Resistance to QoI and DMI Fungicides do not Reduce Virulence of *C. beticola* Isolates in North Central USA

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Abstract:

Cercospora leaf spot (CLS) is a destructive disease limiting sugar beet production and managed using resistant cultivars, crop rotation, and timely applications of effective fungicides. Since 2016, its causal agent, *Cercospora beticola*, has been reported to be resistant to Quinone outside inhibitors (QoIs) and to have reduced sensitive to Demethylation inhibitors (DMIs) in sugar beet growing areas in North Dakota and Minnesota. Isolates of *C. beticola* resistant to QoIs, DMIs, and both QoIs and DMIs were collected from Foxhome fields, Minnesota in 2017. Fitness of these resistant isolates was compared to that of QoI- and DMI-sensitive isolates in laboratory and greenhouse studies. In the lab, mycelial growth, spore production, and spore germination were measured. The results showed that resistant isolates had significantly less mycelial growth and spore production than sensitive isolates, while no significant difference in spore germination was detected. In the greenhouse, six leaf-stage sugar beets were inoculated with a spore suspension made from each resistant group and then incubated in separate humidity chambers. CLS disease severity was evaluated visually at 7, 14, and 21 days after inoculation (DAI) and the areas under disease progress curve (AUDPC) were calculated. Resistant isolates had significantly smaller AUDPC but still caused high disease severity as the sensitive ones at 21 DAI. Although QoI and/or DMI resistant isolates had a relatively slower disease development, they still caused high disease severity and need to be factored in disease management practices.

Keyword: *Beta vulgaris*, sugar beet, Cercospora leaf spot, fungicide resistance, triazoles, strobilurins

In North Dakota and Minnesota, sugar beet (*Beta vulgaris* L.) is an economically important crop but its sustainability is threatened by the foliar disease Cercospora leaf spot (CLS) (Bangsund et al., 2012; Weiland and Koch, 2004). Successful management of CLS is usually achieved by multiple fungicide applications from July through September during the peak growing season (Secor et al., 2010). Sugar beet producers are advised to apply effective fungicides in a timely manner based on scouting, calendar-based schedule, and forecasting models (Shane and Teng, 1983; Windels et al., 1998). Fungicides approved for use on sugar beet belong to different chemical groups with a specific code assigned by Fungicide Resistance Action Committee (FRAC): thiophanate-methyl [Methyl Benzimidazole Carbamates; FRAC group 1], tri-phenyltin hydroxide [Organo tin compounds; FRAC group 30], tetraconazole [DeMethylation Inhibitors (DMIs); FRAC group 3], pyraclostrobin [Quinone outside Inhibitors (QoIs); FRAC group 11], trifloxystrobin [QoIs; FRAC group 11], difenoconazole [DMIs; FRAC group 3], and prothioconazole [DMIs; FRAC group 3] (FRAC, 2020; Secor et al., 2010).

The extensive use of QoI and DMI fungicides for over two decades has resulted in resistant field isolates of *C. beticola* in the United States (Kirk et al., 2012; Secor et al., 2016), Canada (Trueman et al., 2013), Serbia (Trkulja et al., 2017), and Greece (Karaoglanidis et al., 2000; Nikou et al., 2009). Field isolates with a dual resistance or multiple resistances were reported by Trkulja et al. (2017) and Secor et al. (2016, 2020). Both QoI and DMI fungicides are specific inhibitors with a medium to high inherent risk for selecting resistant isolates of targeted fungal pathogen population (McGrath, 2004; FRAC, 2019). Moreover, CLS is a polycyclic disease, and the causal fungal pathogen *Cercospora beticola* can have multiple infection cycles that produce plenty of asexual conidia as well as possible sexual recombination (Bolton et al., 2012b), resulting in increased mutation potential for resistance development. Resistance to QoI

