First Report of Cercospora Leaf Spot Caused by *Cercospora chenopodii* on *Spinacia oleracea* in the USA.

T. Synoground, **A. Batson**, and **M. L. Derie**, Washington State University Mount Vernon Northwestern Washington Research and Extension Center, Mount Vernon, WA 98273; **L. B. Koenick** and **S. J. Pethybridge**, Cornell AgriTech at The New York State Agricultural Experiment Station, Cornell University, Geneva, NY 14456; and **L J. du Toit**, Washington State University Mount Vernon Northwestern Washington Research and Extension Center, Mount Vernon, WA 98273.

In November 2017, leaf spots were observed in a fresh market spinach crop of cv. SV2157VB in New Jersey at ~20% incidence but not in the adjacent crop of Unipack-151. Pale tan lesions (3 to 8 mm in diameter) had gray sporulation at the centers. Symptomatic leaf pieces were surface-sterilized in 0.6% NaOCl for 60 s, triple-rinsed in sterilized water, dried, and plated onto PDA and water agar (each with 100 µg chloramphenicol/mL). Seven isolates all produced hyaline, straight to slightly curved conidia, mostly 2- to 3-septate, 20.0 to 82.0 µm (45.2 ± 11.3 µm mean ± standard deviation) × 6.0 to 11.0 µm (8.1 ± 1.0 µm) ($n = 312$), with no significant differences among isolates. Spore characteristics matched those of *Cercospora* spp. (Swiderska Burek 2015). The consensus sequence of the calmodulin gene (*cmdA*) amplified from all seven isolates using primers CALDF1 and CALDR (Lawrence et al. 2013) (GenBank Accession No. MN422444) was 99.78% identical to that of *Cercospora* cf. *chenopodii* (JX142839.1), which is not differentiated from *C. chenopodii* by this gene (Groenewald et al. 2013). Actin (*actA*) primers ACT-512F and ACT-783R generated an identical sequence for the isolates (MN422442) that was 99.55% identical to that of *C. chenopodii* (JX143081.1) (Carbone and Kohn 1999). The translation elongation factor 1-α (*TEF1- α*) sequence of the isolates (MN422445) had 99.45%

homology to the sequence of a voucher specimen of *C. chenopodii* (JX143327.1). *C. beticola*-specific primers CbCALF and CbCALR did not amplify DNA of these isolates, only DNA of control isolates of *C.beticola* (Knight et al. 2019). Based on a phylogenetic tree inferred from concatenated *actA*, *cmdA*, ITS rDNA, and *TEF1- α* sequences, the isolates clustered with the voucher strain *C. chenopodii* CBS 132620. Six isolates were each tested for pathogenicity on four 33-day-old plants of each of spinach cvs. Mandolin, SV2157VB, Unipack-151, and Viroflay; sugar beet cv. KDH4-9 (USDA National Plant Germplasm System PI 683513, homozygous susceptible to *C. beticola*), red beet cvs. Red Ace and Ruby Queen, and Swiss chard cv. Silverado. Plants were enclosed in plastic bags for 24 h, atomized with a spore suspension (1×10^4 conidia/ml) prepared from clarified V8 agar plates of each isolate, and returned to the bags for 24 h. Four non-inoculated control plants of each cultivar were atomized with water as control treatments. Beet and chard plants were placed in a greenhouse, and spinach plants in a growth chamber with 10 h lighting/day to avoid bolting under 16-h summer days in Washington. Symptoms had not developed after 7 days, at which time spinach plants were moved to the greenhouse. By 9 days, leaf spots resembling those on the original leaves were observed on Mandolin and SV2157VB. By 14 days, there were leaf spots on all four spinach cultivars. Symptoms were most severe on SV2157VB (18.3 ± 2.0% mean ± standard error of leaf area), followed by Mandolin and Viroflay (8.8 ± 1.6%), and Unipack-151 (2 ± 0.5%). Symptoms did not develop on non-inoculated spinach or inoculated and non-inoculated sugar beet, table beet, and Swiss chard. *Cercospora* could only be re-isolated from symptomatic spinach plants, with the isolates confirmed as *C. chenopodii* based on *actA* sequences. Although *C. beticola* has been reported to cause Cercospora leaf spot of spinach (Knight et al. 2018), this is the first report of *C. chenopodii* causing a leaf spot of spinach in the USA. Research is needed to evaluate impacts of this species on additional spinach cultivars, and management options.

