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Response of *Cercospora beticola* from Sugarbeet to a DMI-QoI fungicide combination

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Abstract
Cercospora leaf spot (CLS), caused by *Cercospora beticola*, is a major foliar disease of sugar beet (*Beta vulgaris* L.) that can significantly reduce yield and quality. Fungicides, particularly demethylation inhibitors (DMIs) and quinone outside inhibitors (QoIs), are central to disease management, yet resistance to both classes has been reported across multiple sugar beet growing regions. This study represents one of the first systematic evaluations of the in vitro activity of a DMI-QoI fungicide combination against *C. beticola* isolates. The sensitivity of 40 isolates to a mixture of prothioconazole (DMI) and pyraclostrobin (QoI) was assessed across concentrations of 10-800 µg/mL. The mean EC_{50} for the combination was 111 µg/mL, with isolate-specific values ranging from 9 to 214 µg/mL. These findings demonstrate substantial inhibition of mycelial growth, suggesting additive or synergistic effects of the mixture, while also highlighting that some isolates retain reduced sensitivity. The results provide valuable insights for developing integrated CLS management strategies and underscore the need for ongoing resistance monitoring.

Keywords: *Cercospora beticola*, sugarbeet, prothioconazole, pyraclostrobin, fungicide

Introduction
Cercospora leaf spot (CLS), caused by *Cercospora beticola*, is one of the most economically significant foliar diseases of sugar beet (*Beta vulgaris* L.) worldwide. Severe epidemics can reduce root yield and sucrose content by 30-40%, leading to substantial economic losses in major sugar beet-producing regions (Khan, 2018) [8]. While partial resistance is available in some cultivars, genetic resistance alone has not been sufficient to provide effective control, making fungicides an indispensable component of CLS management programs (Kelman, 1989) [6].

Demethylation inhibitors (DMIs; FRAC Group 3) and quinone outside inhibitors (QoIs; FRAC Group 11) represent the primary fungicide classes used to suppress CLS (Bolton *et al.*, 2013) [1]. These chemistries are valued for their effectiveness and are often applied sequentially or in mixtures to maximize disease suppression (Secor *et al.*, 2010) [14]. However, intensive use of DMIs and QoIs has resulted in reduced sensitivity in field populations of *C. beticola*. Resistance to both fungicide classes has been documented in several sugar beet growing regions resistance to both DMI and QoI fungicides has been confirmed in multiple sugar beet-producing regions in the United States (Kirk *et al.*, 2012) [9], Canada (Trueman *et al.*, 2017) [17], Serbia (Trkulja *et al.*, 2017) [15], and Greece (Karaoglanidis *et al.*, 2002; Nikou *et al.*, 2009) [4, 10]. Field populations displaying resistance to two or more fungicide classes have also been reported (Secor *et al.*, 2016; Trkulja *et al.*, 2017) [13, 15].

Combining fungicides with different modes of action has been proposed as a strategy to improve efficacy and slow resistance development (Secor *et al.*, 2020) [12]. Mixtures may act additively or synergistically, enhancing pathogen inhibition compared with single active ingredients, while also distributing selection pressure across multiple target sites (Khan *et al.*, 2007; Kaiser *et al.*, 2010) [7, 2]. Nevertheless, the success of such approaches depends on the baseline sensitivity of pathogen populations and the extent of existing resistance, as isolates with elevated resistance to one fungicide may also show reduced sensitivity to the mixture (Russel, 2004) [11].

Although mixtures of DMI and QoI fungicides are widely used in sugar beet production, few studies have systematically evaluated their combined activity against *C. beticola* isolates under controlled laboratory conditions. Understanding isolate-level variability in response to such mixtures is essential for designing sustainable fungicide resistance management

strategies. Therefore, the objective of this study was to assess the in vitro sensitivity of *C. beticola* isolates to a combination of prothioconazole (DMI) and pyraclostrobin (QoI), and to evaluate the extent to which fungicide mixtures enhance efficacy or reveal cross-resistance patterns within pathogen populations.