fungicides in *C. beticola* isolates was reported from a field failure in Michigan that was due to the substitution of glycine with alanine at position 143 in the cytochrome b (*cytb*) gene (G143A point mutation) (Bolton et al., 2013; Kirk et al., 2012). The DMI resistance in filamentous fungi is effected through the functioning of the CYP51 gene (Erg11), which belongs to the cytochrome P450 monooxygenase (CYP) superfamily and mediates a crucial step of the synthesis of a fungal-specific sterol ergosterol (Zhang et al., 2019). DMI resistance has been associated with any of the three principal mechanisms; (1) target site modification by point mutations (2) overexpression of the target gene(s) *Cyp51* or its paralogs. and (3) increased efflux by overexpression of membrane-bound drug transporters, reducing the accumulation of fungicide at the target site (Mair et al., 2016). Reduced sensitivity to DMI fungicides in *C. beticola* isolates is associated with overexpression of the cytochrome P450 sterol C-14 alpha-demethylase (*cyp51*) gene (Bolton et al., 2012a).

The fitness of resistant strains in a population of fungi plays a crucial role in the development and stability of fungicide resistance (Cox et al., 2007; Dekker, 1976). In general, the fitness of fungal plant pathogens is the ability to compete with other strains and to survive under environmental conditions. Specifically, the fitness can be measured as several components during a pathogen's life cycle including spore production, spore dispersal, aggressiveness, mycelial growth, spore germination, and the ability to overwinter for long-term survival (Mikaberidze and McDonald, 2015). Fungicide resistance may provide a selective advantage for the resistant isolates compared to sensitive isolates but resistance-conferring mutations may also affect negatively important physiological and biochemical processes (Anderson, 2005). Plant pathologists study fitness costs of fungal pathogens in mycelial growth, spore productivity, and germination ability in laboratory (in vitro) as well as pathogenicity and aggressiveness in

greenhouse experiments (in vivo) (Antonovics and Alexander 1989; Mikaberidze and McDonald, 2015). The measurement of fitness costs in resistant fungal population will help us predict the resistance stability in order to determine whether growers can expect to reuse these fungicides after their withdrawal for years. Theoretically, this information can also help determine the optimal proportion of high- and low- risk fungicides in the mixture (Mikaberidze and McDonald, 2015).

The results of an annual monitoring program sponsored by sugar beet growers in North Dakota and Minnesota indicated that the sensitivity to both QoI and DMI fungicides of *C. beticola* isolates decreased over the years (Secor et al., 2020). Furthermore, the resistance to QoI fungicides resulted in a CLS control failure in sugar beet fields, leading to an epidemic in 2016 (Khan, 2018). The objective of this research was to determine the fitness cost of *C. beticola* isolates resistant to QoIs, DMIs, and both QoI and DMI fungicides and their impact on disease severity.

Materials and methods

Selecting C. beticola isolates. We used 16 *C. beticola* isolates in this study (Table 1). Out of these, 4 QoI- resistant, 4 DMI-resistant, and 4 with both QoI and DMI resistance were recovered from sugar beet fields in Minnesota in 2017; and 4 isolates that were sensitive to both QoI and DMI (obtained from Dr. Gary Secor, North Dakota State University, Fargo, ND) were collected in 1998 and 2016 from sugar beet infected leaf samples submitted from growers in North Dakota and Minnesota. The recovered *C. beticola* isolates were tested for their sensitivity to 1) DMIs, (difenoconazole, prothioconazole, and tetraconazole) using a radial growth procedure (Secor and Rivera, 2012) and 2) QoIs, using a PCR-based molecular procedure to test

the presence of G143A conferring the QoI resistance (Bolton et al., 2013). These isolates were grown on CV-8 media (15g Agar, 100 ml V8 juice, and 900 ml dH_2O) and evaluated for their sensitivities to QoIs and/or DMIs following the methodology described by Secor and Rivera (2012) and Bolton et al. (2013). This research evaluated the fitness cost of each isolate group in terms of mycelial growth, conidial production, and spore germination in the laboratory study (in vitro) and for aggressiveness (amount of disease caused by each isolate group) in the greenhouse study (in vivo).

