazole, mefentrifluconazole, tetraconazole, prothioconazole, pyraclostrobin (with the addition of SHAM proceeding 2024) triphenyltin hydroxide, and thiophanate-methyl. To further characterize *C. beticola* isolate responses, sensitivity thresholds were established in k-means clustering analyses using data from 2021-2022 isolates (Hernandez et al. 2025). Using silhouette and elbow plot visualizations to confirm cluster numbers, natural groupings within the responses were identified based on isolate responses. Isolates clustering in the most sensitive group established the baseline sensitivity for each active ingredient, with any responses greater than the baseline being identified as resistant.

Results: The distributions of EC_{50} values for *C. beticola* were compared from samples tested across 2021 to 2025 (Figure 1). The calculated sensitivity thresholds for the *C. beticola* isolates were: 5.11 ug/ml for difenoconazole, 3.26 ug/ml for mefentrifluconazole, 9.65 ug/ml for prothioconazole, 6.91 ug/ml for tetraconazole, and 2.55 ug/ml for triphenyltin hydroxide (Table 1). Based on previous work conducted, sensitivity thresholds for pyraclostrobin (Bolton et al. 2012b; Secor et al. 2010) and thiophanate-methyl (Jones et al. 1987; Koenraadt et al. 1992) for *C. beticola* were 1 and 5 ug/ml, respectively. The number of clusters ranged from 4-7 for the DMI fungicides, and 10 for triphenyltin hydroxide (Table 1).

