Fungicide resistance monitoring for *Cercospora beticola* populations in Michigan, 2021-23

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Objective 1: Monitor seasonal changes in fungicide resistance of foliar sugarbeet pathogens.

Methods: For Cercospora leaf spot (CLS), leaf samples were collected early-, mid-, and late-season. Approximately 8 lesions were collected at each timepoint, and field site and mono-conidial isolates were obtained from sporulating lesions. Across nine counties in east-central Michigan, 29, 30, and 15 field locations were sampled in 2021, 2022, and 2023, respectively. Concentrations that effectively inhibited 50% of mycelial growth (EC_{50}) were determined through spiral gradient plating (Förster et al. 2004; Torres-Londoño et al. 2016; Rosenzweig et al. 2020). Isolates were tested for sensitivity to pyraclostrobin (FRAC 11, QoI), thiophanate-methyl (FRAC 1, MBC), difenoconazole, tetraconazole, prothioconazole, mefentrifluconazole, and fenbuconazole (FRAC 3, DMI), and triphenyltin hydroxide (FRAC 30).

Results: Resistance to DMI fungicides varied by active ingredient; *C. beticola* isolates exhibited the highest level of *in vitro* resistance to prothioconazole, followed by tetraconazole (Figure 1). High frequencies of resistance to pyraclostrobin were observed across Michigan. Some reduced sensitivity to triphenyltin hydroxide was observed; however, the degree of resistance was lower than for other fungicide classes with no isolates having EC_{50} values >10 ppm (Figure 1). The frequency of *C. beticola* isolates resistant to thiophanate-methyl increased significantly (from 71% to 85%) from 2021 to 2022 ($P < 0.05$). From consecutive timepoint sampling, fungicide resistance was found to be significantly associated with increasing numbers of DMI applications for prothioconazole and tetraconazole and MBC applications for thiophanate-methyl ($P < 0.05$; data not shown).

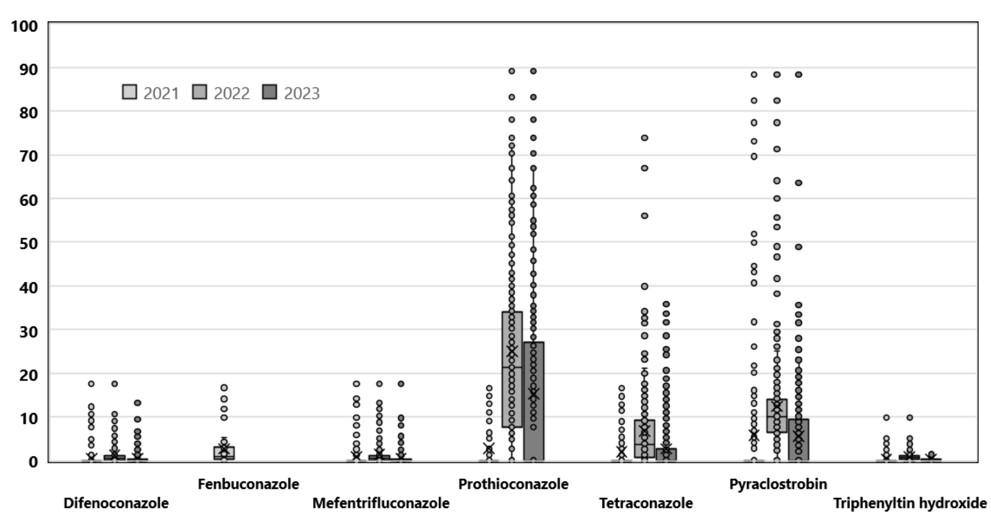

Figure 1. Box plots of the EC_{50} values for each fungicide active ingredient tested for *C. beticola* isolates collected in 2021 (n = 78 isolates), 2022 (n = 304-347), and 2023 (n = 145-156). The box represents the interquartile interval where 50% of the data points are found. The line that divides the box is the median and "X" represents the mean. The lines that extend vertically show variability outside of the interquartile interval. The upper limits were about 18 µg/ml for difenoconazole, fenbuconazole, and mefentrifluconazole, 18 µg/ml (2021) and 89 µg/ml (2022 and 2023) for prothioconazole, 18 µg/ml (2021) and 89 µg/ml (2022 and 2023) for tetraconazole, 88 µg/ml for pyraclostrobin, 89 µg/ml for thiophanate methyl, and 18 µg/ml triphenyltin hydroxide.