In vitro fitness of C. beticola isolates with different fungicide resistance. The 16 *C. beticola* isolates were assessed for mycelial growth in vitro. Under laminar flow (Air science; Fort Myers, FL), agar plugs (5 mm in the diameter) were cut using a sterile cork borer from the leading edge of growth in 14-day-old *C. beticola* cultures and then inverted onto 100 × 15 mm petri dishes containing CV-8 media. The inoculated plates were incubated in the dark at room temperature (22 ± 2°C). After 14 days, when mycelial growth covered 2/3 of the petri dish, two perpendicular measurements of mycelial growth for each isolate were measured using a digital caliper. This trial was conducted twice each with three replicates.

The same 16 *C. beticola* isolates were evaluated for conidial production. First, sporulation plates were made by adding sterile distilled water onto 14-day-old isolates, scraping the mycelia from the isolate surface, transferring the suspension onto petri dishes of CV-8 media, and incubating under continuous fluorescent light at 22 ± 2°C (Secor and Rivera, 2012). After 3 to 4 days of incubation, conidial spores were abundantly induced. Five ml sterile distilled water was added to the plates and a bent sterile glass rod was used to dislodge conidia from each sporulation plate. A 100 µl aliquot of the conidia suspension was applied to a hemocytometer (Hausser Scientific; Horsham, PA, USA), and the conidial concentration was counted using a

compound microscope at × 400 magnification. Each isolate had three replicates and the experiment was conducted twice.

Conidial germination was determined for each of the 16 *C. beticola* isolates. For each isolate, the conidial concentration from a sporulation plate was determined using a hemocytometer as described above and then adjusted to 1×10^4 conidia/ml by adding sterile distilled water. A 120 µl aliquot of the conidia suspension was added onto 1.5% water agar media (15g Agar and 1000 ml dH_2O). The inoculated plates were incubated at 22 ± 2°C in the dark for 16 h to allow conidial germination. Then, 50 conidia per plate were examined for germination (considered when the germ tube length was at least twice as long as the conidium) under a dissecting microscope at × 50 magnification. In this trial, the percentage of germinated conidia for each isolate was calculated with three replicates in two repeats.

In vivo fitness of C. beticola isolates with different fungicide resistance. This research was conducted in 2019 and 2020 in a greenhouse facility (Agricultural Experiment Station at North Dakota State University; Fargo, ND) where environmental conditions were set with a 16-h photoperiod and at 22 ± 2°C (Argus Control Systems Ltd.; British Columbia, Canada). A CLS-susceptible sugar beet cultivar (Niehaus and Moomjian, 2019), MA 504 (proprietary material, Maribo, Mapleton, ND, USA), was used, and three seeds were planted in each pot (10 × 10 × 12 cm) filled with peat mix (Sunshine mix 1, Sun Gro Horticulture Ltd.; Alberta, Canada). Each pot was watered as needed, thinned to have one vigorous seedling, and grown to be used at the 6-leaf stage.

The aggressiveness of the 16 *C. beticola* isolates previously used in in vitro experiments was evaluated under greenhouse conditions. The *C. beticola* isolates were separated into four groups according to their fungicide resistance status (e.g., QoI-resistant, DMI-resistant, both QoI

and DMI resistant, and sensitive to both QoI and DMI). Conidial suspension was made from the same group using a bent sterile glass rod to free conidia from each sporulation plate into sterile distilled water. The conidial suspension was then mixed from the isolates of the same resistant group. The number of conidial spores was counted and adjusted to 4×10^4 conidia/ml using a hemocytometer. Inoculation was conducted using a Preval paint-spray gun (Preval Sprayer Division, Precision Valve Corporation, Yonkers, NY) to spray the conidial suspension until run-off onto five fully expanded leaves of each plant at the 6-leaf stage. On the same day, the plants inoculated with the *C. beticola* isolates of the same resistance group were transferred together into confined humidity chambers (100 × 90 × 140 cm) to prevent interference from the other resistant groups. All the inoculated plants were incubated at 95 to 100% relative humidity with a 16-h photoperiod and average temperature of 28 ± 2°C. At 7 days after inoculation (DAI), the plants were moved back into the greenhouse and watered as needed at the base of each plant. CLS disease severity was evaluated visually at 7, 14, and 21 DAI by estimating the number of leaf spots on the five inoculated leaves (five subsamples). A disease scale from 1 to 10 was used as follows: 1 = 1-5 spots/leaf; 2 = 6-12 spots/leaf; 3 = 13-25 spots/leaf; 4 = 26-50 spots/leaf; 5 = 51-75 spots/leaf; 6 = 76-99 spots/leaf; 7 = 100-124 spots/leaf; 8 = 125-149 spots/leaf; 9 = 150-200 spots/leaf; and 10 = >200 spots/leaf (Jones and Windels, 1991). The experiment was conducted twice as a CRD with four replicates each.