References:

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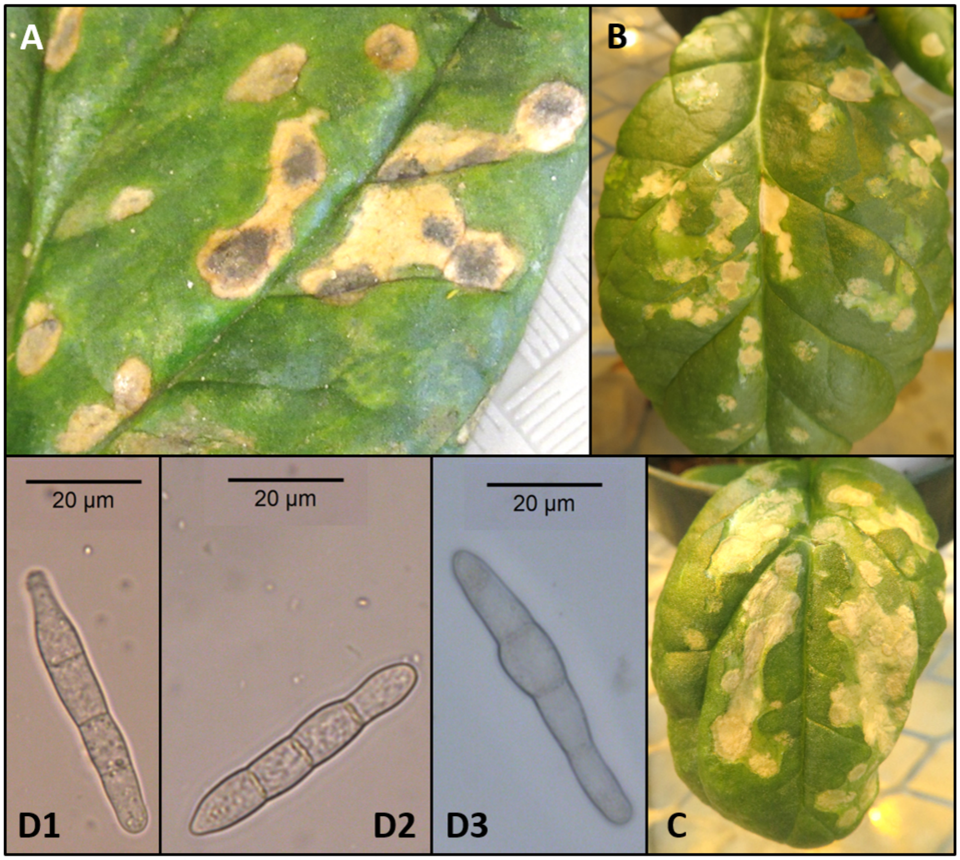
eXtra Figure 1. Symptoms on a leaf sampled from a crop of the spinach cv. SV2157VB in New Jersey, caused by *Cercospora chenopodii* (**A**), with gray sporulation in the centers of older lesions. Symptoms on the spinach cvs. Mandolin (**B**) and SV2157VB (**C**) inoculated with single-spore isolates of the fungus in a greenhouse. Hyaline, 1- to 3-septate conidia of *C. chenopodii* produced in leaf spots on inoculated spinach plants. Leaf spots did not develop on inoculated sugar beet, table beet, and Swiss chard plants.

eXtra Figure 2. A phylogenetic tree inferred from a concatenated alignment of partial sequences of four loci [actin (*actA*), calmodulin (*cmdA*), translation elongation factor 1-α (*TEF1-α*), and the internal transcribed spacer (ITS) region of rDNA] of isolate Crc022 of *Cercospora* obtained from symptomatic leaves of the spinach cv. SV2157VB in New Jersey in 2017, compared with the same four loci for a sample of 47 *Cercospora* spp. from Groenewald et al. (2013). Seven *Cercospora* isolates from the symptomatic spinach plants in New Jersey had identical sequences for these loci, so sequences of a representative isolate, Crc022, were used for phylogenetic analysis. Sequences were aligned for each locus with ClustalOmega, and each alignment was inspected manually for misalignments. ModelTest-NG was used to assess the best model of evolution for each locus, and a maximum likelihood tree was inferred for the concatenated data set using RAxML-NG. The concatenated alignment of *actA*, *cmdA*, ITS rDNA, and *TEF1-α* was partitioned by locus with evolutionary models K80 + G, TN93 + G, TIM1, and K80 + G, respectively. The values on the branches indicated clade support as a percentage of 1,000 bootstrap replicates. The tree was rooted to *C. zeae-maydis* strains CBS 132668 and 132678, and visualized with FigTree. The scale bar represents the number of substitutions per site. Crc022 resembled voucher strain *C. chenopodii* CBS 132620 most closely, and *C. chenopodii* strains were most closely related to *Cercospora* cf. *chenopodii*.



