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Mancozeb sensitivity of DMI and QoI-resistant and susceptible *Cercospora beticola* isolates from sugar beet

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Abstract
Cercospora leaf spot, caused by *Cercospora beticola*, is a major foliar disease of sugar beet (*Beta vulgaris* L.) that can substantially reduce yield and sugar quality. Effective management of CLS relies heavily on fungicides, particularly demethylation inhibitors (DMIs) and quinone outside inhibitors (QoIs). However, repeated and intensive use of these single-site fungicides has led to the emergence of resistant *C. beticola* populations, prompting increased reliance on multi-site protectants such as mancozeb. This study evaluated the sensitivity of 20 *C. beticola* isolates with varying resistance profiles to DMI and QoI fungicides, using mancozeb as a model multi-site fungicide. A randomized complete block design (RCBD) was implemented with two replicates per treatment, testing mancozeb concentrations ranging from 0.01 to 100 µg/mL. Mycelial growth was measured after 14 days, and the effective concentration required to inhibit 50% of fungal growth (EC_{50}) was calculated. Results indicated that isolates resistant to both DMI and QoI fungicides exhibited higher mean EC_{50} values (15 µg/mL), suggesting reduced sensitivity to mancozeb, while isolates resistant to only one fungicide class or susceptible isolates showed lower EC_{50} values (5 µg/mL). These findings highlight that dual resistance may contribute to emerging tolerance against multi-site fungicides. Although mancozeb remains broadly effective, the observed variability underscores the need for continuous monitoring of pathogen populations. Integrating fungicide rotation, tank-mixing strategies, and cultural practices within CLS management programs is essential to preserve the efficacy of available chemistries and mitigate resistance development.

Keywords: Manzate Max, mancozeb, *Cercospora beticola*, sugar beet, pathogen, management

Introduction
The Red River Valley is a leading sugar beet-producing region in North America, where yield and sugar quality are critical for economic returns (Farahmand *et al.*, 2013) [1]. One of the most significant threats to production in this region is Cercospora leaf spot (CLS), caused by the fungus *Cercospora beticola*. This foliar pathogen produces characteristic small, circular to oval lesions with tan centers and dark reddish-brown borders on sugar beet leaves. Lesion coalescence can result in extensive leaf necrosis, premature defoliation, and reduced photosynthetic capacity, ultimately lowering root yield and sucrose content (Secor *et al.*, 2010; Khan *et al.*, 2007) [4, 2]. Infected leaves also serve as a reservoir for conidia, facilitating polycyclic disease development throughout the season (Bolton *et al.*, 2012) [6].
C. beticola overwinters in infected leaf debris and plant residues in the soil, providing inoculum for the following growing season (Secor *et al.*, 2010) [4]. Disease severity is influenced by environmental conditions, with warm, humid periods favoring spore germination and lesion development (Kirk *et al.*, 2012) [3]. Effective management typically combines crop rotation, resistant cultivars, and fungicide applications (Secor *et al.*, 2021) [5]. Among fungicides, multi-site protectants such as mancozeb are crucial for mitigating disease spread and slowing resistance development, particularly when used in combination with single-site fungicides like DMIs or QoIs (Trkulja *et al.*, 2017; Rosenzweig *et al.*, 2015) [7, 11].
Given the economic importance of CLS and the emergence of fungicide-resistant *C. beticola* populations, this study aimed to evaluate the sensitivity of isolates to mancozeb and other commonly used fungicides. Insights from this research are expected to guide more sustainable management strategies, preserve fungicide efficacy, and reduce the impact of CLS on sugar beet production.

Methodology

In vitro Sensitivity of DMI and/or QoI Resistant *C. beticola* Isolates to Mancozeb

An experiment was carried out to assess the sensitivity of 20 *C. beticola* isolates obtained from Yangxi Liu, which displayed varying levels of resistance to DMI and QoI fungicides. The isolates were evaluated for their response to Manzate® Max (UPL; 37% mancozeb, FRAC Group M03, multisite dithiocarbamate). The study employed a randomized complete block design (RCBD) with two independent trials and two replicates per treatment. Mancozeb was tested at concentrations of 0.01, 0.1, 1, 10, and 100 µg/mL, alongside an untreated control.