Data analyses. In the in vitro study, Levene's test was conducted to test the homogeneity of variance across the two data repeats of mycelial growth, spore production, and spore germination before they were combined for analysis of variance. For each fitness parameter, an analysis of variance (ANOVA) was performed among *C. beticola* isolates of four fungicide resistance status. The fitness parameters for the isolates with different fungicide resistance status

were separated by the post hoc test of Fisher's Least Significant Difference (LSD) at $P = 0.05$. The calculation process was achieved using the general linear model procedure (Proc GLM) in the Statistical Analysis System software (Version 9.3, SAS Institute Inc.; Cary, NC, USA).

In the in vivo study, the disease severity caused by *C. beticola* isolates of each fungicide resistant status was evaluated using the disease scale from 1 to 10 on inoculated leaves at 7, 14, and 21 DAI. The disease rating data were analyzed by a non-parametric analysis using SAS procedures of Proc Rank and Proc Mixed with LSMEANS. The relative effect of disease severity for each fungicide resistance status with its confidence interval was calculated using LD-CI macro in SAS (Shah and Madden, 2004). The area under the disease progress curve (AUDPC) was calculated using the SAS general linear models (Proc GLM) procedure, following the formula:

$$\text{AUDPC}= \sum_{i=1}^{n-1}[(y_i + y_{i+1})/2](t_{i+1} - t_i)$$

where y_i= disease severity at the i^{th} observation, t_i= time (days) at the i^{th} observation, and n = total number of observations. The homogeneity for variances between the two data repeats of AUDPC was tested via Levene's test before combining the data for an analysis of variance. Analysis of variance (ANOVA) of AUDPC resulted from resistant status isolates was performed, the means of treatments were separated by Fisher's Least Significant Difference (LSD) calculated at $P= 0.05$ using the SAS general linear models (Proc GLM) procedure.

Results

In vivo fitness of C. beticola isolates with different fungicide resistance. The Levene's test was conducted for the homogeneity of variances, indicating that there were no significant differences between the two data repeats of mycelial growth ($P = 0.19$), spore production ($P =$

195 0.76), and spore germination (P = 0.30) for *C. beticola* isolates. Therefore, a combined analysis
196 was performed on the two repeats of these datasets.

197 In the in vitro study, the fitness traits were evaluated as mycelial growth, spore
198 germination, and spore production of *C. beticola* isolates with four fungicides resistance status:
199 (1) both QoI- and DMI-sensitive, (2) QoI-resistant, (3) DMI-resistant, and (4) both QoI- and
200 DMI-resistance isolates (Table 2). Two significant separations in mycelial growth between
201 fungicide resistance groups were observed. The sensitive isolates had the most mycelial growth
202 but did not significantly differ from the QoI-resistant isolates. The isolates resistant to both QoI
203 and DMI were similar to DMI-resistant isolates, sharing a significantly lower mycelial growth
204 compared to the other two isolate groups. For spore production, the sensitive isolates produced
205 the highest number of conidia estimated at 68,600 spore/ml. Under the same conditions, QoI
206 or/and DMI resistant isolates produced between 26,400 and 33,500 spore/ml, which was
207 significantly lower (P = 0.05) compared to the sensitive isolates. In the spore germination test, all
208 isolates had germination percentages > 98% without significant differences among different
209 resistance groups.

