Direct Polymerase Chain Reaction-Based Detection of *Cercospora beticola* in Field Soils

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ABSTRACT

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Cercospora beticola, the causal agent of Cercospora leaf spot of sugar beet, survives as pseudostromata in infected sugar beet residues in the soil. Under optimal conditions, overwintering propagules germinate and produce conidia that are dispersed as primary inoculum to initiate infection in sugar beet. We developed a polymerase chain reaction (PCR) technique for rapid detection of *C. beticola* in field soils. Total DNA was first isolated from soil amended with *C. beticola* culture using the PowerSoil DNA Kit. The purified DNA was subjected to PCR in Extract-N-Amp PCR mix with CBACTIN primers over 35 cycles. The amplified products were resolved and compared by electrophoresis in 1% agarose gels. The PCR fragment size of *C. beticola* from the amended field soil correlated in size with the amplicon from control *C. beticola* culture DNA extract. Additionally, sample soils were collected from sugar beet fields near Sidney, MT and Foxholm, ND. Total DNA was extracted from the samples and subjected to PCR and resolved as previously described. The amplicons were purified from the gels and subjected to BigDye Terminator Cycle sequencing. All sequences from field soils samples, *C. beticola*-amended field soil, and pure culture were compared by alignment with a *C. beticola* actin gene sequence from GenBank. The result of the alignment confirmed the amplicons as products from *C. beticola*. Rapid screening for the presence of *C. beticola* in the soil by PCR will improve research capabilities in biological control, disease forecasting, and management of this very important sugar beet pathogen.

Cercospora beticola Sacc. causes leaf spot diseases of sugar beet (*Beta vulgaris* L.) (2,19,21), related species in the family Chenopodiaceae (6), and, in recent reports, on safflower (*Carthamus tinctorius* L.) (11,12) and German statice (*Goniolimon tataricum*) (3), belonging to plant families outside of the Chenopodiaceae family. Cercospora leaf spot (CLS) is the most important foliar disease of sugar beet (17). Bleiholder and Welzien (1) indicated that the disease occurs wherever sugar beet is grown. According to Weiland and Koch (19), beet crops in warm, humid growing regions are most acutely affected by CLS and these constitute greater than 30% of the area under sugar beet cultivation. *Cercospora beticola* can survive in soil for at least 20 months (16) and recent evidence by Khan et al. (10) indicates survival of up to at least 22 months. They (10) demon-

strated further that *C. beticola* survives for over 22 months at a depth of 10 cm from the soil surface but for only about 10 months at a depth of 20 cm from the soil surface. Wherever the crop is grown, prevailing weather appears to play a major role in the incidence of CLS. Indeed, according to Windels et al. (21), severity of CLS varies from year to year, depending upon weather conditions and the effectiveness of disease management practices.

The primary inoculum of *C. beticola* in a sugar beet field begins as conidia and pseudostromata in infested plant debris where the pathogen overwinters (9,21). Under favorable conditions, which are characterized by temperature between 20 and 26°C and heavy dew or relatively humidity above 98%, conidiophores and conidia are produced on the pseudostromata (9,18). The current CLS infection model (15,17,21) indicates that conidiophores and conidia that serve as primary inoculum are dispersed by wind, irrigation, rain water, and insects to sugar beet to initiate primary infection. In this manner, the pathogen can be transmitted from the surviving inoculum reservoir to the subsequent crop during the following growing season (9).

Among the cultural practices tested to manage CLS, McKay and Pool (15), early

in the 20th century, recommended sugar beet fields be sown at least 100 m from fields planted to sugar beet the previous year to avoid spread of conidia from infested debris, a practice that is still recommended (21). It also has been suggested, among other measures, to include deep tillage to turn under residues and to use rotation with nonhost crops, with a minimum of 3 years between beet crops, because of survival of *C. beticola* in infested beet leaf residues in the soil (21).