Stock solutions were prepared based on the active ingredient concentration in each formulation to achieve the desired treatment levels in CV8 agar. Under sterile conditions in a laminar flow hood (Air Science, Fort Myers, FL), 5 mm agar plugs were excised from the actively growing margins of 14-day-old cultures and placed inverted onto 100 × 15 mm petri dishes containing fungicide-amended medium. Mycelial growth was recorded after 14 days, once colonies reached approximately two-thirds of the plate diameter, by measuring two perpendicular diameters with a six-inch caliper. Each experiment was repeated twice with two replicates per treatment and an unamended control.

Data Analysis

Colony diameters were averaged across replicates, isolates, and trials to calculate percentage growth inhibition relative to the non-amended control. Non-linear regression was applied to determine the concentration required to inhibit 50% of mycelial growth (EC_{50}). Levene's test was conducted to assess homogeneity of variance across trials, allowing combination of data within each fungicide group. The Generalized Linear Mixed Model (GLIMMIX) procedure was used for analysis of variance, and treatment means were compared using the Tukey-Kramer post hoc test at p=0.05.

Results

Analysis using Levene's test confirmed that variances between the two experimental trials for mycelial growth under mancozeb treatments were not significantly different (p=0.53). Mancozeb at 10 µg/mL inhibited approximately 85% of the isolates, yielding a mean EC_{50} of 9 µg/mL (Figures 1–3). Isolates exhibiting resistance to both DMI and QoI fungicides showed the highest mean EC_{50} of 15 µg/mL, indicating a notable reduction in sensitivity to mancozeb (Table 1). Within this dual-resistant group, four isolates had EC_{50} values above 15 µg/mL, whereas the remainder averaged around 5 µg/mL. Isolates resistant solely to QoIs demonstrated a mean EC_{50} of 5 µg/mL, with one exceptional isolate recording an EC_{50} of 0.8 µg/mL. For other groups, DMI-resistant isolates and isolates susceptible to both fungicide classes both averaged EC_{50} values of 5 µg/mL. These findings suggest that resistance to QoI alone does not markedly influence mancozeb sensitivity, while combined DMI and QoI resistance may compromise its effectiveness.

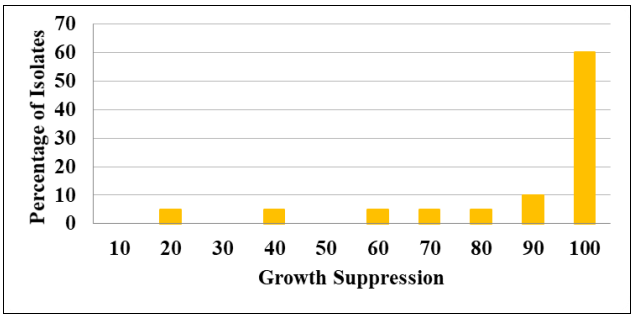

Fig 1: Frequency distribution of *Cercospora beticola* growth suppression in response to mancozeb at 10 µg/mL after 14 days post inoculation.

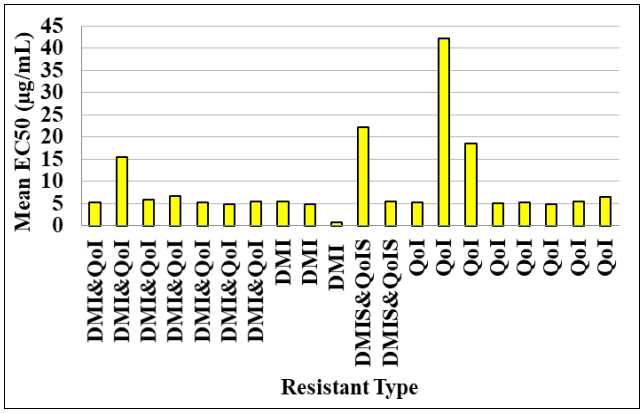

Fig 2: Effective Concentration at 50% inhibition (EC_{50} in µg/mL) of 20 *Cercospora beticola* isolates with different Demethylation Inhibitor (DMI) and Quinone outside Inhibitor (QoI) resistance in response to mancozeb.

