Alternative Hosts of *Cercospora beticola* in Field Surveys and Inoculation Trials

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Abstract

Cercospora beticola, the cause of Cercospora leaf spot (CLS) of sugar beet and table beet, has a broad range of potential alternative hosts. The role of these hosts as inoculum sources in the field is unclear and has had limited investigation since the advent of DNA-based pathogen identification. The presence of *C. beticola* on alternative hosts associated with table beet fields of New York was assessed in field surveys during 2016. Lesions were collected, and 71 cercosporoid conidia were isolated for phylogenetic comparison. *C. beticola* was identified from *Solanum ptycanthum* ($n = 4$), *Chenopodium album* ($n = 2$), and *Spinacia oleracea* ($n = 1$), whereas

C. chenopodii was identified on *Chenopodium album* ($n = 51$). Artificial inoculation of 21 plants species demonstrated that *C. beticola* was pathogenic to *Brassica kaber*, *Chenopodium album*, *Carthamus tinctorius*, *Rumex obtusifolius*, and *Spinacia oleracea*. These results indicate that although *C. beticola* may be pathogenic to a range of plant species, the role of symptomatic tissue for inoculum production on alternative hosts in the field appears limited. Observations of *C. beticola* on necrotic and naturally senescent tissue suggest saprophytic survival on plant debris of a range of species, which has implications for CLS epidemics and disease management.

A comprehensive knowledge of potential sources of inoculum for epidemic initiation is vital for the design of effective disease management strategies. *Cercospora beticola* Sacc., the cause of the foliar disease Cercospora leaf spot (CLS) on sugar beet and table beet (*Beta vulgaris* subsp. *vulgaris* L.) (Weiland and Koch 2004), survives on alternative hosts and colonized plant debris, which are believed to be the primary sources of inoculum for CLS epidemics (Jacobsen and Franc 2009; Khan et al. 2008; McKay and Pool 1918; Pool and McKay 1916; Solel 1970). External sources of inoculum, such as infested beet seed, have also been suggested to harbor *C. beticola* inoculum (Knight et al. 2018; Vaghefi et al. 2017); however, direct evidence is limited (McKay and Pool 1918). The ability of *C. beticola* to colonize a broad range of alternative hosts plays an important role in the disease cycle of CLS. Potential alternative hosts for *C. beticola* include crop and weed species, such as sugar beet, table beet, fodder beet, Swiss chard (*B. vulgaris* subsp. *vulgaris*) (McKay and Pool 1918; Vestal 1933), *B. vulgaris* subsp. *maritima* (Jacobsen and Franc 2009), safflower (*Carthamus tinctorius*) (Lartey et al. 2005), lettuce (*Lactuca sativa*) (Hotezni Houessou et al. 2011), and spinach (*Spinacia oleracea*) (Vestal 1933), along with species in the genera *Acanthus*, *Amaranthus*, *Apium*, *Atriplex*, *Chenopodium*, *Chrysanthemum*, *Cycloloma*, *Goniolimon*, *Limonium*, *Malva*, and *Plantago* (Bobe et al. 2009; Groenewald et al. 2006; Jacobsen and Franc 2009; Rooney-Latham et al. 2011).

Investigations of the host range of *C. beticola* based on pathogenicity trials have yielded conflicting results (McKay and Pool 1918; Vestal 1933), whereas other studies have implied potential hosts based on isolation of *C. beticola* without pathogenicity assessment (Groenewald et al. 2006, 2013). For example, evaluations of the pathogenicity of *C. beticola* to lettuce have either reported 50% disease incidence (Vestal 1933) or described lettuce as a nonhost (McKay and Pool 1918). Additionally, pathogenicity of *C. beticola* on lambsquarters (*Chenopodium album*) was reported (Nagel 1932 as cited in Vestal 1933) but paradoxically declared a nonhost by others (McKay and Pool 1918; Schmidt 1928 as cited in Vestal 1933). Lambsquarters is a weed common to table beet fields in New York and is frequently found with CLS-like symptoms, leading to the assumption that it was a dominant source of inoculum contributing to CLS epidemics (Vaghefi et al. 2017). However, investigation of these symptoms on lambsquarters growing within table beet fields in New York indicated that *C. chenopodii* is the predominantly associated species (Vaghefi et al. 2017). This emphasizes the importance of comprehensive surveys of leaf spots associated with plants in table beet fields for investigating the prevalence and incidence of *C. beticola* on species other than *B. vulgaris*.

The foundational work on the host range of *C. beticola* (McKay and Pool 1918; Vestal 1933) was performed prior to the molecular characterization of *C. beticola* (Groenewald et al. 2005, 2006). Traditionally, morphological characteristics and host range were used to describe and identify *Cercospora* species (Chupp 1954); however, the identification of clades within the morphologically indistinguishable *C. apii* complex using DNA sequences of multiple phylogenetically informative loci has modified species boundaries and classifications (Bakhshi et al. 2015b; Cai et al. 2011; Crous et al. 2015; Groenewald et al. 2005). For example, phylogenetic assessments have suggested the separation of *C. beticola* from morphologically indistinguishable species such as *C. apii* and *C. apiicola* (Groenewald et al. 2005, 2006). Therefore, the accuracy of species identification in studies without DNA sequencing is uncertain, and investigation of the traditionally reported host range requires *C. beticola* isolates identified using DNA sequence-based techniques.

Understanding the host range of *C. beticola* is a priority for CLS disease management in table beet fields in New York. The survival of *C. beticola* on alternative hosts may contribute to the initiation of CLS epidemics in table beet fields and may affect the genetic diversity of *C. beticola* populations by disrupting genetic drift and selection pressures. The aim of this study was to determine the frequency of *C. beticola* associated with foliar disease of potential

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*The e-Xtra logo stands for “electronic extra” and indicates that four supplementary tables are published online.