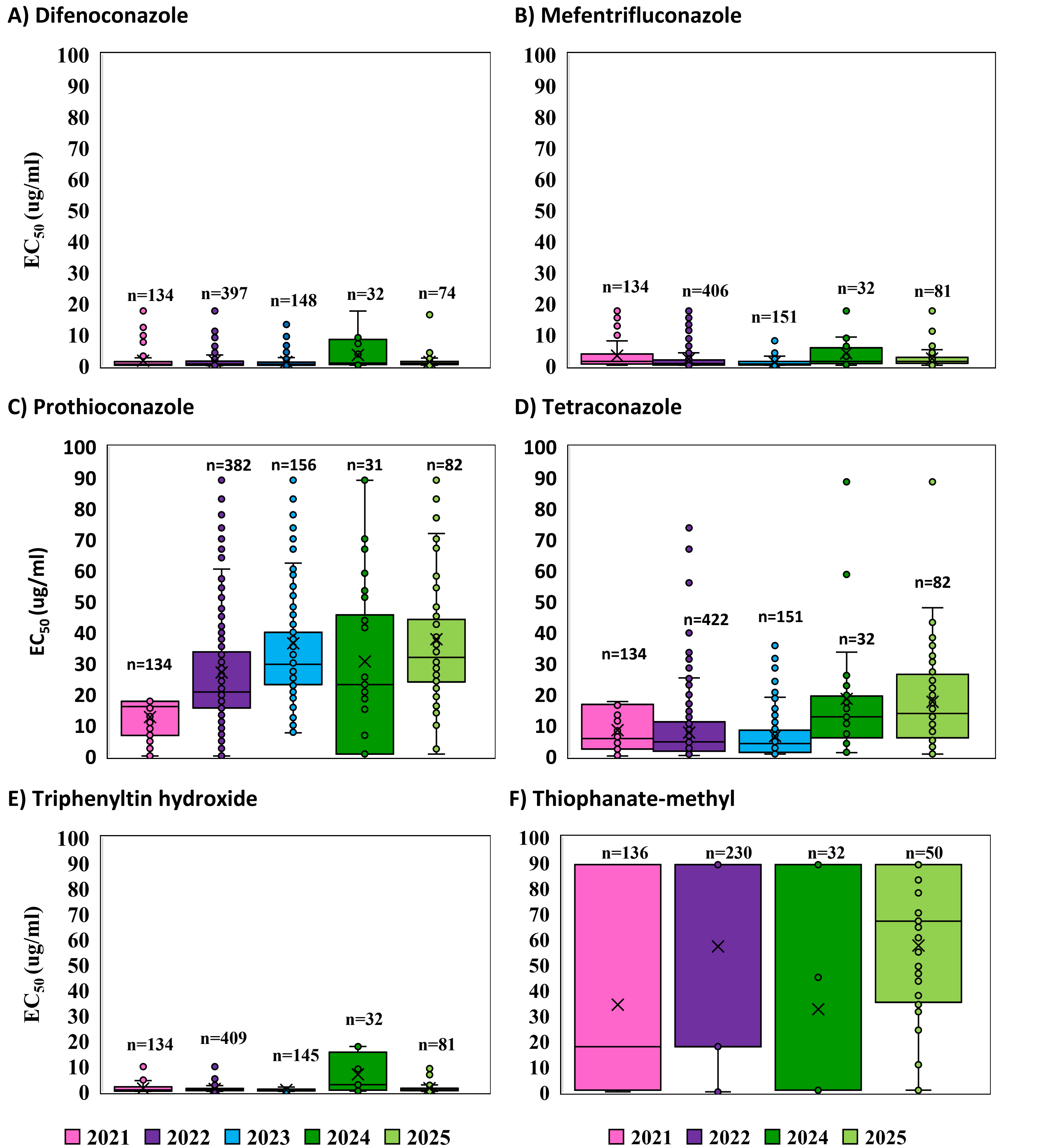

Figure 1: Boxplots illustrating the distribution of ***C. beticola*** fungicide sensitivities of **A)** difenoconazole (n=785), **B)** mefentrifluconazole (n=804), **C)** prothioconazole (n=785), **D)** tetraconazole (n=821), **E)** triphenyltin hydroxide (n=801), and **F)** thiophanate-methyl (n=466) from 2021 to 2025. The boxes represent the interquartile interval where 50% of the data points were found. The line that divides the box is the median and "X" represents the mean. The extended vertical lines show variability outside of the interquartile interval. The "n" number of isolates screened are shown.

Table 1: Percentage of 2025 ***C. beticola*** isolates assigned least and most sensitive groups based on k-means cluster analysis conducted using isolates from 2021-2022.

			2025	
Active Ingredient	# of clusters	Sensitivity threshold (µg/ml)	% in most sensitive cluster	% in least sensitive cluster
Difenoconazole	4	5.11	99%	0%
Mefentrifluconazole	7	3.26	79%	2%
Prothioconazole	4	9.56	2%	95%
Tetraconazole	5	6.91	30%	66%
Pyraclostrobin (+SHAM)[a]	-	1.0[b]	6%	94%
Thiophanate-methyl	-	5.0[c]	24%	76%
Triphenyltin hydroxide	10	2.55	88%	5%

[a] Isolates were screened with pyraclostrobin without SHAM in 2021 and 2022.

[b] Pyraclostrobin threshold was based on prior research (Bolton et al. 2012b; Secor et al. 2010).

[c] Thiophanate-methyl threshold was based on prior research (Jones et al., 1987; Koenraadt et al., 1992).

Objective 2: Characterize fungicide sensitivity in *Alternaria* spp. isolates. Across three years (2022, 2023, and 2025), *Alternaria* spp. isolates were collected from symptomatic sugarbeet tissue sourced from fields in six Michigan counties. The fungicide sensitivities to difenoconazole, mefentrifluconazole, prothioconazole, tetraconazole, thiophanate methyl, triphenyltin hydroxide, and pyraclostrobin (with SHAM) were determined using a similar gradient spiral dilution method as the one previously described in the first objective. Additionally, isolate responses were used in a k-mean clustering analysis to establish sensitivity thresholds as described previously for *C. beticola* isolates.

