Fungicide resistance screening for *Alternaria* spp. causing Alternaria leaf spot of sugar beet, 2022-23

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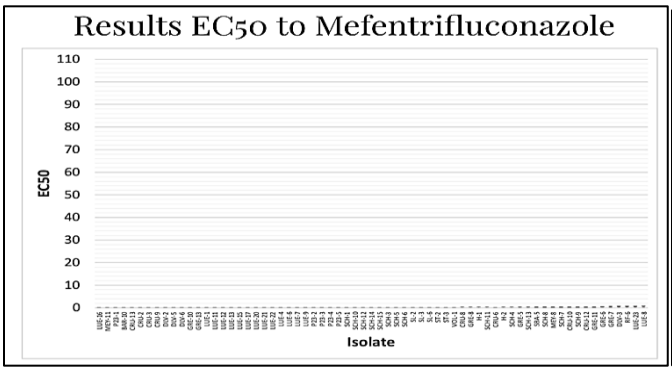
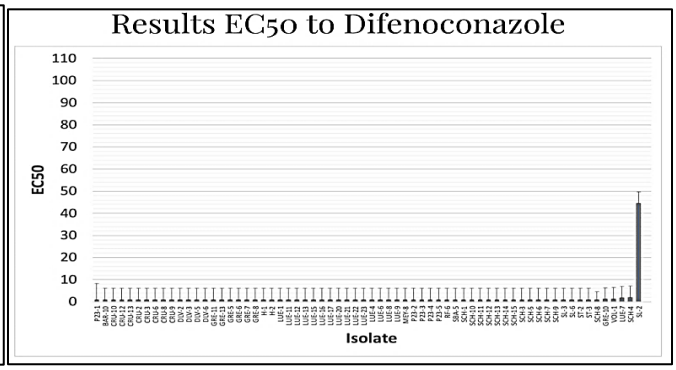
Background: While generally considered a minor disease of sugar beets, reports of Alternaria leaf spot (ALS) disease prevalence and severity have been on the rise. In 2015, Michigan growers reported significant yield reduction because of premature defoliation caused by ALS (Rosenzweig et al., 2017). Increased *in vitro* resistance has subsequently been reported for *Alternaria* spp. from sugar beet (Rosenzweig et al., 2017; Rosenzweig et al., 2019). Interestingly, sensitivity was found to increase after DMI-resistant isolates of *C. beticola* were exposed to prolonged cold temperatures of -20°C (Karaoglanidis and Thanassoulopoulos, 2002; Arabiat et al., 2017). Studies of potential biological trade-offs in resistant *Alternaria* spp. are lacking. Further investigations will improve understanding of pathogen biology and diversity and guide management of beet leaf spot diseases in Michigan.

Objective 1: Characterize virulence and fungicide resistance of *Alternaria* spp. isolates from sugar beet. Spore suspensions were collected from symptomatic sugar beet leaves from Michigan fields across six counties in 2022. Suspensions were adjusted to $1x10^4$ conidia/ml using a hemocytometer. The collected 74 isolates were tested for virulence using a detached leaf-assay using 2-month-old sugar beets grown in the MSU Plant Science Greenhouses of the susceptible beet variety, CR-059. Lesion developments were recorded daily after two days post inoculation for five days. This experiment was repeated twice.

Initial *in-vitro* fungicide sensitivity was collected for six fungicide active ingredients registered for management of leaf spot diseases in sugar beet in Michigan. These included four demethylation inhibitor (DMI) fungicides (FRAC 3) difenoconazole, mefentrifluconazole, prothioconazole, and tetraconazole, as well as triphenyltin-hydroxide (FRAC 30), and thiophanate methyl (FRAC 1) (Rosenzweig et al, 2017; Rosenzweig et. 2019). Plates were fungicide amended using a gradient spiral dilution method (Förster et al, 2004) and spore suspensions were streaked onto them. The effective concentrations to inhibit mycelial growth by 50% (EC_{50}) were determined four days post-inoculation. This experiment was repeated twice.

Results: In the virulence assay, lesion diameters ranged from 0.49 mm to 18.96 mm, and 57 isolates resulted in more severe symptoms than a previously characterized virulent *A. alternata* isolate, P23 (Jayawardana, 2022). In the initial fungicide sensitivity screening, isolates were phenotypically categorized as previously defined by Rosenzweig et al. (2019) as resistant (EC_{50} >100 ppm), insensitive (EC_{50} = 50-100 ppm), moderately insensitive (EC_{50} = 10-50 ppm), reduced sensitive (EC_{50} = 1-10 ppm), and sensitive (EC50 <1 ppm). Percentages of isolates characterized as insensitive were: 0% for difenoconazole, 0% for mefentrifluconazole, 22% for prothioconazole, 37% for tetraconazole, 81% for thiophanate-methyl, and 18% for triphenyltin hydroxide (Figure 1).