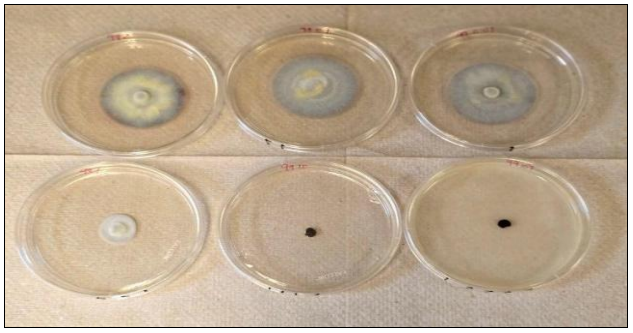

Fig 3: Growth suppression of the most sensitive *Cercospora beticola* isolate in response to mancozeb, with the first plate (top left) as the control and the sixth plate (bottom right) representing the highest concentration (100 µg/ml).

Table 1. Effective Concentration at 50% (EC_{50}) of *Cercospora beticola* isolates in response to mancozeb across different resistance types (Demethylation Inhibitor [DMI]and Quinone outside Inhibitor [QoI]).

Group	Mean EC_{50} (µg/mL)	Number of Isolates
DMI & QoI Resistant	16	7
QoI Resistant	5	8
DMI Resistant	5	3
DMI Susceptible & QoI Susceptible	5	2

Discussion

The declining number of effective fungicides has increased the reliance on broad-spectrum, multi-site fungicides such as mancozeb, which carry a lower risk of resistance development due to their multi-site mode of action (Khan and Smith, 2005; Trkulja *et al.*, 2017) [13, 7]. While multi-site fungicides are generally less potent than certain single-site products in controlling Cercospora leaf spot on sugar beet (Trueman and Burlakoti, 2014) [8], they remain essential for resistance management, particularly when used in rotational or tank-mix programs alongside single-site fungicides (Khan and Smith, 2005; Rosenzweig *et al.*, 2015) [13, 11]. Consistent with guidelines from the Michigan Sugar Company (2020), tank-mixing DMI fungicides with mancozeb or copper-based products and rotating chemistries is recommended to extend fungicide efficacy and minimize resistance risks.

Mancozeb has maintained long-term effectiveness against fungal pathogens due to its multi-site activity, making it a cornerstone of integrated disease management strategies. With the limited availability of alternative fungicides, growers increasingly depend on compounds like mancozeb and copper hydroxide, emphasizing the importance of strategic and responsible use to preserve their effectiveness. According to Weiland (2001) [10], *C. beticola* isolates exhibiting growth at 5 µg/mL mancozeb are considered tolerant, a benchmark later applied by Tümbek *et al.*, (2011) [9]. In this study, most isolates approached or slightly exceeded this threshold, suggesting a gradual increase in tolerance over time, consistent with previous observations (Tümbek *et al.*, 2011) [9].

Isolates resistant to both DMI and QoI fungicides displayed a substantially higher mean EC_{50} of 16 µg/mL, indicating that a subset of isolates has developed tolerance to mancozeb. However, variability within this group where some isolates exceeded 15 µg/mL while others remained near 5 µg/mL suggests that factors such as genetic diversity or distinct resistance mechanisms may influence tolerance levels. In contrast, isolates resistant only to QoI exhibited a mean EC_{50} like susceptible groups (5 µg/mL), indicating that QoI resistance alone does not significantly impact sensitivity to mancozeb. The single QoI-resistant isolate with an exceptionally low EC_{50} (0.7 µg/mL) further supports this observation. These results collectively indicate that tolerance to mancozeb is more strongly associated with dual resistance to both DMI and QoI fungicides rather than single-class resistance. Overall, these findings underscore the potential for repeated use of DMI and QoI fungicides to select for *C. beticola* isolates with reduced sensitivity to mancozeb.

Conclusion

While mancozeb continues to provide effective disease control for most isolates, the variability in EC_{50} values, particularly among isolates with dual resistance, indicates that tolerance is emerging within certain populations. This study emphasizes the critical need for continuous monitoring of pathogen populations to detect shifts in sensitivity early and prevent further resistance development. The findings further reinforce the importance of implementing integrated disease management strategies. Approaches such as rotating fungicides with different modes of action, tank-mixing multi-site fungicides with single-site chemistries and incorporating cultural and genetic control measures are essential to mitigate selection pressure and prolong the effectiveness of available fungicides. Future research should aim to identify and dive deeper into the underlying genetic and biochemical mechanisms driving reduced sensitivity, as this knowledge will support the development of targeted management approaches.

Acknowledgment

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