210 In vivo fitness of C. beticola isolates with different fungicide resistance. The
211 homogeneity of variance was tested with Levene's test between two repeats of AUDPC. No
212 significant differences were found (P =0.81) so that the data repeats of AUDPC were combined
213 for analysis.

214 The increase in CLS severity between 7 DAI and 14 DAI was much greater than that
215 between 14 DAI and 21 DAI. On 7 DAI, DMI-resistant isolates caused the lowest severity
216 compared to other groups, and resulting in the lowest value of AUDPC. This was followed by
217 the QoI-resistant isolates with intermediate AUDPC values. The sensitive isolates and the

isolates resistant to both QoI and DMI did not differ significantly and had the highest AUDPC values. However, on 21 DAI, all the isolate groups with different fungicide resistant status resulted in a similarly high disease severity.

Discussion

C. beticola populations with resistance to multiple fungicide classes has been reported in sugar beet growing regions around the world, where warm and humid environmental conditions warrant repeated fungicide applications for effective disease management. The North Dakota and Minnesota *C. beticola* isolates used in this study were confirmed to be resistant to QoIs and DMIs. These compounds were extensively used during the previous decade but a survey conducted between 2016 and 2019, showed that QoI-resistant *C. beticola* isolates remained at a high frequency (up to 90%) while the sensitivity of DMI-resistant isolates continued to decrease (Secor et al., 2019). Our study was to assess the variation in fitness components of *C. beticola* isolates with different resistance status to QoIs and/or DMIs. Knowledge of the parasitic fitness is critical for implementing fungicide-resistance management strategies because resistant strains may suffer fitness penalties that could affect the stability of resistance in the absence of fungicide selection pressure (Ishii, 2015).

The specific point mutation conferring QoI resistance exerts different effects on fitness parameters of fungi. In this study, QoI-resistant *C. beticola* isolates possessing G143A mutation showed no significant differences in spore germination, mycelial growth or aggressiveness compared to the sensitive isolates but produced significantly fewer conidia. This contrasts with *Alternaria solani* field isolates with a F129L point mutation which resulted in lower spore germination but increased aggressiveness to potato plants (Pasche and Gudmestad, 2008).

Malandrakis et al. (2006) studied laboratory-produced QoI-resistant *C. beticola* isolates and observed reduced spore production and pathogenicity compared with wild-type isolates; however, the fitness cost was likely due to pleiotropic effects of accumulated mutations induced by ultraviolent mutagenesis (Karaoglanidis et al., 2011). In North Dakota and Minnesota, Secor et al. (2019) demonstrated that the percentage of QoI-resistant isolates with G143A mutation was still stable in field populations three years after growers stopped applying QoI fungicides to manage CLS. The lack of fitness penalty on the aggressiveness of the QoI resistant isolates evaluated in this study explain in part this stability. Alternatively, usage of QoI fungicides in the region, although reduced may still play an important role in selecting resistant strains. Other researchers have indicated that QoI resistance was relatively stable in *A. alternata* isolates (Vega and Dewdney, 2014) and that resistant strains recovered rapidly if QoI fungicide was applied again (Genet et al., 2006; Ishii et al., 2007).

The reduced fitness associated with DMI resistance may not be large enough to affect the stability of resistant populations. In our study, field isolates of *C. beticola* were confirmed to be resistant against difenoconazole, prothioconazole, and tetraconazole. These isolates, were significantly less fit in mycelial growth (radial growth rate) and sporulation in vitro as well as in overall aggressiveness compared to the DMI sensitive isolates. However, the latter may have been an artifact produced by a slow start of the disease caused by DMI resistant isolates since the difference in severity observed 7 DAI was quickly erased and by 14 DAI, the severity of disease caused by DMI resistant isolates was like that of DMI sensitive isolates. It is likely, the AUPDC would be similar as well if the study would have been conducted for seven additional days. Further, there does not seem to be consensus among the scientific community on the significance of fitness penalties associated with DMI resistance on disease development. Some researchers

report reduced growth/sporulation and pathogenicity of *C. beticola* isolates (Moretti et al., 2003; Karaoglanidis et al., 2001),while others observed increased aggressiveness (Bolton et al. 2012c) and that tetraconazole (DMI) applications still provided disease control compared to the non-treated check and speculated that the cold winter lasting for five months in North Dakota and Minnesota may lead to a shift in the sensitivity of DMI-resistant strains. Arabiat (2015) and Karaoglanidis et al. (2002) reported the instability of DMI-resistant *C. beticola* strains after cold exposure, although the treatments of low temperature and exposure period were different.

