Diversity and fungicide resistance of *Cercospora beticola* populations on table beet in New York

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INTRODUCTION

New York (NY) is the second largest producer of processing and fresh market table beet in the United States. Cercospora leaf spot (CLS), caused by the fungus *Cercospora beticola*, incurs substantial losses to table beet production by reducing the green leaf area, thus, limiting the supply of nutrients to the developing root and reducing its size and quality. Moreover, when beets are harvested by top-pulling machinery, loss of foliage may lead to the harvester being unable to pull out the roots from the ground.

CLS epidemics start when the overwintering structures, *i.e.*, pseudostromata, give rise to asexual spores that infect leaves. Lesions develop within a few days and produce more spores that are disseminated via rain splash and cause new infections throughout the growing season resulting in rapid disease spread. The ability of *C. beticola* to undergo sexual reproduction is not yet known. However, the genes that regulate sexual reproduction, *MAT1-1* and *MAT1-2*, have been identified in the *C. beticola* genome. If these genes are functional, *C. beticola* isolates carrying the alternate *MAT1* genes will be able to mate and sexually reproduce. Sexual reproduction is a major source of genetic variation through recombination, and increases the evolutionary potential of a pathogen. Therefore, sexually reproducing fungal populations with high genetic diversity due to recombination evolve and become resistant to fungicides more rapidly.

Effective management of CLS in table beet fields primarily depends on the regular application of fungicides. The fungicides currently registered for controlling CLS on table beet in NY have single-site modes of action, and belong to groups 11 (Quadris®, Cabrio® EG, Reason® 500 SC, and Gem® 500 SC) and 3 (PropiMax® and Tilt®), with high to medium risk of resistance development, respectively. Resistance to both these fungicide groups has been reported in *C. beticola* populations on sugar beet in ND, MI, and NE. In a NY table beet field in 2012, a failure to control CLS was observed despite the repeated use of Quadris®. This was later attributed to strobilurin resistance within the *C. beticola* population in this field.

The objectives of this study were to: (1) understand the evolutionary potential of *C. beticola* population in table beet fields in NY; (2) quantify the frequency of strobilurin resistance in *C. beticola* populations in NY; and (3) identify efficacious fungicides from various fungicide resistance action (FRAC) groups for the control of CLS on table beet.

MATERIALS AND METHODS

***Cercospora beticola* populations.** 150 *C. beticola* isolates were collected from two processing beet fields (cv. Ruby Queen) in Batavia, NY in 2014. Genomic DNA of all isolates was extracted, and molecular markers were used to confirm their identity.

Genetic diversity and potential for sexual reproduction of the *C. beticola* populations. 48 isolates of *C. beticola* (24 from each field) were arbitrarily chosen to assess the population genetic diversity using 5 microsatellite markers. The potential for sexual reproduction in the *C. beticola* populations was investigated using two approaches. First, the *MAT1* genes for the whole population were identified, and the hypothesis of random mating was tested by assessing the MAT1-1:MAT1-2 ratio. A random association of microsatellite alleles was also tested.

Q_oI resistance in *C. beticola* populations. Sensitivity of *C. beticola* isolates to azoxystrobin was tested using a conidial germination assay. Technical grade azoxystrobin was added to water agar medium to obtain concentrations of 0.001, 0.01, 0.1, 1.0, 10, 25 and 100 µg/ml. All fungicide-amended plates contained salicylhydroxamic acid (60 µg/ml) to suppress the alternative respiration pathway. The effective concentration required to cause 50% inhibition (EC_{50}) of conidial germination was calculated.

Since strobilurin fungicides act by targeting the mitochondrial cytochrome b complex in fungi, mutations in the *cytb* gene that change the amino acid sequence of the cytochrome b have been shown to render these fungicides ineffective. Thus, the *cytb* region of 59 *C. beticola* isolates was also sequenced.

Quantifying fungicide efficacy for the control of CLS on table beet. A replicated small plot trial was conducted to quantify the efficacy of fungicides from various resistance groups (Table 1).

Table 1. Fungicides included in the trial to quantify efficacy for the control of Cercospora leaf spot in table beets at Geneva, NY in 2015.