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Table 1. *Cercospora* isolates collected in field surveys during 2016 in New York and reference isolates included for phylogenetic comparisons^a

Species	Isolate	Host	Source	GenBank accession number				
				<i>act</i>	<i>cmdA</i>	<i>hisH3</i>	ITS	<i>tefl-α</i>
<i>C. beticola</i>	CBS 116456; CPC 11557	<i>Beta vulgaris</i>	Italy	AY840458	AY840425	AY840392	AY840527	AY840494
<i>C. beticola</i>	CBS 117.47	<i>B. vulgaris</i>	Czech Republic	DQ233374	DQ233400	DQ233426	DQ233322	DQ233348
<i>C. beticola</i>	ICMP 21691; Tb14-081	<i>B. vulgaris</i> subsp. <i>vulgaris</i>	USA	MF680889	MF681401	MF680991	MF681093	MF681195
<i>C. beticola</i>	ICMP 21692; Tb14-085	<i>B. vulgaris</i> subsp. <i>vulgaris</i>	USA	MF680890	MF681402	MF680992	MF681094	MF681196
<i>C. beticola</i>	ICMP 21693; Tb14-140	<i>B. vulgaris</i> subsp. <i>vulgaris</i>	USA	MF680894	MF681406	MF680996	MF681098	MF681200
<i>C. beticola</i>	ICMP 21694; Tb15-259	<i>B. vulgaris</i> subsp. <i>vulgaris</i>	USA	MF680942	MF681454	MF681044	MF681146	MF681248
<i>C. beticola</i>	Lq16-006	<i>Chenopodium album</i>	Field 8, NY	MK272177	MK272269	MK271917	MK271993	MK272129
<i>C. beticola</i>	Lq16-010	<i>Chenopodium album</i>	Field 9, NY	MK272181	MK272273	MK271921	MK271997	MK272130
<i>C. beticola</i>	Ns16-001	<i>Solanum ptycanthum</i>	Field 3, NY	MK272225	MK272317	MK271963	MK272041	MK272133
<i>C. beticola</i>	Ns16-002	<i>Solanum ptycanthum</i>	Field 3, NY	MK272226	MK272318	MK271883	MK272042	MK272134
<i>C. beticola</i>	Ns16-005	<i>Solanum ptycanthum</i>	Field 3, NY	MK272229	MK272321	MK271964	MK272045	MK272137
<i>C. beticola</i>	Ns16-006	<i>Solanum ptycanthum</i>	Field 3, NY	MK272230	MK272322	MK271965	MK272046	MK272138
<i>C. beticola</i>	Sp16-004	<i>Spinacia oleracea</i>	Field 2, NY	MK272243	MK272335	MK271970	MK272059	MK272151
<i>C. cf. flagellaris</i>	CBS 132646; CPC 10681	<i>Cichorium intybus</i>	South Korea	JX143113	JX142867	JX142621	JX143601	JX143359
<i>C. cf. flagellaris</i>	CBS 132670; CPC 14487	<i>Sigesbeckia pubescens</i>	South Korea	JX143117	JX142871	JX142625	JX143605	JX143363
<i>C. cf. flagellaris</i>	ICMP 21686; Tb15-020	<i>B. vulgaris</i> subsp. <i>vulgaris</i>	USA	MF680899	MF681411	MF681001	MF681103	MF681205
<i>C. cf. flagellaris</i>	Ns16-003	<i>Solanum ptycanthum</i>	Field 3, NY	MK272227	MK272319	MK271884	MK272043	MK272135
<i>C. cf. flagellaris</i>	Ns16-004	<i>Solanum ptycanthum</i>	Field 3, NY	MK272228	MK272320	MK271885	MK272044	MK272136
<i>C. cf. flagellaris</i>	Pg16-001	<i>Amaranthus retroflexus</i>	Field 5, NY	MK272231	MK272323	MK271886	MK272047	MK272139
<i>C. cf. flagellaris</i>	Pg16-002	<i>A. retroflexus</i>	Field 5, NY	MK272232	MK272324	MK271887	MK272048	MK272140
<i>C. cf. flagellaris</i>	Pg16-003	<i>A. retroflexus</i>	Field 5, NY	MK272233	MK272325	MK271888	MK272049	MK272141
<i>C. cf. flagellaris</i>	Sp16-001	<i>Spinacia oleracea</i>	Field 2, NY	MK272240	MK272332	MK271891	MK272056	MK272148
<i>C. cf. flagellaris</i>	Sp16-002	<i>Spinacia oleracea</i>	Field 2, NY	MK272241	MK272333	MK271892	MK272057	MK272149
<i>C. cf. flagellaris</i>	Sp16-003	<i>Spinacia oleracea</i>	Field 2, NY	MK272242	MK272334	MK271893	MK272058	MK272150
<i>C. cf. flagellaris</i>	Sp16-005	<i>Spinacia oleracea</i>	Field 2, NY	MK272244	MK272336	MK271894	MK272060	MK272152
<i>C. cf. flagellaris</i>	Sp16-006	<i>Spinacia oleracea</i>	Field 2, NY	MK272245	MK272337	MK271895	MK272061	MK272153
<i>C. cf. flagellaris</i>	Sp16-007	<i>Spinacia oleracea</i>	Field 2, NY	MK272246	MK272338	MK271896	MK272062	MK272154
<i>C. chenopodii</i>	CBS 132620; CPC 14237	<i>Chenopodium album</i>	France	JX143081	JX142835	JX142589	JX143571	JX143327
<i>C. chenopodii</i>	CCTU 1033	<i>Chenopodium album</i>	Iran	KJ885954	KJ885793	KJ886115	KJ886437	KJ886276