Results: The distributions of EC_{50} values for *Alternaria* spp. were compared from samples tested across 2022, 2023, and 2025 (Figure 2). The calculated sensitivity threshold for the *Alternaria* spp. isolates were: 0.46 µg/ml for difenoconazole, 0.16 µg/ml for mefentrifluconazole, 7.33 µg/ml for prothioconazole, 18.19 µg/ml for tetraconazole, 70.18 µg/ml for thiophanate-methyl, 17.05 µg/ml for pyraclostrobin and SHAM, and 2.56 µg/ml for triphenyltin hydroxide (Table 2). The number of clusters for each active ingredient were: 4 clusters for mefentrifluconazole and tetraconazole, 5 clusters for difenoconazole and prothioconazole, and 9 clusters for triphenyltin hydroxide. Thiophanate-methyl and pyraclostrobin responses grouped into two and three clusters, respectively (Table 2).

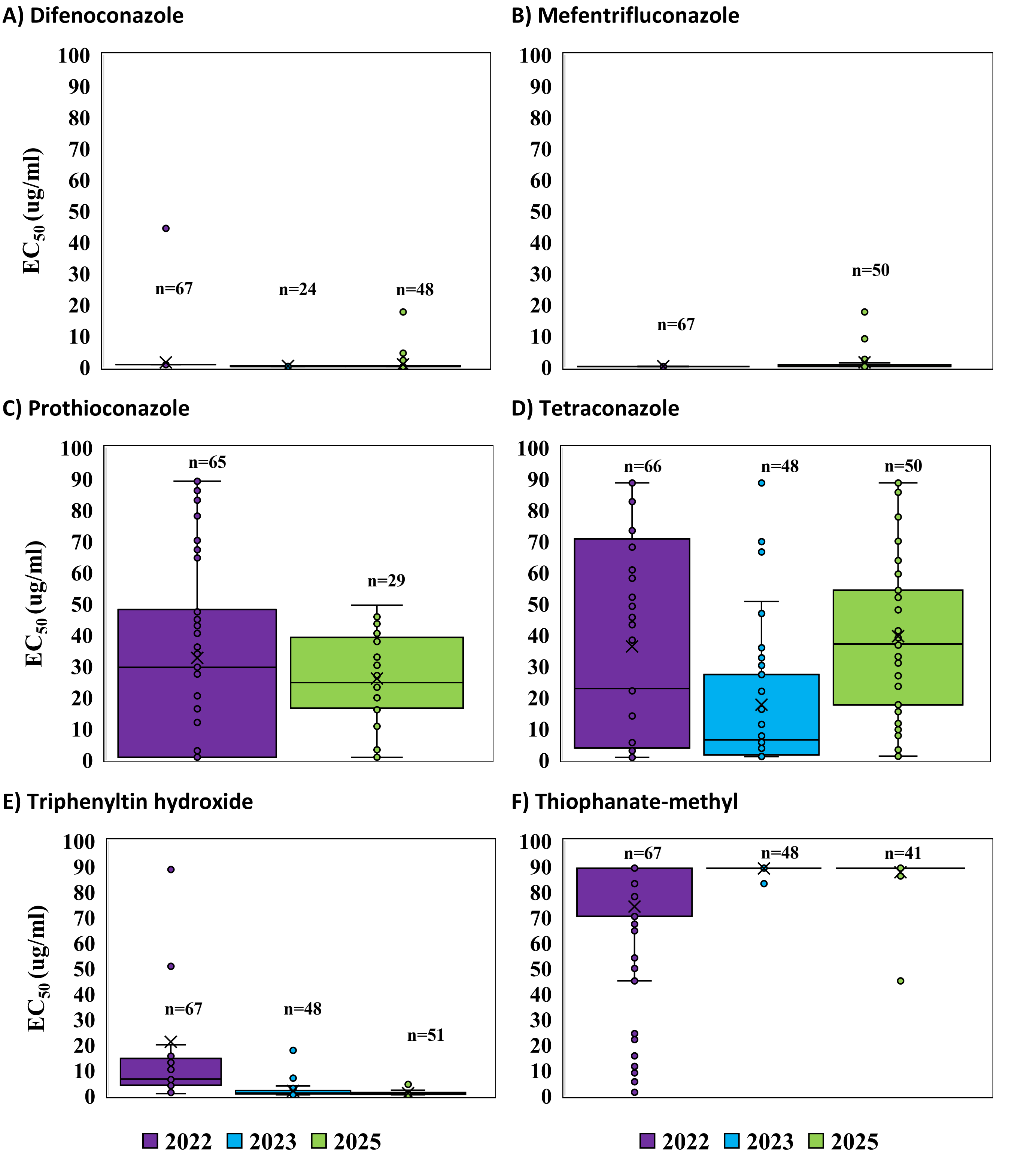

Figure 2: Boxplots illustrating the distribution of ***Alternaria* spp.** fungicide sensitivities of **A)** difenoconazole (n=139), **B)** mefentrifluconazole (n=117), **C)** prothioconazole (n=114), **D)** tetraconazole (n=164), **E)** triphenyltin hydroxide (n=166), and **F)** thiophanate-methyl (n=156) across 2022-2025. The boxes represent the interquartile interval where 50% of the data points were found. The line that divides the box is the median and "X" represents the mean. The extended vertical lines show variability outside of the interquartile interval. The "n" number of isolates screened are shown.

Table 2: Percentages of 2022-2023 and 2025 ***Alternaria* spp.** isolates assigned into each least and most sensitive groups, based on k-means cluster analyses conducted with isolates from 2022-2023.

			2022-2023		2025	
Active ingredient	# of clusters	Sensitivity threshold (µg/ml)	% in most sensitive cluster	% in least sensitive cluster	% in most sensitive cluster	% in least sensitive cluster
Difenoconazole	5	0.46	23%	1%	79%	6%
Mefentrifluconazole	4	0.16	81%	6%	40%	24%
Prothioconazole	5	7.33	47%	22%	8%	8%
Tetraconazole	4	18.19	55%	21%	26%	16%
Pyraclostrobin (+SHAM)	3	17.05	39%	52%	6%	80%