In addition to cultural practices, applications of fungicides have played a major role in the control of CLS. However, an issue in the management of CLS is the well-documented occurrence of fungicide tolerance in *C. beticola* populations (7). As a consequence, the control of leaf spot disease necessitates the judicious rotation of fungicide with diverse modes of action as a means of preventing or delaying the development of resistant strains or reducing their prevalence in populations (19). In addition, forecasting models that predict the likelihood of *Cercospora* spp. infection and severe crop loss in conjunction with timely application of fungicides to manage outbreaks of the disease have become an important complement to genetic resistance in CLS management (5,20,22). Prediction models have contributed significantly toward improved management of CLS in recent years. However, these models lack information about the actual presence of the pathogen in the target environment even under weather conditions favoring infection, thus leading to a recommendation for application of a fungicide. Currently, a polymerase chain reaction (PCR) technique is available for direct detection and identification *C. beticola* in infected plant tissues (13) and, consequently, can enhance detection and identification of various hosts that may serve as potential sources of inoculum. In addition, an enzyme-linked immunosorbent assay (ELISA)-based protocol that targets *C. beticola* protein-antigen has been developed to enable direct detection of *C. beticola* in the soil (4). These two protocols can improve prediction of the incidence of CLS under weather conditions favorable to the disease. Together with scouting for lesions (8), their use could further complement current weather information to improve prediction models, thus enhancing

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the likelihood that fungicides will be applied judiciously in the management of CLS.

The objective of this research was to develop a PCR technique that is based on amplification of a unique segment of the genomic DNA to enable rapid detection of *C. beticola* in field soil. It could also serve as an alternate soil detection method to the ELISA technique (4) that could be applied to examine the incidence of the pathogen in the field under diverse conditions and provide additional information for sugar beet producers to make fungicide application decisions. This could also be used to examine the efficacy of chemical or biological agents to control the inoculum in a new target area, the soil, prior to planting of sugar beet and other host crops.

MATERIALS AND METHODS

Detection of *C. beticola* in amended field soil. The initial experiment was designed to detect *C. beticola* directly from soil without prior isolation or subculturing of the pathogen.

Samples. Samples consisted of field soil amended with a mycelial culture of *C. beticola* isolate S1 that was isolated by Anthony J. Caesar, United States Department of Agriculture–Agricultural Research Service (USDA-ARS) NPARRL, Sidney, MT, from infected sugar beet at Sidney, MT. For soil amendment, *C. beticola* was first cultured on potato dextrose agar (PDA) (Difco Laboratories, Detroit, MI). A single 0.6-cm mycelial plug from the culture was then transferred into 125 ml of potato dextrose broth (PDB) (Difco Laboratories) in Erlenmeyer flasks. The cultures were incubated on a Lab-Line Model 4628 Force Environmental Orbital Shaker (Barnstead/Thermolyne, Melrose Park, IL) at 120 rpm at 25°C for 1 week. The mycelia were harvested after 1 week by pouring through cheesecloth and air dried under a SteriGARD III (The Baker Co, Sanford, ME) laminar flow hood for 24 h. The dried mycelia were transferred into the grinding bowl of a SmartGrind CBG5 Coffee Bean Grinder (Black & Decker Corp., Towson, MD) and subjected to grinding for 10 s. Based on the reported relationship between field depth and longevity of *C. beticola* survival in soil (10), deep field soil was collected from a depth of 60 to 100 cm from a sugar beet field near Sidney, MT. For sampling, soils were collected from five different locations in the field within 10 m² using a hydraulic probe truck. The soils were combined and thoroughly mixed by hand shaking in sterile plastic bags. The soil was amended with the ground dried mycelial culture of *C. beticola* at 0.5 mg of mycelium per 400 g of air-dried soil. Soil and mycelia were thoroughly mixed by hand and used for the experiment.

