Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Alternaria alternata* Causing Leaf Spot on *Menispermum dauricum* in China

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Menispermum dauricum DC. is an ornamental plant used in traditional Chinese medicine (Tang et al. 1992). In September 2019, a leaf spot on *M. dauricum* was first found in a medicinal plant plantation in Harbin city (45.80°N, 126.53°E), Heilongjiang Province, China. The incidence was 76 to 90% on the 0.02-ha plantation. The initial symptoms were irregular black and brown spots on the leaves. The lesions expanded and coalesced, eventually leading to blight. Fresh leaf samples from 10 *M. dauricum* plants with typical symptoms were collected. The areas of leaf between symptomatic and healthy tissue (5 × 5 mm) were cut and surface disinfested in 75% ethanol for 2 min, and with 1% HgCl₂ for 1 min, and then rinsed three times with sterile water. Small lesion pieces were incubated on potato dextrose agar (PDA) for 7 days at 25°C, in the dark. Ten fungal isolates were obtained and transferred onto new PDA and potato carrot agar (PCA) plates to establish pure cultures. After 8 days, the colonies on PDA were 75 to 86 mm in diameter, circular, with distinct concentric rings and a whitish aerial-mycelium margin, cottony, light gray to dark bluish brown. The colonies on the PCA were olive-green and bordered by white aerial hyphae. A total of 150 conidia were single or in short chains, obclavate, oval or inverted pear, light brown to brown, smooth or slightly spiny, with one to six transverse septa, zero to four longitudinal or oblique septa, not narrow or slightly narrowed at the separation, 22.5 to 42.5 × 7.5 to 15.5 μm, and rostrate. Conidiophores were simple, erect,

or ascending, dark brown, geniculate, septate, and with one or several conidial scars, 32.5 to 77.5 × 3.0 to 5.0 μm. Beaks were columnar or conical, 7.5 to 22.5 × 2.5 to 3.5 μm. Morphologically, all 10 isolates were most similar to *Alternaria alternata* (Simmons 2007). For further identification of the fungus at the molecular level, internal transcribed spacer rDNA regions (ITS), RNA polymerase second largest subunit gene (RPB2), and *Alternaria* major allergen (Alt a 1) were amplified and sequenced using the primers ITS1 and ITS4, RPB2-5F2 and RPB2-7CR, and Alt-for and Alt-rev (Woudenberg et al. 2015). The resulting sequences were deposited in GenBank (ITS, MT995193, MZ150794; RPB2, MT999483, MZ170963; Alt a 1, MT802122, MZ170962). BLAST search of these sequences showed 99 to 100% homology with the ITS (FJ196306), RPB2 (KC584375), and Alt a 1 (KT315515) of the type strain CBS 916.96 of *A. alternata*, respectively. Thus, the fungus was identified as *A. alternata* based on the morphology and molecular analysis. For the pathogenicity test, spore suspensions (1 × 10⁶ spores/ml) of the representative isolates BFG001 and BFG002 were sprayed onto the leaves of six healthy plants, separately. As a control, six plants were treated with sterile distilled water. The plants used in the experiment were covered with plastic bags and incubated at 25°C for 10 days. Eight days after inoculation, irregular, slightly sunken black leaf spots appeared at the leaf margin. The experiment was repeated three times with the same method. The same fungus was successfully reisolated from the leaves of the inoculated plants, fulfilling Koch's postulates. No symptoms were observed on control plants. To our knowledge, this is the first report of leaf spot disease on *M. dauricum* caused by *A. alternata* in the world. The appearance of leaf spot disease reduces the yield and quality of Chinese medicinal materials. This report has laid the foundation for the further research and control of leaf spot disease.

References:

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