Objective 2: Optimize early-season techniques to monitor foliar pathogen sensitivity to critical fungicide groups.

Methods: *In vitro* fungicide sensitivity testing was compared to polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) assays which detect point mutations associated with resistance to major fungicide groups: G143A for QoI EC_{50} values > 100 ppm (Rosenzweig et al. 2015), E198A for MBC EC_{50} values ≥ 60 ppm (Rosenzweig et al. 2015), and Glu169 for DMI EC_{50} values of 65-115 ppm (Nikou et al. 2009). A total of 78 and 373 *C. beticola* isolates were screened in 2021 and 2022, respectively.

Results: The benzimidazole PCR marker predicted resistance to thiophanate-methyl (>60 µg/ml) with 99% accuracy. The mutation was present in 68% of isolates screened in 2021 and 74% in 2022. All isolates screened possessed the mutation associated with QoI resistance; however, *in vitro* EC_{50} values for pyraclostrobin ranged from 0.8 ppm (lower limit of assay) to 88.4 ppm (upper limit). Others have also observed that the G143A mutation confers a relatively low level of QoI resistance in *C. beticola* (Bolton et al. 2013). Therefore, this mutation may not meaningfully represent field performance of QoI products for CLS.

The frequency of *C. beticola* isolates with the mutation associated with DMI resistance was 21% in 2021 and 13% in 2022. Based on linear mixed model analyses, difenoconazole and mefentrifluconazole EC_{50} values significantly increased when the mutation was present (Figure 2), while tetraconazole EC_{50} values were significantly reduced, and fenbuconazole and prothioconazole responses were not affected. Resistance to triazoles is a complex trait controlled by multiple genes (Rangel et al. 2020) and further studies are needed to explore the explanatory potential of other mutations associated with DMI resistance (Spanner et al. 2021).

County-level frequencies of *C. beticola* resistance to several active ingredients also revealed interesting spatial trends (Figure 3). For example, central and eastern sugar beet producing counties tended to have higher frequencies of resistance to tetraconazole (Figure 3A) and thiophanate-methyl (Figure 3D). The opposite was observed for difenoconazole where central and western counties tended to have higher frequencies of resistance and eastern counties the lowest (Figure 3B). Interestingly, frequencies of tin resistance were higher in the western-most counties (Figure 3C). Observations were likely driven by regional management decisions.

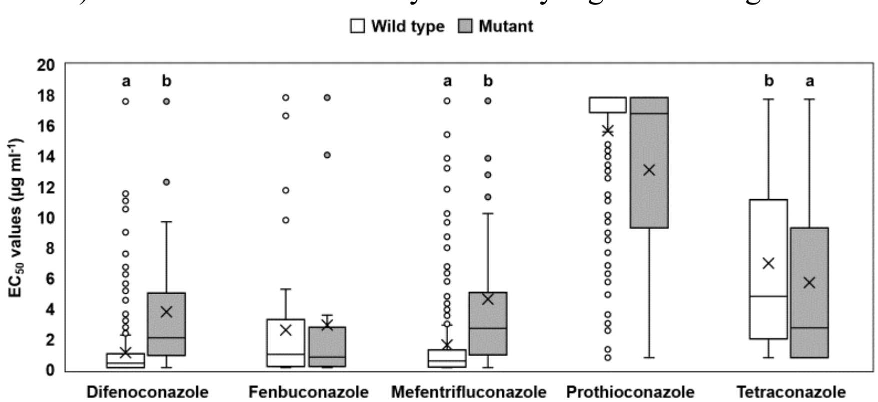

Figure 2. Box plots of *Cercospora beticola* EC_{50} values with (mutant) and without (wild type) the Glu169 mutation associated with DMI resistance for each active ingredient tested in 2021 and 2022. Significant differences are indicated by letters assigned using pairwise comparison with *P*-values adjusted for multiple testing by the Bonferroni method (α=0.05).