Controls consisted of purified DNA from a pure culture of *C. beticola* isolate C2 (19) (provided by John J. Weiland,

USDA-ARS/RRVARC, Fargo, ND) and blank reaction mixture without DNA template. For the control culture, a single 0.6-cm mycelial disc of the *C. beticola* isolate C2 was transferred to the center of fresh PDA plates and incubated at 22°C for 3 weeks. Mycelial plugs (0.6 cm in diameter) were removed from the PDA cultures. The plugs were then rinsed in deionized water to remove excess agar, air dried under the SteriGARD III laminar flow hood for 24 h, and stored at 4°C. Other controls consisted of unamended deep field soil samples from Froid, MT which had never been previously planted with sugar beet. Froid field soil samples were collected from a depth of 60 to 100 cm as previously described for the amended Sidney soil.

DNA templates purification. Total DNA was extracted from soil samples using the PowerSoil DNA Isolation Kit (MO BIO Laboratories, Inc., Carlsbad, CA) with minor modification of the manufacturer's instructions. For DNA purification, 0.25 g of each test soil sample was transferred to manufacturer-provided PowerBead Tubes. Lysis solution C1 of the DNA isolation kit (60 µl) was then added to each soil sample and vortex mixed using Vortex-Genie 2 (Daigger & Co, Vernon Hills, IL) at 120 rpm for 10 s and then at 240 rpm maximum speed for an additional 10 min. The PowerBead Tubes containing the samples then were centrifuged for 30 s at 10,000 × g at room temperature using a Heraeus Biofuge Fresco Microcentrifuge (Kendro Laboratory Products, Newtown, CT). The 450 µl of supernatant was then transferred to manufacturer-provided clean 2-ml collection tubes. Inhibitor removal solution C2 (250 µl) was added to each sample, vortex mixed at maximum speed for about 10 s, incubated at 4°C for 5 min, and centrifuged at room temperature for 1 min at 10,000 × g. Next, 600 µl of supernatant was carefully transferred to a clean 2-ml collection tube and 200 µl of additional inhibitor remover, solution C3, was then added to each sample in the tube, vortex mixed for 10 s, and incubated at 4°C for 5 min. Tubes containing the reaction mixture were centrifuged at room temperature for 1 min at 10,000 × g.

About 750 µl of supernatant was then carefully transferred into clean 2-ml collection tubes. For the DNA in the reaction solution to bind exclusively to silica, 1,200 µl of solution C4 was added to the samples. The tubes were vortex mixed at maximum speed for 5 s. About 675 µl of the sample was loaded onto a spin filter and centrifuged at 10,000 × g for 1 min at room temperature for the DNA to bind to the silica membrane of the spin filter. The filtrate was discarded and an additional 675 µl of supernatant was transferred to the spin filter and centrifuged again for 1 min at room temperature at 10,000 × g. After discarding the filtrate, the remaining

supernatant was transferred onto the spin filter and centrifuged at 10,000 × g for an additional 1 min.

The pellets were then cleaned by transferring 500 µl of solution C5 into tubes containing the spin filter and centrifuging at room temperature for 30 s at 10,000 × g to remove residual salts, humic acid, and other contaminants. Filtrates were discarded and the pellets were centrifuged again at room temperature for an additional 1 min at 10,000 × g to remove residual solution C5. The spin filters were carefully transferred to clean 2-ml collection tubes. Purified total DNA was finally eluted with 50 µl of solution C6 that was added to the center of the white filter membrane and centrifuged at room temperature for 30 s at 10,000 × g and stored at –20°C prior to PCR analysis.

Template from the pure *C. beticola* C2 culture was prepared by modification of the protocol for detection of *C. beticola* from infected sugar beet leaves (13) using Extract-N-Amp Plant PCR Kits (Sigma-Aldrich, St. Louis). Dried fungal mycelium was homogenized in 100 µl of extraction solution of the kit and incubated at 95°C for 10 min. Dilution solution (100 µl) was added to the reaction, vortex-mixed at maximum speed for 10 s, and stored at 4°C until ready to use.