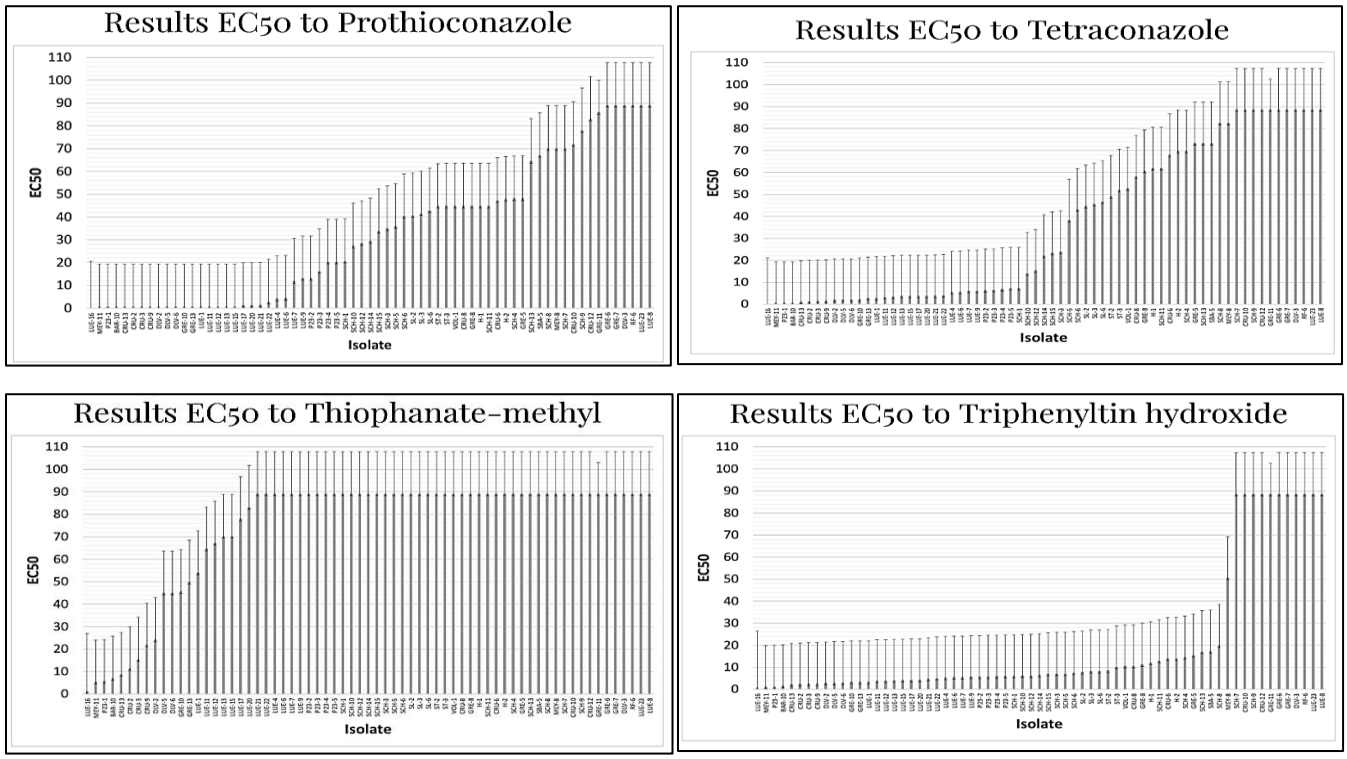


Figure 1: Initial fungicide sensitivity across 74 *Alternaria spp.* from 2022. The p-values denoting differences between isolates were: 0.004 (mefentrifluconazole), 0.5 (difenoconazole), <0.0001 (prothioconazole), <0.001 (tetraconazole), <0.0001(thiophanate-methyl), and <0.001 (triphenyltin hydroxide).

Objective 2: Evaluate potential cold temperature effects on fluctuations in fungicide sensitivity. Seven *Alternaria spp.* isolates (including the previously characterized *A. alternata* isolate P23 (Jayawardana, 2022)), and seven *C. beticola* from 2022 were placed into three temperature-controlled environments (20°C, 4°C, and -20°C) using a split-plot design. Fungicide sensitivity was tested using a gradient spiral dilution method (Förster et al, 2004) against difenoconazole, tetraconazole, thiophanate-methyl, or triphenyltin hydroxide. Screening began an initial two weeks and then continued every subsequent month for seven months.

Results: Preliminary data suggest that exposure to 4°C for two months has significantly increased sensitivity to tetraconazole in *Alternaria* spp. isolates (P=0.003). However, no significant changes were detected in isolate sensitivity to difenoconazole after exposure to any of the temperatures (P=0.145) (shown in Figure 2). As expected, significant differences in sensitivity were also not detected for thiophanate-methyl or triphenyltin hydroxide. Data collection for the remaining timepoints is ongoing for both *Alternaria* spp. and *C. beticola*.

