Diseases Caused by Fungi and Fungus-Like Organisms

First Report of Alfalfa Root Rot Caused by *Plectosphaerella cucumerina* in Inner Mongolia Autonomous Region of China

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Alfalfa (*Medicago sativa* L.) is an important forage crop with high nutrition for animal feed. In May 2016, a disease showing brown root rot was observed on alfalfa collected from several farms in Tongliao City (44°17' N; 121°29' E), Inner Mongolia Autonomous Region of China. The incidence of brown root rot was approximately 50 to 70% in the 2-year-old alfalfa field. Infected alfalfa exhibited varying degrees of decay in the tap root. Symptomatic roots were cut into 0.5-cm pieces, surface disinfected with 70% ethanol for 5 s and 0.1% HgCl₂ for 35 s, rinsed with sterilized distilled water three times, and placed onto potato dextrose agar (PDA) at 26°C in the dark. After 5 days, hyphal tips of the growing colonies were transferred onto PDA plates for purification. Forty-four isolates belonging to five fungal species were obtained from 20 diseased root samples. Six of the isolates resembled the genus *Plectosphaerella*. Colonies of these isolates were white to cream in color with sparse aerial mycelium, and then gradually became salmon pink with slimy or moist mycelium. The hyphae were transparent and branched. Colonies produced numerous hyphal coils with conidiophores. Conidiogenous cells and conidia were both hyaline, solitary, and smooth. Conidia were 4 to 8.5 × 1.2 to 4.8 μm ($n = 100$), zero to one septum, elliptical and ovoid, and aggregating to form a head (Palm et al. 1995). According

to these morphological characteristics, the fungus was identified as *Plectosphaerella cucumerina* (Lindf.) (Carlucci et al. 2012). To confirm the identification, the genomic DNA of two representative isolates was extracted, and their internal transcribed spacer (ITS) region was amplified and sequenced with the primer pair ITS1/ITS4 (White et al. 1990). The ITS sequences of the two isolates were deposited in GenBank (accession nos. MN915126 and MN915127). The two ITS sequences showed 99 to 100% identity to known *P. cucumerina* strains CBS 131739 (accession no. KY662258.1) (Su et al. 2017) and MP313 (accession no. KC756835.1) from alfalfa in China (Wen et al. 2015). To test for pathogenicity, a set of 15 alfalfa seedlings (cv. Aohan) were root-dipped in the conidial suspension of one of the isolates (1×10^5 conidia/ml) prepared from 7-day-old cultures on PDA. Inoculated seedlings were transplanted in three pots (10 × 15 cm) with sterilized nursery soil. Another set of five alfalfa seedlings inoculated with sterile water only served as the controls. Treated alfalfa seedlings were maintained in a greenhouse at 25 to 28°C under a 12-h photoperiod. After 25 days, the roots of all inoculated plants showed brown lesions. *P. cucumerina* was reisolated from symptomatic tissue. No symptoms were observed on the control plants. *P. cucumerina* was previously reported on alfalfa in the fields of Huanxi County (36°20' N; 107°21' E), Gansu Province, China (Wen et al. 2015). To our knowledge, this is the first report of *P. cucumerina* causing root rot of alfalfa in Inner Mongolia Autonomous Region, China. This disease may cause serious economic losses in the region. It is needed to develop effective management strategies for control of this disease.

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