PCR amplification. The primer set CBACTIN959 (13) that was designed from the *Cercospora* actin gene sequence (GenBank accession no. AF443281) was used for the PCR amplification. The primer set, consisting of CBACTIN959L (5' AGCA CAGTATCATGATTGGTATGG 3') and CBACTIN959R (5' CACTGATCCAGACGGAGTACTTG 3'), was designed to amplify about a 959-bp DNA fragment of the *C. beticola* actin gene sequence. Amplifications were carried out using the Extract-N-Amp PCR mix as described by Lartey et al. (13). The PCR reactions consisted of 10 µl of Extract-N-Amp PCR mix, 4 µl of the DNA extract (above), and 1.5 µm each of the forward and reverse primers in distilled-deionized water. The mixture was adjusted to the final volume of 20 µl with the distilled-deionized water. The 35-cycle amplification was carried out using Master gradient Thermal Cycler (Eppendorf Scientific Inc., Westbury, NY) at 94°C for 1 min of denaturation, 52°C for 30 s of annealing, and 72°C for 1 min of extension. The PCR products were electrophoresed through 1% agarose gels in Loening E buffer (14). The PCR product sizes were estimated by comparing fragment mobilities to a 1-kb ladder in adjacent lanes.

Detection of *C. beticola* from sugar beet field soils by PCR. To test the viability of the soil detection protocol, field soil samples were collected from different locations and subjected to detection of *C. beticola* by PCR. In addition to the *C. beticola* S1 amended field soil that was

previously described, test soils were collected from two dissimilarly irrigated sugar beet fields near Sidney, MT that were under mid-elevation spray application (MESA) and low-energy precision application (LEPA) irrigation systems, respectively. Finally, a sample soil was collected from a field near Foxholm, ND that was last planted with sugar beet in 2007 (1 year before the soil was sampled). As in the earlier experiment, the deep field soil sample from Froid, MT was included as a control. Field soils from the three locations were collected from the upper depth of 5 to 10 cm of each soil profile, where the pathogen survives for over 22 months (10). For sampling, soils were collected at five different locations within 10 m² of the field using a hand probe. The soils were combined and thoroughly mixed by hand shaking in sterile plastic bags and used for the experiment. Other than the field soil which was amended with *C. beticola*, other test soils received no additional *C. beticola* amendment. The DNA extract from pure mycelial culture of *C. beticola* C2 and the blank reaction mixture from the amended soil experiment served as additional controls. All soil samples were prepared and subjected to PCR as described in the section entitled "Detection in Amended Field Soil."

PCR amplicons purification. The cloning and sequencing PCR amplicons were carefully excised from the agarose gel with a sharp scalpel, weighed, and purified with QIAquick Gel Extraction Kit (Qiagen Inc., Valencia, CA) with minor modification of the manufacturer's protocol. As per the manufacturer's instructions, 3 volumes of Buffer QG were added to 1 volume of gel (300 µl per 100 mg) and incubated at 50°C for 10 min (or until the gel slice was completely dissolved). To accelerate the dissolution process, tubes containing the mixture were vortex mixed for 5 s at the maximum speed every 3 min during the incubation period. Samples were then transferred to QIAquick spin columns in manufacturer-provided 2-ml collection tubes and centrifuged at 10,000 × g for 1 min for the DNA to bind to the column. The flow-through from the centrifugation was discarded. The QIAquick columns were then returned to their original collection tubes.

To remove all traces of agarose, 0.5 ml of Buffer QG was added to the QIAquick column and centrifuged at maximum speed

for 1 min. The DNA was finally rinsed by adding 0.75 ml of Buffer PE to the QIAquick column and centrifuging after 3 min at 10,000 × g for 1 min. The flow-through was discarded, after which the QIAquick columns were centrifuged for an additional 1 min at 10,000 × g.

To elute DNA, the QIAquick columns were transferred into clean 1.5-ml microcentrifuge tubes. Distilled-deionized water (30 µl) was then added to the center of the QIAquick membrane and centrifuged at 10,000 × g for 1 min. The purified DNA was stored at -20°C prior to sequencing.