<i>C. chenopodii</i>	CCTU 1060	<i>Chenopodium album</i>	Iran	KJ885955	KJ885794	KJ886116	KJ886438	KJ886277
<i>C. chenopodii</i>	CPC 15763	<i>Chenopodium</i> sp.	Mexico	JX143085	JX142839	JX142593	JX143575	JX143331
<i>C. chenopodii</i>	Lq16-001	<i>Chenopodium album</i>	Field 2, NY	MK272172	MK272264	MK271914	MK271988	MK272080
<i>C. chenopodii</i>	Lq16-002; ICMP 22619	<i>Chenopodium album</i>	Field 2, NY	MK272173	MK272265	MK271915	MK271989	MK272081
<i>C. chenopodii</i>	Lq16-003	<i>Chenopodium album</i>	Field 4, NY	MK272174	MK272266	MK271879	MK271990	MK272127
<i>C. chenopodii</i>	Lq16-004	<i>Chenopodium album</i>	Field 4, NY	MK272175	MK272267	MK271880	MK271991	MK272128
<i>C. chenopodii</i>	Lq16-005	<i>Chenopodium album</i>	Field 4, NY	MK272176	MK272268	MK271916	MK271992	MK272082
<i>C. chenopodii</i>	Lq16-007	<i>Chenopodium album</i>	Field 8, NY	MK272178	MK272270	MK271918	MK271994	MK272083
<i>C. chenopodii</i>	Lq16-008	<i>Chenopodium album</i>	Field 8, NY	MK272179	MK272271	MK271919	MK271995	MK272084
<i>C. chenopodii</i>	Lq16-009	<i>Chenopodium album</i>	Field 8, NY	MK272180	MK272272	MK271920	MK271996	MK272085
<i>C. chenopodii</i>	Lq16-011	<i>Chenopodium album</i>	Field 10, NY	MK272182	MK272274	MK271922	MK271998	MK272086
<i>C. chenopodii</i>	Lq16-012	<i>Chenopodium album</i>	Field 10, NY	MK272183	MK272275	MK271923	MK271999	MK272087
<i>C. chenopodii</i>	Lq16-013	<i>Chenopodium album</i>	Field 10, NY	MK272184	MK272276	MK271924	MK272000	MK272088
<i>C. chenopodii</i>	Lq16-014	<i>Chenopodium album</i>	Field 10, NY	MK272185	MK272277	MK271925	MK272001	MK272089
<i>C. chenopodii</i>	Lq16-015	<i>Chenopodium album</i>	Field 5, NY	MK272186	MK272278	MK271926	MK272002	MK272090
<i>C. chenopodii</i>	Lq16-016	<i>Chenopodium album</i>	Field 5, NY	MK272187	MK272279	MK271927	MK272003	MK272091
<i>C. chenopodii</i>	Lq16-017	<i>Chenopodium album</i>	Field 5, NY	MK272188	MK272280	MK271928	MK272004	MK272092
<i>C. chenopodii</i>	Lq16-018	<i>Chenopodium album</i>	Field 2, NY	MK272189	MK272281	MK271929	MK272005	MK272093
<i>C. chenopodii</i>	Lq16-019	<i>Chenopodium album</i>	Field 2, NY	MK272190	MK272282	MK271930	MK272006	MK272094
<i>C. chenopodii</i>	Lq16-020	<i>Chenopodium album</i>	Field 2, NY	MK272191	MK272283	MK271931	MK272007	MK272095
<i>C. chenopodii</i>	Lq16-021	<i>Chenopodium album</i>	Field 2, NY	MK272192	MK272284	MK271932	MK272008	MK272096
<i>C. chenopodii</i>	Lq16-022	<i>Chenopodium album</i>	Field 2, NY	MK272193	MK272285	MK271933	MK272009	MK272097
<i>C. chenopodii</i>	Lq16-024	<i>Chenopodium album</i>	Field 3, NY	MK272194	MK272286	MK271934	MK272010	MK272098
<i>C. chenopodii</i>	Lq16-025	<i>Chenopodium album</i>	Field 3, NY	MK272195	MK272287	MK271935	MK272011	MK272099
<i>C. chenopodii</i>	Lq16-027	<i>Chenopodium album</i>	Field 3, NY	MK272196	MK272288	MK271936	MK272012	MK272100
<i>C. chenopodii</i>	Lq16-028	<i>Chenopodium album</i>	Field 3, NY	MK272197	MK272289	MK271937	MK272013	MK272101
<i>C. chenopodii</i>	Lq16-029	<i>Chenopodium album</i>	Field 3, NY	MK272198	MK272290	MK271938	MK272014	MK272102
<i>C. chenopodii</i>	Lq16-030	<i>Chenopodium album</i>	Field 3, NY	MK272199	MK272291	MK271881	MK272015	MK272131
<i>C. chenopodii</i>	Lq16-031	<i>Chenopodium album</i>	Field 3, NY	MK272200	MK272292	MK271939	MK272016	MK272103
<i>C. chenopodii</i>	Lq16-032	<i>Chenopodium album</i>	Field 3, NY	MK272201	MK272293	MK271940	MK272017	MK272104
<i>C. chenopodii</i>	Lq16-033	<i>Chenopodium album</i>	Field 3, NY	MK272202	MK272294	MK271941	MK272018	MK272105
<i>C. chenopodii</i>	Lq16-034	<i>Chenopodium album</i>	Field 3, NY	MK272203	MK272295	MK271942	MK272019	MK272106
<i>C. chenopodii</i>	Lq16-035	<i>Chenopodium album</i>	Field 3, NY	MK272204	MK272296	MK271943	MK272020	MK272107
<i>C. chenopodii</i>	Lq16-036	<i>Chenopodium album</i>	Field 3, NY	MK272205	MK272297	MK271882	MK272021	MK272132

