Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Alternaria cinerariae* Causing Leaf Spot on *Tussilago farfara* in China

L. He,^{1,2} H. F. Liu,^{1,2} A. A. Htun,^{1,2} H. Ge,³ J. X. Deng,^{1,2,†} and T. Du^{3,†}

¹ Department of Plant Protection, College of Agriculture, Yangtze University, Jingzhou 434025, China

² Hubei Engineering Research Center for Pest Forewarning and Management, Yangtze University, Jingzhou 434025, Hubei, China

³ Gansu University of Chinese Medicine, Lanzhou 730000, China

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Tussilago farfara is widely distributed in China, India, Iran, Pakistan, Russia, Western Europe, and North Africa. The flower bud of *T. farfara* has medicinal value used for the treatment of cough, asthma, phlegm, and angiocardiopathy (Chen 1999). During the rainy season of July 2019, many small spots were observed on 70% of the leaves of *T. farfara* plants in Hezheng Medicinal Botanical Garden. The botanical garden is affiliated with Gansu University of Chinese Medicine and located in Hezheng County, Linxia Hui Autonomous Prefecture, Gansu, China. Eventually, the center of the lesion turned into light brown as symptoms progressed. Diseased tissues were cut into small pieces and put onto moist filter papers within Petri dishes and incubated at 25°C (humidity around 90%). After 1 day, *Alternaria* spores developed from the edge of necrotic tissues. Spores were singly picked up and transferred to potato dextrose agar (PDA). Pure cultures were deposited in the Fungi Herbarium of Yangtze University (YZU), Jingzhou, Hubei, China. All strains were similar to each other, and two (YZU 191293 and YZU 191294) were selected for further studies. Three- to 4-day-old mycelial plugs (6 mm in diameter) were transferred onto fresh PDA plates (90 mm in diameter) incubated at 25°C in the dark. After 7 days, colonies were 65 to 67 mm in diameter, hazel to buff from the center to edge, and reverse side fuscous black to creamy white. The isolated fungus was cultured on V8 juice agar at 22°C under an 8-h photoperiod for 7 days for microscopic observations (Simmons 2007). Conidiophores were straight or curved, 30 to 90 µm long, and 4 to 7 µm wide. Conidia were solitary or two to three in a chain, long ellipsoid to obclavate, one to eight transverse septa, 40 to 125 µm long, and 8 to 36 µm

wide; blunt-tapered beak variable in length 14 to 32 µm long, 6 to 10 µm wide. Morphologically, the fungus was identified as *Alternaria cinerariae* according to previous descriptions (Nishikawa and Nakashima 2015; Simmons 2007). Genomic DNA was extracted from 3-day-old mycelium grown on PDA (Cenis 1992). Three gene regions including rDNA ITS (ITS4/ITS5), RPB2 (RPB2-5F/RPB2-7cR), and EF-1α (EF1-728F/EF1-986R) were amplified and sequenced according to the method of Woudenberg et al. (2013). The resulting sequences were 100% similar to those of the representative strain CBS 116495 (GenBank nos. KC584190 for ITS, KC584389 for RPB2, and KC584648 for EF-1α) of *A. cinerariae* in GenBank database during BLAST searches. Maximum likelihood analysis was performed using the combined dataset of the three gene regions after alignment under 1,000 bootstrap replicates with the model of SYM+I+G. The phylogenetic tree showed that the present strains and *A. cinerariae* CBS 116495 fell into a clade with 100% bootstrap value support. Based on morphological characteristics and phylogenetic analysis, the isolated fungus was identified as *A. cinerariae*. To confirm its pathogenicity, 20 µl of conidial suspension (3×10^5 spores/ml) was dropped on living leaves ($n = 3$) of *T. farfara* plants ($n = 3$), which were cultivated for 1 month after transplantation as seedlings. Controls were treated with sterile distilled water. The plants were cultivated in a greenhouse under supplemental fluorescent light at 25°C and covered by plastic bags to maintain humidity around 90%. The experiment was repeated three times. After 2 days, brown spots, 6 to 8 mm in size, were observed on the inoculated leaves. Gradually, the lesions enlarged to the leaf edge, and leaves became blighted and shrunk. All control plants were symptomless. The same fungus was reisolated from the lesions of symptomatic plants and identified using the EF-1α gene sequence. To our knowledge, this is the first report of *A. cinerariae* causing leaf spot on *T. farfara*.

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[†]Indicates the corresponding author.

E-mail: djxin555@hotmail.com (J.X.D.) and gslzdt@163.com (T.D.)