Isolates resistant to both DMIs and QoIs have been detected and collected in North Dakota and Minnesota (Secor et al., 2016) and were used in this study. Multiple fungicide resistance has been reported on *C. beticola* before (Trkulja et al., 2017) as well as in other fungal pathogens, such as *Botrytis cinerea* (De Miccolis Angelini et al., 2014) and *Venturia inaequalis* (Chapman et al., 2011). *C. beticola* resistant to QoI and DMI did not show a reduction in aggressiveness compared to the sensitive isolates. Other researchers have listed as potential explanation for this is the ability of *C. beticola* to produce more phytotoxins and degradative enzymes that play an important role in infection progression (Steinkamp et al., 1979). Also, it is possible that the mutations that confer resistance to both fungicide classes did not affect other pathways required for virulence or pathogenicity.

In conclusion, compared to sensitive *C. beticola* isolates, the QoI and/or DMI resistant isolates had a relatively slower disease development on sugar beet leaves due to their fitness penalty in sporulation and mycelial growth. However, the fitness cost was not large enough as to reduce the ability of the fungus to cause high levels of disease severity. Currently, QoI fungicides are not highly recommended to sugar beet growers for controlling CLS because the resistance is easy to reoccur once used again. A similar case occurs with another site-specific

chemical benzimidazole, where the resistant *C. beticola* strains associated with two amino acid replacements can be rapidly selected if the fungicide was applied again (Karaoglanidis et al., 2003; Trkulja et al., 2013). DMI fungicides are slightly different in chemical structures (Kuck et al., 1995) and there are multiple modes of DMI resistance (Mair et al., 2016), therefore, individual DMI fungicide might still work on controlling CLS. There is a need to consider using multi-site fungicides, such as copper-based chemicals, as management options for CLS control. More importantly, it is wise to use effective fungicides belonging to a different mode of action in alternation or mixture fungicide application programs to better CLS control as well as for fungicide resistance management for the sugar beet industry. Field sanitation, crop rotation with non-host crops, and planting resistant cultivars are always recommended for growers to suppress field population of various *C. beticola* strains. The recent availability of CR+ varieties with improved tolerance to *C. beticola* that requires less fungicides without significant reduction in tonnage and sucrose concentration will be a boon to the sugar beet industry.

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Table 1: *Cercospora beticola* isolates with different fungicide resistance used in the fitness study.

Isolates[a]	*C. beticola* isolates	QoIs (G143A point mutation)[b]	DMIs (ug/ml)[c]		
			Difenoconazole	Prothioconazole	Tetraconazole
16-41	QoI- and DMI-sensitive	S	<1	<1	<1
16-65	QoI- and DMI-sensitive	S	<1	<1	<1
98-25	QoI- and DMI-sensitive	S	<1	<1	<1
98-46	QoI- and DMI-sensitive	S	<1	<1	<1
17-157	QoI-resistant	R	0.393	0.301	0.184
17-183	QoI-resistant	R	0.034	0.149	0.130
17-193	QoI-resistant	R	0.031	0.008	0.224
17-211	QoI-resistant	R	0.041	0.009	0.189
17-192	DMI-resistant	S	2.248*	>10*	>10*
17-258	DMI-resistant	S/r	10*	>10*	>10*
17-179	QoI- and DMI-resistant	R	10*	7.812*	>10*
17-244	QoI- and DMI-resistant	R	1.832*	2.161*	4.152*
17-256	QoI- and DMI-resistant	R	1.638*	1.094*	4.988*
17-287	QoI and DMI resistant	R	1.292*	10*	>10*
17-292	QoI- and DMI-resistant	S	5.133*	1.205*	3.162*
17-300	QoI- and DMI-resistant	S	>10*	>10*	>10*

[a]12 *C. beticola* isolates were collected from the Minn-Dak factory district near Foxhome, Minnesota in 2017. Dr. Secor from North Dakota State University, Fargo, North Dakota provided five isolates as follows: 16-33, 16-41, 16-65, 98-25, and 98-46; these isolates were collected from factory district areas of sugar beet crop in North Dakota and Minnesota.