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^a Reference isolates are indicated in bold. *act* = actin, *cmdA* = calmodulin, *hisH3* = histone, ITS = internal transcribed spacer, and *tefl-α* = translation elongation factor 1-alpha.

alternative hosts in table beet fields of New York and to investigate the host range of *C. beticola* across selected crop and weed species using pathogenicity testing.

Materials and Methods

Field surveys and isolations. Leaves of weed and crop species with foliar symptoms as either CLS-like spots or necrotic lesions were sampled opportunistically from 10 fields during September 2016 (Supplementary Table S1). Fields included broad-acre and mixed-cropping systems that had grown table beet in 2014, 2015, or 2016 (Supplementary Table S2). Sampling of weed and crop species varied according to in-field frequency and the incidence and severity of disease.

Following sampling, all lesions on a plant were inspected for cercosporoid conidia under 63× magnification using a SZX10 stereo microscope (Olympus, Tokyo, Japan) with a KL 1600 LED light source (Schott, Elmsford, NY). Single-conidium cultures for individual lesions were produced using the method described by Knight et al. (2018), with the exception of lambsquarters. When numerous lesions were present on diseased lambsquarters, 10 to 20 lesions were randomly selected for morphological observation of conidia. Conidia exhibiting morphology similar to *C. beticola* (Jacobsen and Franc 2009) were transferred to 2% water agar (20 g/liter; Hardy Diagnostics, Santa Maria, CA).

DNA extraction, PCR, and sequencing. Mycelial growth and DNA extraction were performed as described in Knight et al. (2018). DNA of isolates from field surveys (Table 1) was amplified using five primer pairs (Supplementary Table S3) previously described in phylogenetic studies of *Cercospora* species (Bakhshi et al. 2018; Groenewald et al. 2005, 2013; Vaghefi et al. 2018). The primer pairs amplified partial sequences of internal transcribed spacers of the nuclear ribosomal DNA including the 5.8S region (ITS), actin (*act*), calmodulin (*cmdA*), histone (*hisH3*), and translation elongation factor 1- α (*tef1- α*) genes. PCR was performed as described by Groenewald et al. (2005) using a T100 Thermal Cycler (Bio-Rad, Hercules, CA). PCR products were visualized after electrophoresis on a 10 g/liter agarose gel in Tris-acetate-EDTA containing 0.001% (vol/vol) GelRed (Biotium Inc., Hayward, CA). Product size was determined against a 100-bp DNA ladder (Axygen Inc., Union City, CA). PCR products were subsequently treated with

exonuclease I and shrimp alkaline phosphatase (New England BioLabs, Ipswich, MA) and were Sanger sequenced at the Genomics Facility at the Cornell University Institute of Biotechnology (Ithaca, NY) with the forward and reverse primers used for the original PCR. Consensus sequences were obtained by pairwise alignment of forward and reverse sequences in Geneious version 11.1.5 (<https://www.geneious.com/>; Kearse et al. 2012). Sequences were deposited in the GenBank nucleotide database (Table 1).

Phylogenetic analyses. Sequences of reference isolates were obtained from the National Center for Biotechnology Information nucleotide database (Table 1) and aligned with DNA sequences of the field isolates. Multiple sequence alignments for each locus were conducted using MAFFT version 7.388 (Katoh and Standley 2013; Katoh et al. 2002) in Geneious. Sequences of the five loci were subsequently concatenated as “*act + cmdA + hisH3 + ITS + tef1- α* ” and phylogenetic trees were generated using the approximately maximum likelihood method of FastTree version 2.1.5 (Price et al. 2009, 2010) in Geneious. Node support values were computed using the Shimodaira-Hasegawa test (Shimodaira and Hasegawa 1999) in the FastTree plug-in in Geneious. Alignments and phylogenetic trees were deposited in TreeBASE (<https://www.treebase.org/> and <http://purl.org/phylo/treebase/phyloids/study/TB2:S23738>).

***C. beticola* isolates and inoculum preparation.** Two isolates of *C. beticola* (Tb14-081 [ICMP:21691] and Tb14-085 [ICMP:21692]) from CLS of table beet in New York, representing two clades within *C. beticola* (Vaghefi et al. 2018), were selected for inoculation experiments. Inoculum was prepared according to Secor and Rivera (2012) with slight modifications. Briefly, 10 ml of sterile distilled water was added to a 2-week-old culture grown on potato dextrose agar (Hardy Diagnostics), and mycelium was disrupted from the surface using the edge of a microscope slide. Three-hundred microliters of the mycelial suspension was spread onto each of 20 clarified V8 agar plates (10% [vol/vol] clarified V8 broth, 20 g/liter agar) (Miller 1955; Secor and Rivera 2012). The plates were subsequently dried for 30 min in a laminar flow cabinet, sealed with Parafilm (Bemis Co., Neenah, WI), and incubated for 6 days with an 8-h photoperiod. Conidia were collected by flooding plates with 5 ml of sterile distilled water and disrupting the surface with a glass spreader before filtering through two layers of cheesecloth. The concentration of the conidial suspension was quantified using

Table 1. (Continued from previous page)