Cycle sequencing and sequence comparison. Purified DNA fragments were sequenced using BigDye Cycle Terminator Sequencing (v. 3.1 kit; Applied Biosystems, Inc., Foster City, CA). The cycle sequencing reactions were performed in sterile 0.2-ml thin-walled microfuge tubes (Eppendorf North America, Westbury, NY). The reaction consisted of 1 to 3 µl (1.3 µg) of purified DNA, 5 pmol of either CBACTIN959L primer for sense sequencing or CBACTIN959R primer for antisense sequencing, 1 µl of BigDye terminator ready reaction premix containing AmpliTaq DNA polymerase, 3 µl of 5× sequencing buffer, and MilliQ water (Millipore, Bedford, MA) for a total reaction volume of 10.35 µl. Tubes were preheated at 96°C for 1 min followed by 25 cycles of PCR, each consisting of denaturation at 96°C for 10 s, annealing at 50°C for 5 s, and extension at 60°C for 4 min using Mastercycler Gradient (Eppendorf Scientific Inc.) thermal cyclor.

After the PCR sequencing reaction, excess dye terminators and salts were cleaned from the reaction products with Centri-Sep columns (Princeton Separation Inc, Adelphia, NJ) packed with G-50 Sephadex (GE Healthcare Bio-Sciences Corp., Piscataway, NJ). Purified extension products were dried for 1 h or until completely dried in a Speed Vac (Savant, Holbrook, NY), and resuspended in 10 µl of Hi-Di Formamide. The samples were loaded on an ABI 3130 Genetic Analyzer (Applied Biosystems, Inc.) with a four-capillary array and a 96-well plate. Base calling was done automatically with the manufacturer's sequencing analysis software (version 5.2).

Total DNA was extracted three times from each sample and PCR was also run three times each on the *C. beticola* pure

culture and *C. beticola*-amended soil, and each DNA extract from the field soil samples and purified amplicons from gels of each treatment was sequenced at least four times. The sequences were imported into the Vector NTI V.11 sequence and data management suite (Invitrogen Corp, Carlsbad, CA) to generate consensus sequences for the *C. beticola* pure culture, *C. beticola*-amended soil and each tested field soil. Sequences have been deposited in GenBank and can be accessed under the numbers shown in Table 1.

For analysis and comparison, the sequences, together with a *C. beticola* actin sequence from GenBank (accession no. AF443281), were aligned using the Vector NTI software.

RESULTS

Detection of *C. beticola* in amended field soil. At all times in the primary PCR experiments (Fig. 1), the expected *C. beticola* amplicon was amplified from total DNA extracted from amended Froid field soil with CBACTIN 959 primers (lane 4). The amplified fragment of about 1 kb was comparable in size with the amplicon from DNA extract amplified from pure *C. beticola* C2 culture (lane 3). No amplification was observed in the deep control soil from Froid (lane 5) or in the blank control (lane 2) after PCR amplification.

Detection of *C. beticola* from sugar beet field soils by PCR. Detection of *C.*

Fig. 1. Detection of *Cercospora beticola* in amended natural field soil by polymerase chain reaction visualized on a 1% agarose stained with ethidium bromide. Lane 1, kilobase ladder; lane 2, control blank reaction mixture without DNA template; lane 3, control *C. beticola* C2 culture; lane 4, *C. beticola* S1-amended field soil; and lane 5, Froid control deep field soil.

Fig. 2. Polymerase chain reaction detection of *Cercospora beticola* in amended and natural field soils. Lane: 1, kilobase ladder; lane 2, control blank reaction mixture without DNA template; lane 3, control *C. beticola* C2 culture; lane 4, *C. beticola* S1-amended field soil; lane 5, low-energy precision application irrigated sugar beet field soil; lane 6, mid-elevation spray application irrigated sugar beet field soil; lane 7, Foxholm, ND sugar beet field; and lane 8, Froid control deep field soil.