Methodology

Evaluation of Fungicide Sensitivity in *C. beticola* Isolates to DMI and QoI Fungicide Mixtures

The in vitro response of the 40 isolates was evaluated against the combined activity of prothioconazole and pyraclostrobin. Active ingredients were obtained from commercial formulations: Proline® (Bayer CropScience; 41% prothioconazole, FRAC Group 3; DMI - Demethylation Inhibitor) and Headline® (BASF; 23.6% pyraclostrobin, FRAC Group 11; QoI - Quinone Outside Inhibitor). Stock solutions were prepared according to the concentrations of active ingredient in each formulation and subsequently diluted to achieve the desired working concentrations in the assay medium.

Under aseptic conditions in a laminar flow hood (Air Science, Fort Myers, FL), 5 mm agar plugs were excised from the actively expanding margins of 14-day-old cultures using sterile cork borers. Each plug was placed in an inverted position onto 100 × 15 mm Petri dishes containing CV8 agar amended with the fungicides at concentrations of 0.01, 0.1, 1, 10, or 100 µg/mL.

Plates were incubated in the dark at 22±2 °C. Radial growth was recorded after 14 days, when colonies had extended to approximately two-thirds of the plate surface. For each isolate, colony diameter was determined by taking two perpendicular measurements with a Sangabery six-inch caliper. The study followed a randomized complete block design (RCBD) and was conducted twice. Each trial included two replicate plates per treatment concentration as well as untreated controls.

Results

In Vitro Sensitivity of *C. beticola* Isolates to DMI-QoI Fungicide Mixtures

Analysis of variance indicated no significant difference between the two experimental trials (Levene's test, $P = 0.99$), allowing the datasets to be pooled for subsequent evaluation. Across the tested concentration range (10-800 µg/mL), the average effective concentration required to inhibit 50% of mycelial growth (EC_{50}) was estimated at 111 µg/mL (Figure 1). Considerable variation in response was detected among isolates. The most sensitive isolate displayed an EC_{50} of 9 µg/mL (Figure 2.14), reflecting strong inhibition at low concentrations, whereas the least sensitive isolate required 214 µg/mL to achieve comparable suppression (Figure 2), indicative of markedly reduced sensitivity.

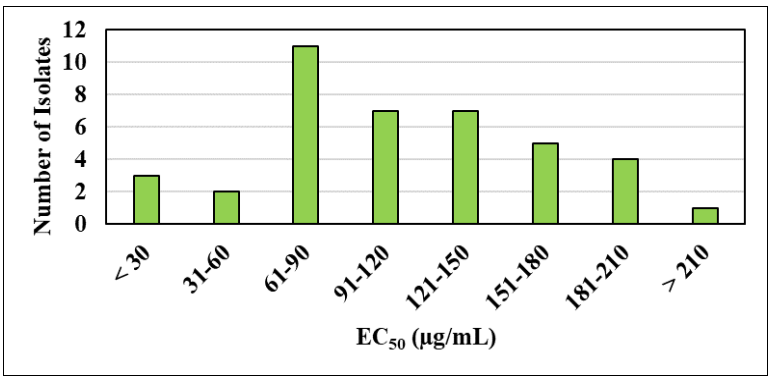

Figure 1. Effective concentration (EC_{50}) required to inhibit 50% of *Cercospora beticola* growth in response to a prothioconazole-pyraclostrobin mixture, with EC_{50} values representing both active ingredients.