Species	Isolate	Host	Source	GenBank accession number				
				<i>act</i>	<i>cmdA</i>	<i>hisH3</i>	ITS	<i>tef1-α</i>
<i>C. chenopodii</i>	Lq16-037	<i>Chenopodium album</i>	Field 3, NY	MK272206	MK272298	MK271944	MK272022	MK272108
<i>C. chenopodii</i>	Lq16-038	<i>Chenopodium album</i>	Field 3, NY	MK272207	MK272299	MK271945	MK272023	MK272109
<i>C. chenopodii</i>	Lq16-039	<i>Chenopodium album</i>	Field 7, NY	MK272208	MK272300	MK271946	MK272024	MK272110
<i>C. chenopodii</i>	Lq16-042	<i>Chenopodium album</i>	Field 7, NY	MK272209	MK272301	MK271947	MK272025	MK272111
<i>C. chenopodii</i>	Lq16-043	<i>Chenopodium album</i>	Field 7, NY	MK272210	MK272302	MK271948	MK272026	MK272112
<i>C. chenopodii</i>	Lq16-044	<i>Chenopodium album</i>	Field 7, NY	MK272211	MK272303	MK271949	MK272027	MK272113
<i>C. chenopodii</i>	Lq16-045	<i>Chenopodium album</i>	Field 7, NY	MK272212	MK272304	MK271950	MK272028	MK272114
<i>C. chenopodii</i>	Lq16-046	<i>Chenopodium album</i>	Field 7, NY	MK272213	MK272305	MK271951	MK272029	MK272115
<i>C. chenopodii</i>	Lq16-047	<i>Chenopodium album</i>	Field 7, NY	MK272214	MK272306	MK271952	MK272030	MK272116
<i>C. chenopodii</i>	Lq16-048	<i>Chenopodium album</i>	Field 7, NY	MK272215	MK272307	MK271953	MK272031	MK272117
<i>C. chenopodii</i>	Lq16-049	<i>Chenopodium album</i>	Field 7, NY	MK272216	MK272308	MK271954	MK272032	MK272118
<i>C. chenopodii</i>	Lq16-050	<i>Chenopodium album</i>	Field 7, NY	MK272217	MK272309	MK271955	MK272033	MK272119
<i>C. chenopodii</i>	Lq16-051	<i>Chenopodium album</i>	Field 7, NY	MK272218	MK272310	MK271956	MK272034	MK272120
<i>C. chenopodii</i>	Lq16-052	<i>Chenopodium album</i>	Field 7, NY	MK272219	MK272311	MK271957	MK272035	MK272121
<i>C. chenopodii</i>	Lq16-053	<i>Chenopodium album</i>	Field 7, NY	MK272220	MK272312	MK271958	MK272036	MK272122
<i>C. chenopodii</i>	Lq16-054	<i>Chenopodium album</i>	Field 7, NY	MK272221	MK272313	MK271959	MK272037	MK272123
<i>C. chenopodii</i>	Lq16-055	<i>Chenopodium album</i>	Field 7, NY	MK272222	MK272314	MK271960	MK272038	MK272124
<i>C. chenopodii</i>	Lq16-056	<i>Chenopodium album</i>	Field 7, NY	MK272223	MK272315	MK271961	MK272039	MK272125
<i>C. chenopodii</i>	Lq16-057	<i>Chenopodium album</i>	Field 7, NY	MK272224	MK272316	MK271962	MK272040	MK272126
<i>Cercospora</i> sp. G	CPC 5438	<i>Salvia viscosa</i>	New Zealand	JX143196	JX142950	JX142704	JX143682	JX143442
<i>Cercospora</i> sp. G	ICMP 21688; Tb15-004	<i>B. vulgaris</i> subsp. <i>vulgaris</i>	USA	MF680897	MF681409	MF680999	MF681101	MF681203
<i>Cercospora</i> sp. G	Pg16-004	<i>A. retroflexus</i>	Field 7, NY	MK272234	MK272326	MK271889	MK272050	MK272142
<i>Cercospora</i> sp. G	Pg16-005	<i>A. retroflexus</i>	Field 7, NY	MK272235	MK272327	MK271890	MK272051	MK272143
<i>C. zeae-maydis</i>	CBS 117756; A359	<i>Zea mays</i>	USA	DQ185097	DQ185109	DQ185121	DQ185073	DQ185085

a hemocytometer (Hausser Scientific, Horsham, PA) and diluted to 10^5 conidia/ml. Polysorbate-20 (as 0.01% [vol/vol] Tween-20; Agdia, Elkhart, IN) was added to the suspension. The viability of conidia was assessed by spreading 100 μ l of the suspension onto 2% water agar (Hardy Diagnostics) and counting germinated conidia ($n = 50$) at 24 h. Conidia were considered germinated when the length of the germ tube was at least the length of the conidium.

Plant species, growth conditions, and inoculation. Eight- to 10-week-old plants of 21 species (Table 2) were inoculated with each of the *C. beticola* isolates. Seeds of each species (Supplementary Table S4) were planted at a depth of 1 cm in soil medium, SunshineMix No. 8 Fafard-2 (Sungro Horticulture, Agawam, MA), in round pots (15 cm diameter $\times$ 13 cm depth). Glasshouse conditions were

maintained at 21°C/15°C for 14-h/10-h light/dark photoperiod cycles. Germination of eastern black nightshade (*Solanum ptycanthum*) and dock weed (*Rumex obtusifolius*) seeds was induced as described by Zhou et al. (2005). Briefly, seeds were soaked in distilled water at 20°C in the dark for 24 h, then placed on filter paper in a Petri plate moistened with 2 ml of distilled water. Plates were incubated under a 14-h/10-h light/dark photoperiod, with 30°C/25°C alternating temperatures, until germination (approximately 7 days). Germinated seeds were placed in the soil medium at a depth of 1 cm and then placed in the glasshouse.

Three inoculation treatments were applied in each trial, including *C. beticola* isolates Tb14-081 and Tb14-085 and a negative control solution (0.01% [vol/vol] polysorbate-20). Treatments were applied

Table 2. Disease incidence, symptom type, and reisolation frequencies following inoculation of 21 plant species with two isolates of *Cercospora beticola* from *Cercospora* leaf spot in table beet