[b]the resistance to QoIs was tested using a PCR-based molecular procedure to test the presence of G143A point mutation in isolates. The results indicated as follows: R: all spores with G143A mutation; R/s: >50% of spores with G143A mutation; S/R: equal numbers of spores with G143A mutation; S/r: <50% spores with G143A mutation; S: No spores with G143A mutation. The isolates tested to have >50% spores with the mutation were considered as QoI-resistant ones.

[c]the DMIs sensitivity (difenoconazole, prothioconazole, and tetraconazole) was evaluated using a radial growth procedure; the *C. beticola* isolates with an EC_{50} value higher than the discriminate

rate of 1 ug/ml were considered as resistant against DMIs and labeled with an asterisk (*). Representative isolates were phenotyped for tetraconazole, difenoconazole and prothioconazole. Haplotype analysis of CbCY51 identified the E170 and L144F mutations associated with DMI resistance (Spanner et al. 2021).

Table 2: Mycelial growth, spore germination, and spore production of *Cercospora beticola* isolates in different fungicide resistance groups (in vitro).

Reaction to fungicides[a]	Radial growth[b] (mm)	Spore production[b] (10^3 Spores/ml)	Spore germination[b] (%)
QoI- and DMI-sensitive	63.9 a	68.6 a	98.8 a
QoI-resistant	63.8 a	26.4 b	98.6 a
DMI-resistant	60.7 b	32.5 b	98.8 a
QoI- and DMI-resistant	58.9 b	33.5 b	98.7 a
LSD (*P*=0.05)	1.8	7.8	0.5

[a]Four isolates were selected for each of four fungicide resistance groups and a total of 16 *C. beticola* isolates were evaluated in this study.

[b]Means of the treatment with the same letter were not significantly different at $P = 0.05$ using the post hoc test of Fisher's Least Significant Difference (LSD).

Table 3: Evaluation of disease severity and aggressiveness (areas under disease progress curve [AUDPC]) caused by *Cercospora beticola* isolates in different fungicide resistance groups in greenhouse study (in vivo).

	CLS Ratings (disease scale from 1 to 10)[b]			
Reaction to fungicides[a]	**7 DAI[c]**	**14 DAI[c]**	**21 DAI[c]**	**AUDPC**
QoI- and DMI-sensitive	4.8 a	8.7 a	9.1 a	123 a
QoI-resistant	4.1 ab	7.9 a	9.0 a	113 bc
DMI-resistant	3.0 b	8.1 a	8.5 a	107 c
QoI- and DMI-resistant	4.9 a	8 a	9.1 a	120 ab
LSD (*P*=0.05)[d]				10

[a]Spores from four isolates within each fungicide resistance group were combined and used to inoculate plants of CLS-susceptible cultivar MA-504.

[b]A disease scale from 1 to 10 for CLS evaluation was used: 1 = 1-5 spots/leaf; 2 = 6-12 spots/leaf; 3 = 13-25 spots/leaf; 4 = 26-50 spots/leaf; 5 = 51-75 spots/leaf; 6 = 76-99 spots/leaf; 7 = 100-124 spots/leaf; 8 = 125-149 spots/leaf; 9 = 150-200 spots/leaf; and 10 = >200 spots/leaf.

[c]Means of disease scale between treatments with the same letter were not significantly different at $P = 0.05$ using a non-parametric analysis.

[d]Fisher's Least Significant Difference (LSD) was calculated at $P = 0.05$.