Table 1. GenBank accession numbers of polymerase chain reaction amplicons sequences of the partial actin gene of control *Cercospora beticola* C2 culture and *C. beticola* S1 in amended field soil and three sugar beet fields

Sequence, source ^a	Bases	GenBank accession no.
Control C2 culture	855	FJ972813
Foxholm, ND sugar beet field	845	FJ972814
LEPA-irrigated sugar beet field soil	848	FJ972815
MESA-irrigated sugar beet field soil	855	FJ972816
<i>C. beticola</i> S1-amended field soil	848	FJ972817

^a LEPA = low-energy precision application and MESA = mid-elevation spray application.

beticola in natural field soil is presented in Figure 2. The *C. beticola* CBACTIN959 actin primers consistently amplified the expected segments from the purified *C. beticola* C2 culture (lane 3) and the soil amended with *C. beticola* S1 (lane 4) in all replicated experiments. Similarly, fragments were observed in lanes 5, 6, and 7. These indicate amplification of samples that correspond to soils from the LEPA-irrigated sugar beet field, MESA-irrigated sugar beet field, and Foxholm sugar beet field, respectively. In contrast, amplicons were not observed in lanes 2 and 8, the blank control and the deep soil sample from Froid, respectively. As in the first experiment, all amplicons sizes were about 1 kb, as expected.

Sequences and analysis of PCR amplicon fragments. Amplified DNA fragments from the five positive samples were purified with the QIAquick Gel Extraction Kit and subjected to BigDye Terminator Cycle sequencing using the Applied Biosystems 3130 Genetic Analyzer. Between 845 and 855 bp of the amplicons of the expected size for *C. beticola* were sequenced from the five samples. The consensus sequence for each sample was determined from at least four independent sequences. As previously stated, the five sequences from the control *C. beticola* C2 culture, *C. beticola* S1-amended field soil, LEPA-irrigated sugar beet field soil, MESA-irrigated sugar beet field soil, and Foxholm, ND sugar beet field soil have been deposited to GenBank. They can be accessed under the numbers in Table 1.

The sequences were compared for similarity by alignment using Vector NTI sequence analysis software. The comparison of the *C. beticola* (AF443281) actin sequence from GenBank, sequences of amplicons from pure *C. beticola* C2 culture (Cbet C2 culture), soil amended with *C. beticola* S1 (Cbet S1 in Soil), two different irrigated sugar beet fields from Sidney, MT—LEPA (LEPA SB Field) and MESA (MESA SB Field)—and a sugar beet field from Foxholm, ND (Foxholm, ND 07 Soil) all provided 95.2% sequence identity among the samples.

Further comparison of individual sequences with the *C. beticola* actin sequence from GenBank by alignment showed between 97.2 and 99.4% sequence identity. Specifically, the sequence of *C. beticola* from GenBank (AF443281) showed 98.1% identity with *C. beticola* S1 from amended soil and 99.4% with *C. beticola* C2 culture. From field samples, the identity was 97.2% with sequence from Foxholm, ND sample soil, 97.5% with LEPA sugar beet field soil, and 98.9% with MESA sugar beet field soil. The result of the alignments of the sequences confirmed that the amplified PCR fragments were all from *C. beticola* and provided additional evidence for differences among the detected races in the soil.

DISCUSSION

We have developed and present here a PCR protocol for rapid detection of *C. beticola* from naturally infested field soil. The protocol was first tested on deep field soil that was supplemented with a dried ground culture of *C. beticola*. In that phase, we were able to amplify a target segment of the *C. beticola* actin gene with CBACTIN 959 primers. The PCR amplicon from the *C. beticola* supplemented field soil was comparable in size with that from the control *C. beticola* pure culture. No amplified product was observed from a deep soil sample (60 to 100 cm below surface) from a field near Froid, MT that had never been in sugar beet cultivation. These observations provided preliminary evidence that the amplicon from the amended soil sample is from *C. beticola*.