Common name	Species	Cultivar ^a	Isolate	Lesions (%) ^b	Marginal necrosis (%) ^c	Symptomless (%) ^d	Reisolation (%) ^e
Broadleaf plantain	<i>Plantago major</i>	–	Tb14-081	10	40	60	40
			Tb14-085	20	80	10	90
Buckwheat	<i>Fagopyrum esculentum</i>	–	Tb14-081	0	100	0	80
			Tb14-085	0	100	0	70
Carrot	<i>Daucus carota</i> subsp. <i>sativus</i>	Carson	Tb14-081	0	50	50	60
			Tb14-085	0	60	40	60
Celery	<i>Apium graveolens</i> var. <i>secalinum</i>	Cutting	Tb14-081	0	0	100	0
			Tb14-085	0	0	100	0
Dock weed	<i>Rumex obtusifolius</i>	–	Tb14-081	100	0	0	90
			Tb14-085	100	0	0	90
Eastern black nightshade	<i>Solanum ptycanthum</i>	–	Tb14-081	30	40	30	60
			Tb14-085	0	60	40	80
Lambsquarters	<i>Chenopodium album</i>	–	Tb14-081	100	0	0	100
			Tb14-085	100	0	0	100
Lettuce	<i>Lactuca sativa</i>	Great Lakes	Tb14-081	0	100	0	80
			Tb14-085	0	100	0	90
Lucerne	<i>Medicago sativa</i>	Summer	Tb14-081	0	30	70	30
			Tb14-085	0	20	80	20
Pea	<i>Pisum sativum</i>	Bolero	Tb14-081	0	0	100	0
			Tb14-085	0	0	100	0
Pigweed	<i>Amaranthus retroflexus</i>	–	Tb14-081	10	0	90	10
			Tb14-085	0	0	100	0
Ragweed	<i>Ambrosia artemisiifolia</i>	–	Tb14-081	0	0	100	0
			Tb14-085	0	0	100	0
Rye	<i>Secale cereale</i>	Aroostook	Tb14-081	0	0	100	0
			Tb14-085	0	20	30	20
Safflower	<i>Carthamus tinctorius</i>	Centennial	Tb14-081	100	0	0	100
			Tb14-085	100	0	0	100
Snap bean	<i>Phaseolus vulgaris</i>	Huntington	Tb14-081	0	60	40	50
			Tb14-085	20	60	20	70
Spinach	<i>Spinacia oleracea</i>	Bloomsdale	Tb14-081	100	0	0	100
			Tb14-085	100	0	0	100
Sweet potato	<i>Ipomoea batatas</i>	Beauregard	Tb14-081	0	0	100	0
			Tb14-085	0	0	100	0
Velvetleaf	<i>Abutilon theophrasti</i>	–	Tb14-081	0	0	100	0
			Tb14-085	0	0	100	0
Watermelon	<i>Citrullus vulgaris</i> var. <i>lanatus</i>	Crimson Sweet	Tb14-081	0	100	0	70
			Tb14-085	0	100	0	100
Wild mustard	<i>Brassica kaber</i>	–	Tb14-081	100	100	0	60
			Tb14-085	100	100	0	70
Yellow nutsedge	<i>Cyperus esculentus</i>	–	Tb14-081	0	0	100	0
			Tb14-085	0	0	100	0

^a Dashes indicate species that did not have cultivars.

^b Lesions indicate the presence of circular-shaped necrotic tissue after inoculation with *C. beticola*. The percentage incidence of lesions was calculated by dividing the total number of symptomatic plants by the total number of plants, multiplied by 100.

^c Marginal necrosis indicates the presence of necrotic leaf edges after inoculation with *C. beticola*. The percentage incidence of marginal necrosis was calculated by dividing the total number of symptomatic plants by the total number of plants, multiplied by 100.

^d Symptomless indicates the absence of lesions or necrotic leaf edges after inoculation with *C. beticola*. The percentage incidence of symptomless plants was calculated by dividing the total number of symptomless plants by the total number of plants, multiplied by 100.

^e Reisolation indicates the observation of stromata and conidia with morphology resembling *C. beticola*. The reisolation frequency was calculated by dividing the number of plants with isolations resulting in *C. beticola* by the total number of plants, multiplied by 100.

to five plants of three to four species, along with five table beet plants (cultivar Ruby Queen) as positive controls for CLS. For each treatment, plants were arranged in a randomized block design on separate benches. Inoculation of each plant species and fungal isolate was replicated.

Prior to inoculation, each potted plant was placed in a transparent 32-liter plastic bag (ULINE, Pleasant Prairie, WI) in a growth chamber. The adaxial leaf surface of each plant was inoculated with approximately 12 ml of inoculum using a 355-ml TS-12 trigger pump sprayer (Sprayco, Livonia, MI) and was sealed in the plastic bag. After 48 h, the plastic bags were removed, and a misting apparatus was turned on for 4 h on 5 days each week for 4 weeks. Growth chamber conditions were 25°C with 16 h of light at 820 lux (110 W fluorescent mercury bulbs; Philips, Andover, MA) and a mist water temperature of 22.7°C. Relative humidity was maintained at >90% for 4 h per day.

***C. beticola* pathogenicity.** After inoculation, plants were examined for disease symptoms every second day for 4 weeks. Symptomatic tissues were collected, evaluated under 63× magnification using a SZX10 stereo microscope (Olympus) with a KL 1600 LED light source (Schott), and incubated for 24 h in a plastic box containing moist paper. When cercosporoid conidia were observed, 20 µl of sterile distilled water was pipetted onto the symptomatic tissue, conidia were dislodged with the pipette tip, and the suspension was transferred to a microscope slide (VWR, Radnor, PA) for examination under 200 to 400× magnification using a BX53 compound microscope (Olympus). Leaf tissues with conidia resembling the expected morphology and dimensions of *C. beticola* were considered a positive reisolation. A maximum of five isolations per plant were conducted. The disease incidence (number of symptomatic plants divided by total number of plants × 100) and reisolation frequency (number of plants with isolations resulting in *C. beticola* divided by total number of plants × 100) were calculated for each plant species and *C. beticola* isolate. Images of symptomatic leaf tissues were taken using a Nexus 5X (LGE, Seoul, South Korea) or iPhone 6 (Apple, Cupertino, CA) camera.

***C. chenopodii* pathogenicity to table beet.** Two isolates of *C. chenopodii*, Lq15-006 and Lq16-002, were selected for pathogenicity tests on table beet (cultivar Ruby Queen). Inoculum production, inoculation, and growth conditions were as described above. Treatments in the replicated experiment included Lq15-006, Lq16-002, a *C. beticola* positive control (isolate Tb14-085), and a negative control solution (0.01% [vol/vol] polysorbate-20). Each treatment contained five table beet plants, which were examined daily for symptom development over 4 weeks.