Thiophanate-methyl	2	70.18	7%	93%	2%	98%
Triphenyltin hydroxide	9	2.56	36%	1%	96%	0%

Overall Summary:

- Sensitivity thresholds for *C. beticola* and *Alternaria* spp. can differ between active ingredients. The use of k-means analysis could identify potential thresholds that could identify earlier signs of resistance development especially in the DMI fungicides.
- For the DMI fungicides, distributions for difenoconazole and mefentrifluconazole generally followed similar patterns; this was also observed for prothioconazole and tetraconazole.
- A majority (>80%) of *in vitro* screened sensitivities to difenoconazole were below the respective sensitivity thresholds for both pathogens, supporting continued use for leaf spot management.
- The high prevalence of tin-sensitive *C. beticola* (88%) and *Alternaria* spp. (96%) isolates from 2025 supports continued use of organotin fungicides for leaf spot management.
- The high prevalence of thiophanate-methyl resistance in *C. beticola* (76%) and *Alternaria* spp. (98%) isolates indicates this product may be ineffective in leaf spot management programs.

Future Directions: Fungicide sensitivity characterizations for *C. beticola* and *Alternaria* spp. are still ongoing for 2025 isolates. In the future, a panel of current and recent sugarbeet varieties will be used to characterize *C. beticola* isolates collected from the growing season of 2025 to investigate potential changes in morphological characteristics of lesions. Additionally, the inclusion of regional information will be analyzed to investigate potential geographical differences in fungicide sensitivities of both *Alternaria* spp. and *C. beticola* populations across the state.

Acknowledgements: We thank the Michigan sugar beet industry for access to field sites and thank Michigan Sugarbeet Advancement and the Michigan Sugar Company for collection of sample materials.

This work is supported by the Michigan Sugar Company, Michigan State University Project GREEEN, and USDA-ARS.