Subsequently, the protocol was applied to detect *C. beticola* in sugar beet field soils from three different locations with different management strategies. The samples were from MESA irrigation and LEPA irrigation fields near Sidney, MT and a sugar beet field at Foxholm, ND that was last planted to sugar beet a year before the soil was sampled. In all soils, *C. beticola* was detected as indicated by positive PCR application of the actin fragment and comparison of PCR amplicon sizes with that from a cultured control *C. beticola* and *C. beticola*-amended soil in an agarose gel. As additional evidence, the DNA of the amplicons was purified from the gel and sequenced. Comparison of the sequences from the amplicons with that of *C. beticola* from GenBank provided convincing evidence that the amplified products were, indeed, from *C. beticola*. Under current protocols, target soil-inhabiting microbes, including plant pathogens, are isolated from soil on agar media and then subcultured on selective media. DNA is then isolated from the culture before subjecting it to PCR for detection of the target pathogen.

Under the new protocol presented in this research, purification of total DNA templates were carried out on soil without prior isolation and subculture of the *C. beticola*, thus expediting detection of the pathogen. Thus, this technique has the advantage of bypassing the need for growing the pathogen out on media before being tested by PCR. Key elements required for success of current standard protocols are the isolation and culture of the pathogen, which is also an indication of its viability. These factors are not required in this new protocol, thus speeding up the detection of the pathogen even without evidence of its viability. Indeed, current CLS disease forecasting models lack evidence for both availability and viability of *C. beticola*.

Even where crop rotation is a standard practice, as in most growing areas, severe incidence of CLS can occur under suitable

environmental conditions. Additional control measures such as application of fungicides may be necessary to prevent severe economic loss. Our observations further suggest the presence of an inoculum reservoir, detectable in soil where the pathogen has been reported to survive for 20 months and more (10,16). Our protocol also could serve as an alternate soil detection technique or complement the recently developed ELISA-based soil detection protocol (4). It could be applied for rapid evaluation of field soil prior to planting for prospective evidence of *C. beticola*, including identification of which races may be present as a source of inoculum. This information, together with knowledge of real-time weather conditions, should help in improving effective management strategies against *C. beticola*. Furthermore, this protocol combined with the ELISA protocol could be used to enhance evaluation of effectiveness of soil-applied fungicides, biological control agents, or management practices such as deep tillage and different rotation practices (or crops) on *C. beticola* and CLS development. The protocol was developed for detection of *C. beticola* in field soils. With appropriate primers, such as those described in Weiland and Sundsbak (20), it could be adapted for detection of other soilborne fungal pathogens and to study ecological relationships among soil-inhabiting fungi, including impact of biological agents on target fungal pathogens.

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LITERATURE CITED

1. Bleiholder, H., and Weltzien, H.C. 1972. Beiträge zur Epidemiologie von *Cercospora beticola* Sacc. an Zuckerrübe: Geopathologische Untersuchungen. Phytopathol. Z. 73:93-114.
2. Bobba, V., and Conway, K. E. 2003. Competitive saprophytic ability of *Laetisaria arvalis* compared with *Sclerotium rolfsii*. Proc. Okla. Acad. Sci. 83:17-22.
3. Bobev, S. G., Groenewald, J. Z., and Crous, P. W. 2009. First report of *Cercospora beticola* as a pathogen of German statice (*Goniolimon tataricum*) in Bulgaria. Plant Dis. 93:553-553.
4. Caesar-TonThat, T., Lartey, R. T., and Shelper, W. L. 2007. Enzyme-linked immunosorbent assay for *Cercospora beticola* in soil. J. Sugar Beet Res. 44:51-70.
5. Duffus, J. E., and Ruppel, E. G. 1993. Diseases. Pages 347-427 in: The Sugar Beet Crop. D. A. Cooke and A. K. Scott, ed. Chapman and Hall, London.
6. Forsyth, F. R., Unwin, C. H., and Jursic, F. 1963. Cultural and pathogenic studies of an isolate of *Cercospora beticola* Sacc. J. Am. Soc. Sugar Beet Technol. 12:485-491.
7. Ioannidis, P. M., and Karaoglanidis, G. S. 2000. Resistance of *Cercospora beticola* Sacc. to fungicides. Pages 123-145 in: *Cercospora beticola* Sacc. Biology, Agronomic Influence and Control Measures in Sugar Beet. M. J. C. Asher, B. Holschulte, M. Richard Molard, F. Rosso, G. Steinbrücken, and R. Beckers, eds. Advances in Sugar Beet Research 2, International Institute for Beet Research, Brussels, Belgium.
8. Jacobsen, B. J. 2000. Sugarbeet *Cercospora*

