PCR-based fungicide resistance screening in *Cercospora beticola* populations in Michigan, 2021

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Background: There are multiple fungicide groups that are commonly used and registered for CLS management in sugar beet including methyl benzimidazole carbamates (MBC or benzimidazole, FRAC group 1), quinone outside inhibitors (QoI or strobilurin, FRAC group 11), demethylation inhibitors (DMI or triazole, FRAC group 3), and multi-site contact activity (FRAC group M03) fungicide classes. Reduced sensitivity to QoI, MBC, and DMI fungicides has been detected and extensively monitored in *C. beticola* populations in Michigan (Weiland and Halloin 2001, Kirk et al. 2012, Bolton et al. 2012, Rosenzweig et al. 2015, Rosenzweig et al. 2020). PCR-based methods to detect mutations associated with fungicide resistance could provide timely and field specific guidance to improve CLS management.

Methods: Testing was conducted using polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) assays to detect point mutations in the *C. beticola* genome associated with fungicide resistance. Twenty-nine field locations were sampled across nine counties in east-central Michigan. QoI resistance was determined using the G143A point mutation present in the fungal mitochondrial cytochrome b gene of *C. beticola* isolates characterized to be resistant to pyraclostrobin, with EC_{50} values >100 ppm (Rosenzweig et al. 2015). MBC resistance was determined using the E198A point mutation present in the beta-tubulin gene of *C. beticola* isolates characterized to be resistant to benzimidazole, with EC_{50} values $\geq$ 60 ppm (Rosenzweig et al. 2015). DMI resistance was associated with the Glu169 (GAA to GAG) mutation present in the C-14 alpha-demethylase gene of *C. beticola* isolates characterized to be highly resistant to epoxiconazole, with EC_{50} values of 65-115 ppm (Nikou et al. 2009). These results will be validated using *in vitro* methods (Truman et al. 2017, Rosenzweig et al. 2020).

Summary: High frequencies of QoI resistance were observed across all locations and dates; of the 386 isolates tested, 385 were found to contain the G143A mutation. The percentage of resistant isolates detected in a field ranged from 87-100% for QoIs, 0-100% for MBCs and 0-81% for DMIs. By the final sampling of the season, mutations for resistance to QoIs and MBCs were present in 100% of the fields tested, while the mutation conferring high levels of resistance to DMIs was found in 83% of fields. Many fields were sampled several times to investigate changes in fungicide resistance over time (in progress).

Table 1. Frequencies of *C. beticola* resistance detected by Michigan county in 2021

County	No. Locations	Total Samples[a]	Strobilurin (QoI) % Resistant	Benzimidazole (MBC) % Resistant	Triazole (DMI) % Highly Resistant
Arenac	2	16	100.0	62.5	23.1
Bay	7	117	100.0	58.1	29.6
Gladwin	2	16	100.0	80.0	37.5
Gratiot	2	32	100.0	21.9	53.6
Huron	3	32	100.0	87.5	6.7
Midland	1	8	100.0	62.5	50.0
Saginaw	3	48	97.9	47.8	15.2
Sanilac	4	48	100.0	51.1	17.0
Tuscola	5	69	100.0	80.3	28.4

[a] Samples tested were either single sporulating lesions selected from 4-16 leaves per location or mycelial tissue collected from pure cultures after isolation of *C. beticola* from lesions on symptomatic leaves.

Table 2. Frequencies of *C. beticola* resistance detected at final sampling dates (Aug-Oct) in Michigan in 2021

Date	Field Location	County	No. Samples[a]	Strobilurin (QoI) % Resistant	Benzimidazole (MBC) % Resistant	Triazole (DMI) % Highly Resistant
9-Aug	Sandusky	Sanilac	8	100.0	100.0	0.0
16-Aug	Auburn	Bay	16	100.0	75.0	37.5
1-Sep	Ruth	Huron	8	100.0	62.5	0.0
1-Sep	Ubly	Huron	8	100.0	100.0	0.0
1-Sep	Sandusky	Sanilac	8	100.0	100.0	28.6
7-Sep	Cass City	Tuscola	8	100.0	57.1	25.0
7-Sep	Crump	Bay	8	100.0	100.0	25.0
10-Sep	Freeland	Saginaw	8	100.0	83.3	12.5
13-Sep	Gladwin	Gladwin	8	100.0	71.4	12.5
15-Sep	Midland	Midland	8	100.0	62.5	50.0
15-Sep	Gilford	Tuscola	8	100.0	100.0	57.1
16-Sep	Standish	Arenac	8	100.0	62.5	14.3
16-Sep	Auburn	Bay	8	100.0	87.5	25.0
17-Sep	Pinconning	Bay	8	100.0	12.5	0.0
17-Sep	Au Gres	Arenac	8	100.0	62.5	33.3
18-Sep	Brown City	Sanilac	8	100.0	37.5	12.5
18-Sep	Croswell	Sanilac	8	100.0	50.0	12.5
22-Sep	Auburn	Bay	8	100.0	100.0	0.0
22-Sep	Freeland/ Saginaw	Saginaw	8	100.0	62.5	37.5
24-Sep	Unionville	Tuscola	8	100.0	100.0	12.5
24-Sep	Beaverton	Gladwin	8	100.0	87.5	62.5
3-Oct	Munger	Bay	8	100.0	87.5	28.6
3-Oct	Akron	Tuscola	8	100.0	87.5	37.5
10-Oct	Fairgrove	Tuscola	8	100.0	66.7	14.3
15-Oct	Ithaca	Gratiot	8	100.0	37.5	14.3
19-Oct	Auburn	Bay	8	100.0	37.5	25.0
21-Oct	Freeland	Saginaw	8	100.0	12.5	16.7
23-Oct	Caseville	Huron	8	100.0	87.5	14.3
24-Oct	Breckenridge	Gratiot	8	100.0	37.5	20.0
Total	**29 Locations**	**9 Counties**	**240**	**100.0**	**70.0**	**21.7**

[a] Samples tested were either single sporulating lesions selected from 4-16 leaves per location or mycelial tissue collected from pure cultures after isolation of *C. beticola* from lesions on symptomatic leaves.