Diseases Caused by Fungi and Fungus-Like Organisms

First Report of Alfalfa Leaf Spot Caused by *Leptosphaerulina australis* in China

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A disease was observed on alfalfa cultivar WL168 characterized by white to brown leaf spots of regular to round shapes, in Aluhorqin County, Inner Mongolia, China (120°13'23" to 120°29'14" E, 43°27'52" to 43°35'16" N; 281.71 to 360.13 m altitude) during 2019 to 2020. The disease mainly presented in spring 1 month after regreening, and the incidence was 78.30% in this field. Twenty alfalfa plants with severe symptoms were used for pathogen isolation. The infected tissue was cut into 2 × 2-mm pieces, surface sterilized (in 75% ethanol and 5% commercial bleach [NaClO] for 30 s and 2 min, respectively), rinsed five times with sterilized distilled water, and dried between sterile filter paper (Wang et al. 2019). The diseased tissues from each plant sample were cultured on potato dextrose agar (PDA) and incubated at 25°C with 12 h of light per day for 10 days. A fungus was isolated from the diseased leaves at 100% frequency. Fungal growth on PDA was round with a black surface, radial edge, and a dirty white center. The ascocarps were moved to a clean microscope slide to release asci and ascospores. Ascocarps were spheroidal, subglobose brown, 120 to 160 µm × 160 to 180 µm, which contained several asci. The size of asci was

31.0 to 41.6 × 75.0 to 87.5 µm, and each ascus had eight ascospores. Ascospores were ellipsoid to oblong with a gelatinous sheath, brown, 8.8 to 15.0 × 29.9 to 43.0 µm, with two to three horizontal septa and zero to two vertical septa. A phylogenetic tree was constructed after DNA extraction and PCR with primers to amplify the ITS (VG9, 5'-TTACGTCCCTGCCCTTTGTA-3'; and ITS4, 5'-TCCTCCGCTTATTGATATGC-3') and LSU (LR7, 5'-TACTA CCACCAAGATCT-3'; and LROR, 5'-GTACCCGCTGAACCTTAAGC-3') regions. The LSU (SUB8273071) and ITS (SUB8218291) amplicons showed 99% similarity with *Leptosphaerulina australis* (EU754166.1) in GenBank. To verify the pathogenicity, fungal plugs were inverted on three compound leaves of 20 alfalfa WL168 for 2 days. Agar plugs (PDA) were inverted on three compound leaves of another 20 alfalfa WL168, which were a control. All plants were maintained at 22°C and 44% relative humidity in a growth chamber. Similar disease symptoms were observed on infected leaves 10 days after inoculation, whereas control plants showed no symptoms. The same fungus was reisolated from the lesions, and further morphological characterization and molecular assays, as described above. *L. australis* has been reported on various plants, including *Prunus armeniaca*, *Dolichos*, *Poa*, *Lolium*, and *Vitis* in Australia (Graham and Luttrell 1961), and also from Korean soil in 2018 (Weilan et al. 2018). Additionally, *L. briosiana*, which is common in the United States, China, and other countries, causes *Leptosphaerulina* leaf spot (Samac et al. 2015). *L. trifolii* is newly reported to occur in China (Liu et al. 2019). To the best of our knowledge, this is the first report of *L. australis* infecting alfalfa in China. Considering the large planting area in Inner Mongolia, this pathogen may cause losses to alfalfa cultivation. Hence, future studies should explore aspects of effective management of this disease.

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