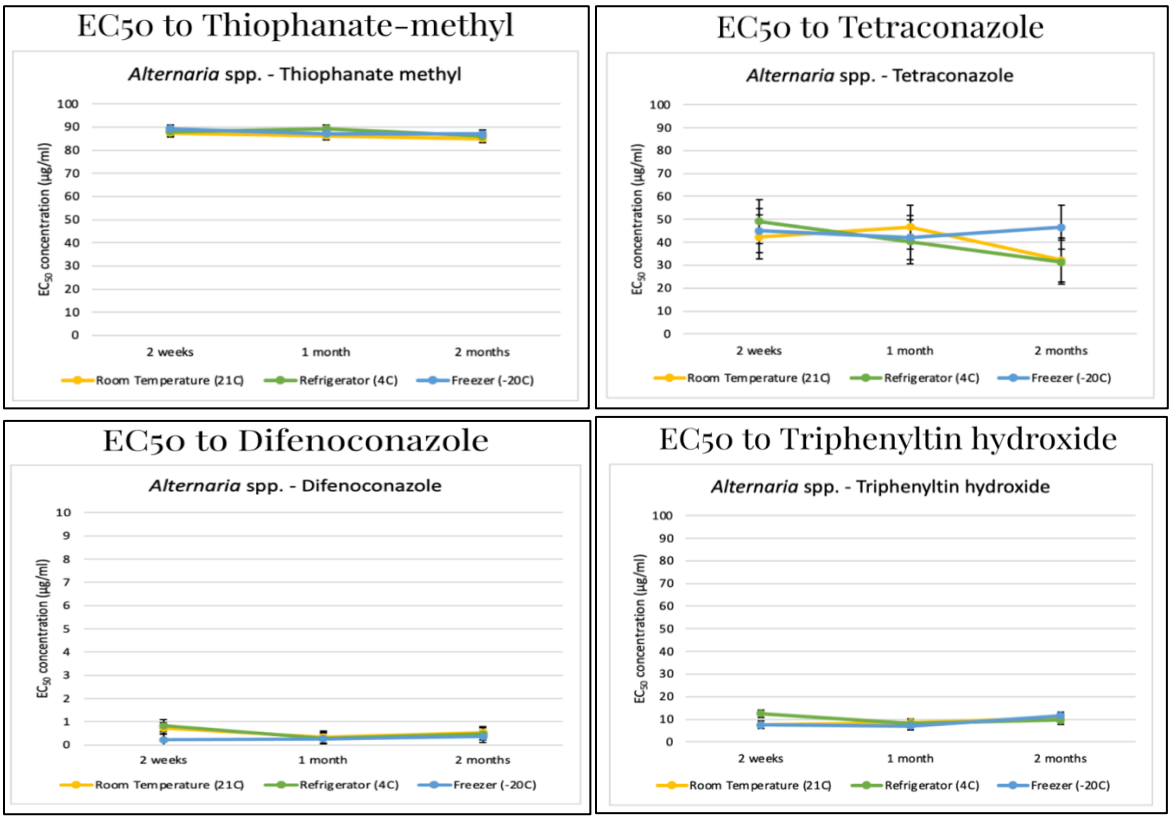


Figure 2: Preliminary EC_{50} values across *Alternaria* spp. isolates after incubation in each environment for two months. Exposure to 4ºC significantly increased sensitivity to tetraconazole in *Alternaria* spp. isolates (P=0.003). Thus far, no significant changes were detected in isolate sensitivity to difenoconazole (P=0.145), thiophanate-methyl (P>0.05), or triphenyltin hydroxide (P>0.05).

Overall Summary:

- Similar levels of insensitivity were observed for tetraconazole and prothioconazole across *Alternaria* spp. isolates. Difenoconazole and mefentrifluconazole also had comparable responses with many isolates being classified as sensitive or reduced sensitive. Most isolates were classified as insensitive for thiophanate-methyl.
- Prolonged cold exposure has potential to significantly affect sensitivity against DMI fungicides according to preliminary results with *Alternaria* species. Further analyses are ongoing for both leaf spot pathogens.

Future Directions: Data collection is ongoing for cold-environment experiments and will be repeated. Both *Alternaria* spp. and *C. beticola* isolates collected from 2023 will be characterized for virulence and fungicide sensitivity. Additionally, *Alternaria* spp. isolates will be further characterized to identify the species present in Michigan fields. Pyraclostrobin (QoI, FRAC 11) with the addition of SHAM (salicylhydroxamic acid), will be added to the fungicide screenings to represent QoI applications used to manage leaf spot diseases.

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