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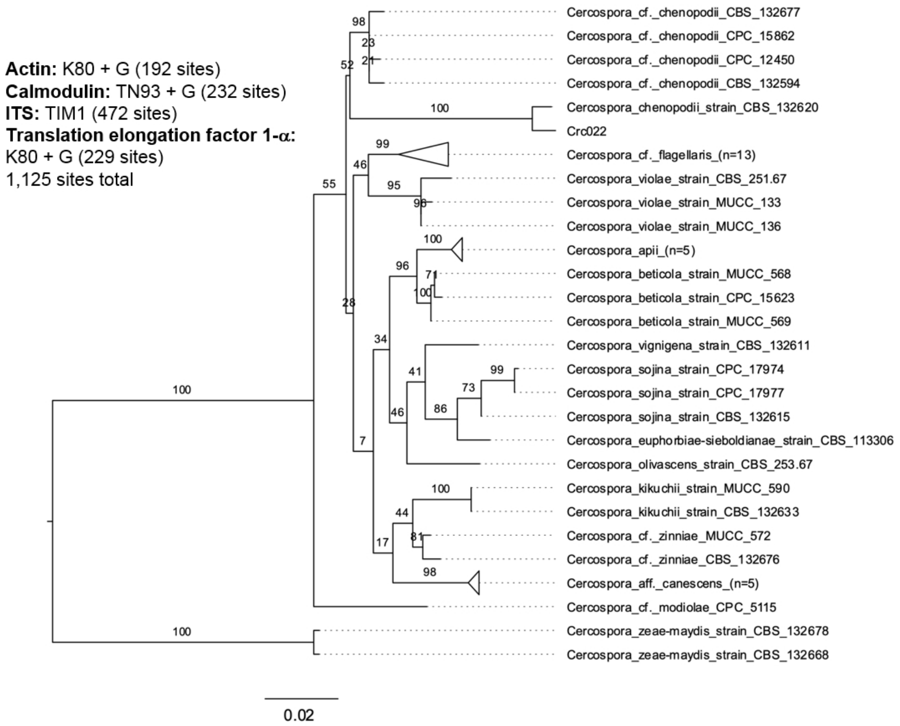

eXtra Figure 2. A phylogenetic tree inferred from a concatenated alignment of partial sequences of four loci [actin (*actA*), calmodulin (*cmdA*), translation elongation factor 1-ɑ (*TEF1-ɑ*), and the internal transcribed spacer (ITS) region of rDNA] of isolate Crc022 of *Cercospora* obtained from symptomatic leaves of the spinach cv. SV2157VB in New Jersey in 2017, compared with the same four loci for a sample of 47 *Cercospora* spp. from Groenewald et al. (2013). Seven *Cercospora* isolates from the symptomatic spinach plants in New Jersey had identical sequences for these loci, so sequences of a representative isolate, Crc022, were used for phylogenetic analysis. Sequences were aligned for each locus with ClustalOmega, and each alignment was inspected manually for misalignments. ModelTest-NG was used to assess the best model of evolution for each locus, and a maximum likelihood tree was inferred for the concatenated data set using RAxML-NG. The concatenated alignment of *actA*, *cmdA*, ITS rDNA, and *TEF1-ɑ* was partitioned by locus with evolutionary models K80 + G, TN93 + G, TIM1, and K80 + G, respectively. The values on the branches indicated clade support as a percentage of 1,000 bootstrap replicates. The tree was rooted to *C. zeae-maydis* strains CBS 132668 and 132678, and visualized with FigTree. The scale bar represents the number of substitutions per site. Crc022 resembled voucher strain *C. chenopodii* CBS 132620 most closely, and *C. chenopodii* strains were most closely related to *Cercospora* cf. *chenopodii*.

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