plant disease

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DISEASE NOTES

First Report of Leaf Spot of Spinach Caused by *Stemphylium beticola* in Italy

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During the spring and autumn of 2017, 40-day-old spinach (*Spinacia oleracea* L.) plants, cultivar Crocodile, grown in a commercial field in the Asti province in Piedmont (Northern Italy), showed symptoms of a previously unknown foliar disease. The first symptoms developed at air temperatures ranging from 10 to 22°C. Five to 25% of plants showed small circular, yellow to pale brown leaf spots (1 to 2 mm in diameter), with a well-defined border, generally surrounded with a yellow halo. As the lesions expanded, they changed from circular to elliptical or irregular, becoming necrotic and broken in the center. Small tissue fragments were excised from symptomatic leaves, dipped in a solution containing 1% sodium hypochlorite for 5 s, washed in sterile water, dried on sterile paper, and plated on potato dextrose agar. After 7 days of incubation under 12-h fluorescent light at 22°C, 80% of fragments were colonized by the same fungus. Examination of the isolates IT27, IT28, and IT50 grown on V8 agar medium (200 ml of V8, 15 g of agar, 0.5 g of $CaCO_3$, and 1 liter of distilled water) revealed multicellular, light brown pigmented, obclavate or subspherical conidia, measuring 19.2 to 31.9 µm long × 10.7 to 21.4 µm wide (length/width ratio 1.1 to 1.4), with two to four transverse and three to four longitudinal septa. Conidia developed on a distinctive brown conidiophore with a terminal enlargement. These morphological characters permitted identifying the fungus as *Stemphylium* sp. (Simmons 1969). Genomic DNA from 7-day-old pure cultures of IT27, IT28, and IT50 were extracted with E.Z.N.A. Plant DNA Kit (Omega Bio-Tek). Polymerase chain reaction (PCR) was performed using primers ITS1/ITS4 to amplify the internal transcribed spacer, the intergenic region between 28 S and 18 S sequences of the ribosomal RNA, including the 5.8 S sequence. The PCR products were purified and sent for sequencing to BMR Genomics (Padova, Italy). BLASTn analysis (Altschul et al. 1997) of the three isolates (483 bp) showed 100% similarity

with *Stemphylium beticola* CBS 141024 (GenBank no. NR_154925.1). Additionally, alignment with the CBS isolates used by Woudenberg et al. (2017) and the isolates from spinach was carried out using Clustal W with Mega 6.0.6 (Tamura et al. 2013). Sequence analysis of the same length sequences was performed using the maximum likelihood method with the Kimura two-parameter model plus gamma distribution. The sequences from IT27, IT28, and IT50 isolates clustered with the *S. beticola* strains CBS 141024, CBS 141025, and CBS 141026 (GenBank nos. KU850520, KU850521, and KU850522, respectively), allowing confirmation of the identity of isolates from spinach as *S. beticola*. The sequences from the isolates IT50, IT27, and IT28 were deposited in GenBank with the accession numbers MG913379, MG913380, and MG91338, respectively. The pathogenicity test was carried out by spraying a conidial suspension of 10^5conidia/ml from a single-spore culture of strain IT50 onto leaves of 30-day-old spinach cultivar Crocodile plants, maintained under >90% relative humidity conditions for 5 days after inoculation. Pots (five per treatment, 10 plants/pot) were kept in a growth chamber at an average temperature of 20°C. The first lesions developed on leaves 8 days after inoculation, whereas control plants remained healthy. The pathogenicity test was conducted three times, providing the same results. Based on morphological and molecular analysis, the same fungus was consistently reisolated from the lesions. This is to our knowledge the first report of *S. beticola* on spinach in Italy and probably in Europe (Farr and Rossman 2018), although the same species has been reported on spinach in the United States (Woudenberg et al. 2017). The importance of the disease is at present limited but could rapidly increase in the cultivation areas where spinach is intensively grown.

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