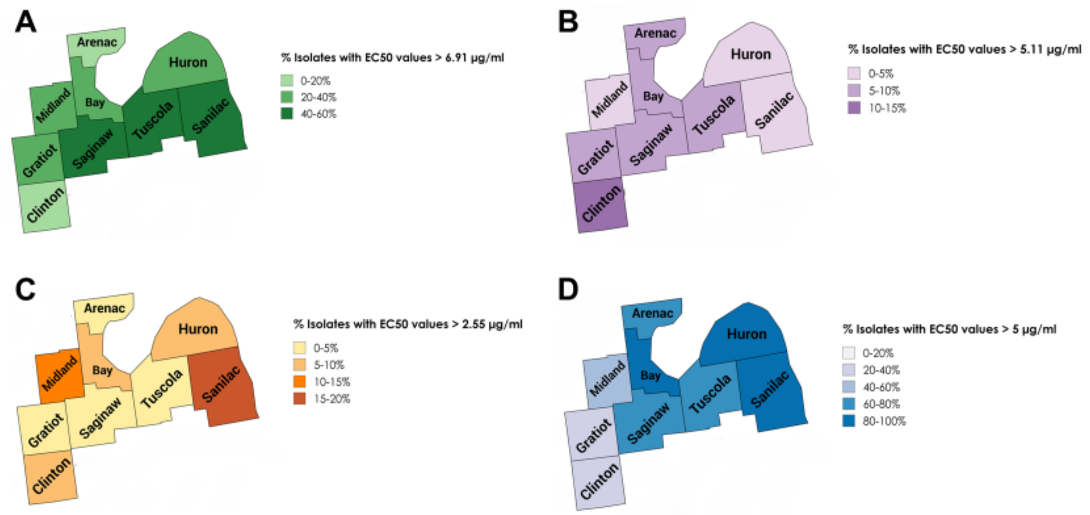

Figure 3. County-level percentages of *Cercospora beticola* isolates with reduced sensitivity based on EC_{50} k-means established thresholds to (A) tetraconazole, (B) difenoconazole, (C) triphenyltin hydroxide, and > 5 µg ml^{-1} (D) thiophanate-methyl (Secor et al. 2010; Bolton et al. 2012b). Isolates were pooled across both sampling years 2021 (29 field locations) and 2022 (30 field locations). Michigan sugarbeet growing counties included in this study were Arenac (n = 12 isolates), Bay (n =124), Clinton (n = 18), Gratiot (n = 24), Huron (n = 77), Midland (n = 4), Saginaw (n = 33), Sanilac (n = 40), and Tuscola (n = 41).

Overall Summary:

- Laboratory-level resistance was particularly widespread for the DMIs prothioconazole and tetraconazole as well as the QoI pyraclostrobin and the MBC thiophanate-methyl.
- While the PCR-RFLP rapid detection technique was highly accurate at predicting MBC resistance, the mutations used in this study were not sufficient for accurately predicting QoI or DMI *in vitro* sensitivity for *C. beticola* isolates.
- County-level fungicide resistance trends may be useful to direct regional management recommendations and decisions to mitigate further resistance development.

Future Directions: Isolates collected in 2024 will be tested using the spiral gradient method and compared to previous years to assess shifts in *C. beticola* populations. Additional mutations associated with DMI resistance will be tested for their ability to predict isolate sensitivity. Newer qPCR techniques (Shrestha et al. 2020) will also be investigated for screening optimization.

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