Results

Field surveys. Samples were selected from 10 symptomatic plant species for fungal isolations. Seventy-one cercosporoid isolates were morphologically identified from lesions collected from four plant species (Table 3). The majority of isolates were collected from leaf spots on lambsquarters (75%), with the remainder isolated from eastern black nightshade (8%), pigweed (*Amaranthus retroflexus*) (7%), and spinach (10%).

Phylogenetic species identification. The phylogenetic tree constructed using the aligned concatenation of five loci (*act* [186 bp] + *cmdA* [216 bp] + *hisH3* [321 bp] + ITS [454 bp] + *tef1-α* [281 bp]) identified seven isolates as *C. beticola* (Fig. 1). These *C. beticola* isolates originated from eastern black nightshade (*n* = 4), lambsquarters (*n* = 2), and spinach (*n* = 1). Fifty-one isolates clustered with *C. chenopodii*, and all originated from lambsquarters. *C. chenopodii* consisted of two separate clusters, consisting of four and 47 isolates, respectively. The remaining isolates clustered with *C. cf. flagellaris* (*n* = 11) and *Cercospora* sp. G (*n* = 2) (Groenewald et al. 2013).

***C. beticola* pathogenicity assessment.** During each experiment, all table beet plants inoculated with either *C. beticola* isolate developed CLS symptoms (Fig. 2), and the reisolation frequency of cercosporoid conidia was 100%. Five of the 21 plant species inoculated with *C. beticola* exhibited 100% incidence of leaf lesions and reisolation frequencies of 60 to 100% (Table 2). These consisted

of dock weed, lambsquarters, safflower, spinach, and wild mustard (*Brassica kaber*) (Fig. 2). Disease incidence ranged from 10 to 30% in broadleaf plantain (*Plantago major*), eastern black nightshade, pigweed, and snap bean (*Phaseolus vulgaris*) following inoculation with the two *C. beticola* isolates.

Nine plant species exhibited marginal leaf necrosis: namely, broadleaf plantain, buckwheat (*Fagopyrum esculentum*), carrot (*Daucus carota* subsp. *sativus*), eastern black nightshade, lettuce, lucerne (*Medicago sativa*), rye (*Secale cereale*), snap bean, and watermelon (*Citrullus vulgaris* var. *lanatus*). This symptom was also observed on control plants and therefore the pathogenicity of *C. beticola* to these species was discounted. However, reisolation from *C. beticola* inoculated plants indicated that cercosporoid conidia were present on the necrotic tissue at frequencies of 20 to 100%. Stromata were also observed on leaf lesions and marginal leaf necroses.

Celery (*Apium graveolens* var. *secalinum*), pea (*Pisum sativum*), ragweed (*Ambrosia artemisiifolia*), sweet potato (*Ipomoea batatas*), velvetleaf (*Abutilon theophrasti*), and yellow nutsedge (*Cyperus esculentus*) did not exhibit disease symptoms after inoculation with either *C. beticola* isolate.

Natural senescence of leaves (distinct from lesion or marginal necrosis) occurred for both inoculated and control treatments over the 4-week period following inoculation. Cercosporoid conidia and stromata were observed on the naturally senescing tissues of each asymptomatic plant species inoculated with *C. beticola*. No sporulation was observed on necrotic or naturally senescent tissue of control plants.

***C. chenopodii* pathogenicity to table beet.** Inoculation of table beet with *C. chenopodii* isolates Lq15-006 and Lq16-002 failed to result in disease, whereas inoculation with *C. beticola* isolate Tb14-085 induced typical CLS symptoms. Inoculum of each *C. chenopodii* isolate had >90% viability on water agar.

Discussion

This study utilized two fungal isolates, identified as *C. beticola* using five loci (Vaghefi et al. 2018), and pathogenicity testing on plant species common to table beet fields in New York to re-evaluate host range. *C. beticola* was pathogenic to five of the 21 plant species tested. Although artificial inoculations provided indications of the pathogenicity of *C. beticola*, information on occurrence and persistence in natural field environments is required to determine the relative contribution of these alternative hosts to CLS epidemics. Field surveys of leaf lesions occurring on plants other than *B. vulgaris* subsp. *vulgaris* in table beet fields resulted in the identification of seven *C. beticola* isolates from lambsquarters, eastern black nightshade, and spinach. The majority of isolates (72%) were identified as *C. chenopodii* on lambsquarters. These findings imply that *C. beticola* does not frequently cause lesions on weeds in table beet fields and challenge the concept that symptomatic weeds are important sources of inoculum for CLS epidemics.

C. beticola was pathogenic on dock weed, lambsquarters, safflower, spinach, and wild mustard following artificial inoculation in a controlled environment. These findings were supported by the isolation of *C. beticola* from lambsquarters and spinach in the field, albeit at low frequencies. A low incidence of pathogenicity to celery, lettuce, sweet potato, watermelon, and broadleaf plantain is in contrast to the results of Vestal (1933); however, direct comparisons are challenging because of the different origins of isolates and flux in *C. beticola* taxonomy. This study also provides the first report of *C. beticola* being pathogenic to dock weed and wild mustard.

Table 3. Plant species from table beet fields in 2016 exhibiting leaf lesions and the number of lesions yielding cercosporoid conidia

Common name	Species	Symptomatic plants sampled	Cercosporoid isolations
Eastern black nightshade	<i>Solanum ptycanthum</i>	6	6
Lambsquarters	<i>Chenopodium album</i>	120	53
Pigweed	<i>Amaranthus palmeri</i>	144	5
Spinach	<i>Spinacea oleracea</i>	7	7

These two weed species were introduced to the United States and are reported to be widespread across the northern states, including New York (U.S. Department of Agriculture 2018). To date, there have been no reports of *C. beticola* being isolated from lesions on these plants in the field; however, targeted surveys are required to investigate their occurrence. The isolation of *C. beticola* from eastern black

nightshade in the field, along with low disease incidence after artificial inoculation, also suggests that this species could harbor *C. beticola* inoculum.