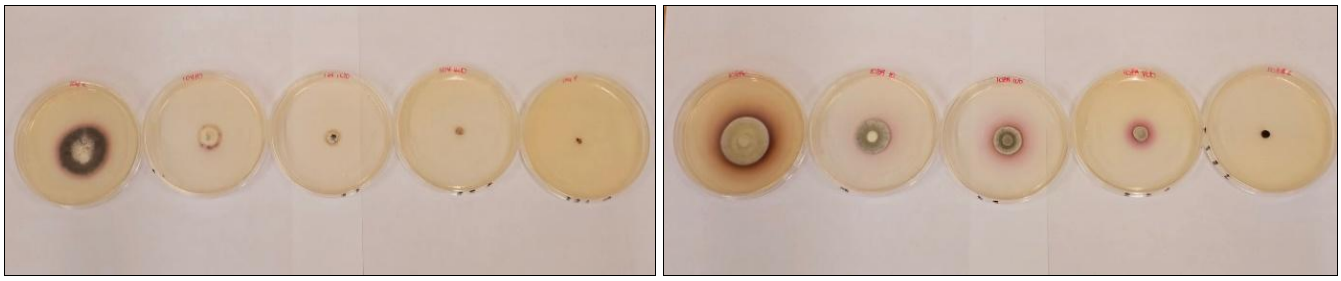

Figure 2. Mycelial growth inhibition of *Cercospora beticola* by a prothioconazole-pyraclostrobin mixture, showing the most sensitive isolate on the left and the least sensitive isolate on the right.

Discussion

Kalika-Singh *et al.,* (2025) [3] reported an average EC_{50} of 496 µg/mL for prothioconazole applied alone; in the present study, this value decreased to 111 µg/mL when combined with pyraclostrobin, representing an almost 4.5-fold increase in sensitivity. The reduction in EC_{50} suggests a potential additive or synergistic interaction between the two fungicides, enhancing overall inhibition of mycelial growth. To our knowledge, this is among the first studies to evaluate the in vitro activity of this DMI-QoI combination against *Cercospora beticola* isolates, providing new insights into pathogen sensitivity and potential management strategies. These findings align with prior reports demonstrating improved disease control in sugar beet through mixtures of DMIs and QoIs, applied in combination with disease forecasting and resistance management strategies (Khan *et al.*, 2007; Kaiser *et al.*, 2010) [2]. The mode of blending and application of these fungicides is critical in shaping

pathogen sensitivity and resistance evolution (Karaoglanidis and Thanassoulopoulos, 2003; Secor *et al.*, 2010) [5, 14], and DMIs have historically contributed to reduced disease severity and increased sugar yield in treated plants (Trueman and Burlakoti, 2014) [16].

Despite the overall enhanced efficacy, variation in isolate response was observed. Strains that exhibited high EC_{50} values to either prothioconazole or pyraclostrobin alone also showed elevated EC_{50} when exposed to the combination, indicating cross-resistance and suggesting that mixtures may not fully overcome existing resistance in all populations. While fungicide combinations are often promoted as a strategy to reduce selection pressure and delay resistance development (van den Bosch *et al.*, 2014; Trkulja *et al.*, 2017) [18, 15], our findings raise the possibility that, in some cases, resistance to multiple fungicides could accumulate more rapidly, particularly in populations already exhibiting partial resistance.

Overall, the combination of prothioconazole and pyraclostrobin can substantially improve efficacy against sensitive *C. beticola* isolates. However, the persistence of resistant phenotypes underscores the importance of continuous monitoring and the adoption of integrated resistance management strategies. Such strategies should incorporate fungicide rotation, optimized application timing guided by disease forecasting, and deployment of cultivars with partial resistance. Future field studies are needed to validate these laboratory results under natural epidemic conditions, quantify the practical benefits of the fungicide mixture, and determine how these findings can be translated into sustainable management practices for CLS.

Conclusion

The combination of prothioconazole and pyraclostrobin significantly enhanced inhibition of *C. beticola* isolates when compared with single-fungicide treatments from previous studies, demonstrating potential additive or synergistic effects. However, the presence of isolates with high EC_{50} values to both fungicides highlights the persistence of resistance and the potential for cross-resistance to compromise mixture efficacy. These results underscore the importance of integrated resistance management strategies that combine fungicide rotation, optimized application timing based on disease forecasting, and the use of partially resistant cultivars. Continuous monitoring of pathogen sensitivity is critical to maintaining the effectiveness of DMI-QoI mixtures. Future field studies are necessary to validate laboratory findings under natural epidemic conditions and to refine practical recommendations for sustainable CLS control in sugar beet production.