Fungicide	Active ingredient	Rate/A	FRAC group	Rationale for inclusion
Quadris® F	Azoxystrobin	9 fl oz	11	Currently NY registered in table beet
Vertisan®	Penthiopyrad	16 fl oz	7	Registered for CLS control in sugar beet
Aprovia™ Top	solatenol + difenoconazole	13 oz	7 + 3	New 3rd generation group 7
Inspire Super®	cyprodinil + difenoconazole	7 fl oz	9 + 3	New fungicide combination
Cannonball® WP	fludioxonil	7 oz	12	Different resistance group
Manzate®	Mancozeb	3 lb	M3	Multi-site; registered on sugar beet
Omega® 500F	Fluazinam	0.85 pt	29	Different resistance group

The trial was planted at the NYSAES, Geneva, NY, on May 22 (var. 'Ruby Queen'), in a completely randomized block design with 5 replicates for each treatment; and 9 non-treated controls. The trial was inoculated twice with a mycelial suspension containing eight *C. beticola* isolates that were representative of the populations found in table beet fields in NY. Five isolates included in the inoculum were resistant to strobilurins, with EC_{50} values of 0.8 to 5.8 µg/ml. The effect of fungicides on disease severity and dry weight of foliage was analyzed.

RESULTS

Genetic diversity and potential for sexual reproduction of the *C. beticola* populations. Both fields showed high diversity, equal ratio of mating types, and linkage equilibrium of microsatellite loci. Taken together, this information suggested the presence of sexual reproduction in the *C. beticola* populations.

Q_OI resistance in *C. beticola* populations. EC_{50} values of the *C. beticola* isolates ranged from 0.003 to 19.397 µg/ml, with 54 isolates (41%) having EC_{50} values > 0.2 µg/ml; and 77 isolates (59%) having EC_{50} values ≤ 0.1 µg/ml. Sequencing the *cytb* in 59 isolates revealed that isolates with EC_{50} values ≤ 0.1 µg/ml carried no mutations. All isolates with EC_{50} ≥ 0.2 µg/ml contained single nucleotide mutations known to be associated with strobilurin resistance.

Quantifying the efficacy of fungicides for the control of CLS on table beet. Prior to fungicide application, plant density and disease severity were not significantly different across the entire trial area (Table 2). Disease severity assessment on September 3 showed the most efficacious treatments were Aprovia™ Top, Quadris®, Manzate®, and Inspire Super®. Application of Vertisan® also significantly reduced the disease severity compared to the nontreated plots but disease severity was significantly higher than all other fungicides. All fungicides had a significant effect on the dry weight of foliage a harvest (September 3) but only Aprovia™ Top and Quadris® increased the dry weight of foliage upon regrowth on October 15 (Table 2).

Table 2. Effect of fungicides on Cercospora leaf spot disease severity and dry weight of foliage in table beet, in a replicated small plot trial at Geneva, NY in 2015.

Fungicide	Plant density (/m)	Average lesion number/leaf		Dry weight foliage/m	
	25 Jun[b]	10 Aug[b]	3 Sept	3 Sept	15 Oct
Quadris® F	34.0	3.6	27.6 e	1027 ab	371 a
Vertisan®	24.8	4.0	152.5 b	780 bc	233 b
Aprovia™ Top	32.4	3.9	18.0 e	1252 a	465 a
Inspire Super®	34.0	3.5	23.4 e	1074 ab	251 b
Cannonball® WP	32.0	2.8	111.6 c	933 ab	235 b
Manzate®	35.0	3.8	55.7 de	791 bc	193 b
Omega® 500F	34.0	4.5	65.9 d	879 bc	255 b
Nontreated	31.4	4.6	232.1 a	548 c	193 b
LSD[a]	-	-	38.1	339.4	115.7
P =	0.226 (ns)	0.86 (ns)	<0.001	0.009	<0.001

[a]LSD = Least significant difference (*P* = 0.05); [b] Prior to fungicide application

DISCUSSION

The high genetic diversity, linkage equilibrium of microsatellite loci, and equal ratios of mating types indicated the probable presence of sexual recombination in the two *C. beticola* populations. This suggested a high potential of the population to evolve and develop resistance to selection pressures, such as fungicides. Resistance to azoxystrobin was found to be prevalent and in 41% of the *C. beticola* isolates sampled from the two processing table beet fields. However, despite the use of strobilurin-resistant *C. beticola* isolates in the inoculum for the small plot replicated field trial one of the most efficacious fungicides was Quadris® (azoxystrobin). This suggests the presence of the strobilurin-resistance mutation within the *cytb* gene may also incur a fitness cost resulting in reduced virulence and/or aggressiveness. Other efficacious fungicides identified in the small plot replicated trial were Aprovia™ Top, Inspire Super®, and Manzate®. Aprovia™ Top contains a new group 7 fungicide called solatenol and difenoconazole (FRAC group 3). Inspire Super® contains the fungicides, difenoconazole and cyprodinil (FRAC group 9). The poor disease control from application of Vertisan® raises concern that the local *C. beticola* isolates may have reduced sensitivity to the group 7 fungicides. It is therefore a high priority to determine whether the solatenol or difenoconazole is providing the effective disease control from the Aprovia™ Top formulation. Cannonball® and Omega® showed moderate disease control and may provide valuable opportunities to rotate with members of different FRAC groups in future programs.

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