The pathogenicity of *C. beticola* has been similarly reported on lambsquarters (Vestal 1933), safflower (Lartey et al. 2005), and spinach (Vestal 1933) in artificial inoculation trials; however the role of

Fig. 1. Phylogenetic tree constructed using an approximately maximum likelihood analysis of the concatenated alignment of five loci (partial sequences of actin, calmodulin, histone, and translation elongation factor 1- α and the internal transcribed spacer region). Four species of *Cercospora* were identified, including *C. beticola*, *C. chenopodii*, *C. cf. flagellaris*, and *Cercospora* sp. G. The tree was rooted to *C. zeae-maydis* (CBS 117756). Node support values were computed using the Shimodaira-Hasegawa test in the FastTree version 2.1.5 plug-in in Geneious version 11.1.5. The scale bar represents the number of estimated changes per position.

these plants in the CLS disease cycle is not well understood. For example, although safflower exhibits lesions during artificial *C. beticola* inoculation trials, the fungus has yet to be isolated from this host in the field (Groenewald et al. 2006). In contrast, lambsquarters is commonly found growing within table beet fields of New York and frequently exhibits lesions during CLS epidemics on table beets (U.S. Department of Agriculture 2018; Vaghefi et al. 2017). However, the identification of predominantly *C. chenopodii* from lesions on lambsquarters, in the current study and by Vaghefi et al. (2017), conflicts with the conventional understanding that *C. beticola* causes the leaf spots on both table beet and lambsquarters (Jacobsen and Franc 2009). *C. chenopodii* has been similarly isolated from lambsquarters in Iran (Bakhshi et al. 2015a) and is considered as a fungal bioherbicide for lambsquarters (Scheepens and van Zon 1982). *C. chenopodii* isolated from lambsquarters was not pathogenic on table beet in artificial inoculation trials. These findings suggest that CLS

on lambsquarters may occur in the same field as CLS of table beet but is initiated by different *Cercospora* spp.

The identification of *C. cf. flagellaris* and *Cercospora* sp. G from lesions on eastern black nightshade, pigweed, and spinach further demonstrates the challenge of identifying *C. beticola*-associated lesions on alternative hosts in the field. Vaghefi et al. (2018) investigated the pathogenicity of *C. apii*, *C. cf. flagellaris*, *Cercospora* sp. G, and *C. zebrina* on table beet. *C. apii* caused typical CLS symptoms, *C. cf. flagellaris* caused infrequent disease or was not pathogenic, and *Cercospora* sp. G and *C. zebrina* were also not pathogenic. More surveys of lesions in table beet-associated fields, during both table beet production and rotations, are required to elucidate the role of alternative hosts in CLS epidemics.

Infrequent isolation of *C. beticola* from lesions on lambsquarters, eastern black nightshade, and spinach in the field may indicate that *C. beticola* does not readily cause lesions on nonhost species. However,

Fig. 2. Lesion formation on **A**, table beet (*Beta vulgaris* subsp. *vulgaris* 'Ruby Queen'), **B**, lambsquarters (*Chenopodium album*), **C**, spinach (*Spinacia oleracea* 'Bloomsdale'), **D**, wild mustard (*Brassica kaber*), **E**, safflower (*Carthamus tinctorius* 'Centennial'), and **F**, dock weed (*Rumex obtusifolius*) after inoculation with *Cercospora beticola*.

a lack of lesions may not necessarily indicate the absence of *C. beticola*. During artificial inoculation experiments, *C. beticola* conidia and conidiophores were reisolated from necrotic and naturally senescing leaf tissues of several plant species that did not exhibit necrotic spots. Furthermore, a low incidence (10 to 30%) of disease resulted in broadleaf plantain, eastern black nightshade, pigweed, and snap bean after artificial inoculation. Although *C. beticola* may have been weakly pathogenic on these species, it is also possible that other factors (e.g., insects, fungi, or physiological reactions) may have played a role in the lesion formation, with *C. beticola* subsequently colonizing the senescing tissue. This finding suggests that, under favorable environmental conditions such as high humidity and temperatures near 25°C, *C. beticola* may be able to survive saprophytically. Vestal (1933) proposed that *C. beticola* may live as a saprophyte after finding growth on dead leaves of plants other than sugar beet, including dandelion, lettuce, and potato. The survival of *C. beticola* on plant debris for 10 to 22 months (Khan et al. 2008) supports the important role of a saprophytic lifestyle for *C. beticola*.

The concept of plant pathogenic fungi surviving on nonhost plants was also proposed by Crous and Groenewald (2005). They outlined the “pogo stick hypothesis,” which states that “host-specific fungal plant pathogens frequently exhibit the ability to colonize nonhost tissue or other substrates, forming fruiting bodies that will produce a limited amount of propagules, and enabling them to disperse further, in an attempt to find the host on which they are pathogenic” (Crous and Groenewald 2005). The potential for *C. beticola* to colonize senescing tissue of nonhost plants in the field, including lesions caused by other pathogens, needs to be considered. The methods of fungal isolation in this study were limited by the requirement for lesions with sporulating fungi. Thus, when a mixture of sporulating fungi was present on a lesion or *C. beticola* was not sporulating but present, *C. beticola* may not have been identified. Detection of *C. beticola* in a range of biological substrates, including alternative hosts, would benefit from improved species-specific detection tools such as quantitative PCR.

Informative host range studies require the detection and isolation of pathogens from field samples along with controlled inoculation experiments, with the selection of plant species for inoculation guided by field results. The infrequent isolation of *C. beticola* from lesions of nonhost plants in the field limited the objective selection of plants species for artificial inoculations. However, lesion formation on five plant species, along with *C. beticola* sporulation on senescing tissues of nonhosts, indicates that a broad range of substrates may play a role in *C. beticola* survival in a field environment. The potential involvement of multiple inoculum substrates in the field would explain the continued challenge of CLS control using the recommended 3-year rotation (Pethybridge et al. 2018).

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