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References

1. Bolton MD, Rivera-Varas V, del Río Mendoza LE, Khan MFR, Secor GA. Efficacy of variable tetraconazole rates against *Cercospora beticola* isolates with differing in vitro sensitivities to DMI fungicides. Plant Dis. 2012;96:1749-1756.
2. Kaiser U, Kluth C, Märländer B. Variety-specific epidemiology of *Cercospora beticola* Sacc. and consequences for threshold-based timing of fungicide application in sugar beet. J Phytopathol. 2010;158:296-306.
3. Kalika-Singh, del Río LM, Khan M. Characterization of the sensitivity of *Cercospora beticola* to fungicides commonly used to manage cercospora leaf spot of sugar beet in the Red River Valley. Int J Plant Pathol Microbiol. 2025;5(2):92-98.
4. Karaoglanidis GS, Ioannidis PM, Thanassoulopoulos CC. Changes in sensitivity of *Cercospora beticola* populations to sterol-demethylation-inhibiting fungicides during a 4-year period in northern Greece. Plant Pathol. 2002;51:55-62.
5. Karaoglanidis GS, Ioannidis PM, Thanassoulopoulos CC. Reduced sensitivity of *Cercospora beticola* isolates to sterol demethylation inhibiting fungicides. Plant Pathol. 2000;49:567-572.
6. Kelman A. Introduction: The importance of research on the control of postharvest diseases of perishable food crops. Phytopathology. 1989;79:1374-1374.
7. Khan J, del Río LE, Nelson R, Khan MFR. Improving the *Cercospora* leaf spot management model for sugar beet in Minnesota and North Dakota. Plant Dis. 2007;91:1105-1108.
8. Khan MFR. Success and limitations of using fungicides to control cercospora leaf spot on sugar beet. Agric Res Technol. 2018;14:555909.
9. Kirk WW, Hanson LE, Franc GD, Stump WL, Gachango E, Clark G, Stewart J. First report of strobilurin resistance in *Cercospora beticola* in sugar beet (*Beta vulgaris*) in Michigan and Nebraska, USA. New Dis Rep. 2012;26:3-3.
10. Nikou D, Malandrakis A, Konstantakaki M, Vontas J, Markoglou A, Ziogas B. Molecular characterization and detection of overexpressed C-14 alpha-demethylase based DMI resistance in *Cercospora beticola* field isolates. Pestic Biochem Physiol. 2009;95:18-27.
11. Russell PE. Sensitivity baselines in fungicide resistance research and management. FRAC Monograph No. 3. Brussels (Belgium); 2003.
12. Secor GA, Rivera V, Bolton MD. Sensitivity of *Cercospora beticola* to foliar fungicides in 2020. Sugarbeet Res Ext Rep. 2020;51:6-200.
13. Secor GA, Rivera V, Bolton MD, Khan MFR. Sensitivity of *Cercospora beticola* to foliar fungicides in 2016. Sugarbeet Res Ext Rep. 2016;46:154-162.
14. Secor GA, Rivera VV, Khan MFR, Gudmestad NC. Monitoring fungicide sensitivity of *Cercospora beticola* of sugar beet for disease management decisions. Plant Dis. 2010;94:1272-1282.
15. Trkulja NR, Milosavljević AG, Mitrović MS, Jović JB, Toševski IT, Khan MF, Secor GA. Molecular and experimental evidence of multi-resistance of *Cercospora beticola* field populations to MBC, DMI and QoI fungicides. Eur J Plant Pathol. 2017;149:895-910.
16. Trueman CL, Burlakoti RR. Evaluation of products for management of *Cercospora* leaf spot in sugar beet. Plant Dis Manag Rep. 2014;9:FC009.
17. Trueman CL, Hanson LE, Somohano P, Rosenzweig N. First report of DMI-insensitive *Cercospora beticola* on sugar beet in Ontario, Canada. New Dis Rep. 2017;36:20-20.
18. van den Bosch F, Paveley N, van den Berg F, Hobbelen P, Oliver R. Mixtures as a fungicide resistance management tactic. Phytopathology. 2014;104:1264-1273.