Diseases Caused by Fungi and Fungus-Like Organisms

Southern Blight on *Medicago sativa* Caused by *Sclerotium rolfsii* in North China

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Alfalfa (*Medicago sativa* L.) is one of the most important perennial leguminous forages in many countries, known for its high feed value and yield potential. With the increasing demand for feed, alfalfa has been planted all over China. However, an increasingly serious alfalfa disease was observed and may restrict the development of the alfalfa industry in North China. In August 2019, an emerging alfalfa disease with symptoms resembling southern blight was observed in Jiaozhou experimental base (Jiaozhou Modern Agricultural Science and Technology Demonstration Park) of Qingdao Agricultural University (Qingdao, Shandong province, China). The infected plants showed dark brown lesions on the stems and yellowing and wilting of the leaves. The pathogen produced white fluffy mycelia, and later sclerotia on stems and roots; the disease affected up to 25% of the plants and caused bare spots filled with weeds. Typical symptomatic tissues were brought back to the laboratory for pathogen isolation and identification. Fragments (3 to 5 mm²) of root tissues were excised from lesions on the symptomatic roots, and their surfaces were disinfested by sequential dipping in 70% ethanol for 30 s and in 2% NaClO for 3 min, and then the fragments were rinsed in sterile water five times and cultured on potato dextrose (PDA) medium amended with streptomycin sulfate (0.1 mg/

ml). Cultures were incubated at 28°C in the dark and purified in PDA medium three times. A representative strain (coded as CZL1) was isolated from the root rot of the diseased plant. After 4 days incubation on PDA, CZL1 formed white fluffy aerial mycelium 5.6 to 6 cm in diameter typical of *Sclerotium rolfsii*. After 15 to 20 days, abundant round sclerotia approximately 1 to 3 mm in diameter were produced on the surface of the culture. The sclerotia were white at first and then gradually turned dark brown. To confirm the identity of the causal fungus, the complete internal transcribed spacer (ITS) rDNA region of the fungus was amplified using the primers ITS1/ITS4 (White et al. 1990), and the elongation factor-1 α gene (*EF1 α*) was amplified using primers EF1-983F/EF1-2218R (Rehner and Buckley 2005). Then the PCR amplicons were cloned into the pCE2 TA/Blunt-Zero vector. The isolate was determined to contain two distinct sequence types for each gene. The results of ITS (MT812692 and MT812693) and *EF1 α* (MT846496 and MT846497) sequences were deposited in GenBank. DNA analysis revealed that the two ITS sequences were more than 99% identical to *Athelia rolfsii* (MN872304) in the NCBI GenBank database, and two *EF1 α* sequences were 99% identical to the *A. rolfsii* *EF1 α* sequence MN702789 and KP982854. To fulfill Koch's postulates, infected sorghum grain was placed near the roots of 15 40-day-old healthy alfalfa seedlings split into three pots with the same number of seedlings receiving a control treatment of sterilized sorghum grain. All plants were incubated in a growth chamber at 24 $\pm$ 1°C with 14-h photoperiod (85% relative humidity). After 10 to 15 days, blight symptoms identical to those in the field were observed on inoculated plants, whereas control plants were symptomless. *S. rolfsii* was successfully reisolated from the inoculated plants and molecularly characterized as described above. Based on disease symptoms, fungal colonies, the ITS and *EF1 α* sequences, and pathogenicity to the host, this fungus was identified as *S. rolfsii* (teleomorph *A. rolfsii*). To our knowledge, this is the first report of *S. rolfsii* as the causal agent of southern blight of alfalfa in North China, and it is also the first report of southern blight on alfalfa caused by *S. rolfsii* in China since 1996 observed in Guizhou province (Mo and Luo 1996).

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