- leaf spot—scouting, disease monitoring and control. *Mont. Crop Health Rep.* 13:1-3.
9. Jacobsen, B. J., and Franc, G. D. 2009. *Cercospora* leaf spot. In: *Compendium of Beet Diseases and Pests*. R. M. Harveson, L. E. Hanson, and G. L. Hein, eds. American Phytopathological Society, St. Paul, MN.
 10. Khan, J., del Rio, L. E., Nelson, R., Rivera-Varas, V., Secor, G. A., and Khan, M. F. R. 2008. Survival, dispersal, and primary infection site for *Cercospora beticola* in sugar beet. *Plant Dis.* 92:741-745.
 11. Lartey, R. T., Caesar-TonThat, T., Caesar, A. J., Shelper, W. L., Sol, N. I., and Bergman, J. W. 2005. Safflower: a new host of *Cercospora beticola*. *Plant Dis.* 89:797-801.
 12. Lartey, R. T., Lenssen, A. W., Evans, R. G., and Ghoshroy, S. 2007. Comparative structural study of leaf spot disease of safflower and sugar beet by *Cercospora beticola*. *Plant Pathol. J.* 6:37-43.
 13. Lartey, R. T., Weiland, J. J., Caesar-TonThat, T., and Bucklin-Comiskey, S. 2003. A PCR protocol for rapid detection of *Cercospora beticola* in infected sugar beet tissues. *J. Sugar Beet Res.* 40:1-10.
 14. Loening, U. E. 1969. The determination of the molecular weight of ribonucleic acid by polyacrylamide gel-electrophoresis. *Biochem. J.* 113:131-138.
 15. McKay, M. B., and Pool, V. W. 1918. Field studies of *Cercospora beticola*. *Phytopathology* 8:119-136.
 16. Nagel, C. A. 1938. The longevity of *Cercospora beticola* in soil. *Phytopathology* 28:342-350.
 17. Ruppel, E. G. 1986. *Cercospora* leaf spot. Pages 8-9 in: *Compendium of Beet Diseases and Insects*. E. D. Whitney and J. E. Duffus, eds. American Phytopathological Society, St. Paul, MN.
 18. Steinkamp, M. P., Martin S. S., Hoefert L. L., and Ruppel E. G. 1979. Ultrastructure of lesions produced by *Cercospora beticola* in leaves of *Beta vulgaris*. *Physiol. Plant Pathol.* 5:13-26.
 19. Weiland, J., and Koch, G. 2004. Sugarbeet leaf spot disease (*Cercospora beticola* Sacc.). *Mol. Plant Pathol.* 5:157-166.
 20. Weiland, J. J., and Sundsbak, J. L. 2000. Differentiation and detection of sugar beet fungal pathogens using PCR amplification of actin coding sequences and the ITS region of the rRNA Gene. *Plant Dis.* 84:475-482.
 21. Windels, C. E., Lamey, A. H., Hilde, H., Widner, J., and Knudsen, T. 1998. A *Cercospora* leaf spot model for sugar beet, in practice by an industry. *Plant Dis.* 82:716-725.
 22. Wolf, P. F. J., and Vereet, J. A. 2002. An integrated pest management system in Germany for the control of fungal leaf diseases in sugar beet: the IPM sugar beet model. *Plant Dis.* 86:336-344.