Diseases Caused by Fungi and Fungus-Like Organisms

First Report of Purple Spot on Dandelion in China Caused by *Alternaria tenuissima*

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Dandelion (*Taraxacum mongolicum*) is a perennial herb of the family Asteraceae with a high edible and medicinal value, and it is widely grown at medium and low altitudes in China. In July 2019, purple spot of dandelion was found in a field near Harbin City, Heilongjiang province, China. The disease incidence regionally reached 95% in fields with yield losses between 10 and 20%, seriously reducing the economic and food value of dandelion. Multiple irregular brown spots were first observed on the leaves of this plant, which later developed into circular or near-circular purple spots with raised centers, or purple lesions along the veins. When the leaf spots coalesced, the value of the commodity was lost. To isolate the pathogen, 5 × 5-mm pieces of leaf tissue from the margins of lesions were surface disinfected in 75% alcohol, rinsed in distilled water, and incubated on potato dextrose agar plates at 28°C until sporulation. Using single-spore isolation, a pure culture (YY-1) was obtained with abundant grayish white hyphae that later turned olive green. The underside of the colonies was brown. Conidia were typically obclavate, had a short beak with one to six, but usually three, transverse septa, and up to three longitudinal septa. The transverse septum was thicker, and the wall of the conidium appeared brick-like. Conidia were pale brown, catenulate, and measured from 25 to 42 µm long by 6 to 10 µm wide. YY-1 was identified as *Alternaria* sp. based on morphological characteristics (Simmons 2007). Molecular identification was performed to detect the fungal species, and included the internal transcribed spacer (ITS), translation

elongation factor 1- α (EF1), actin gene (ACT), plasma membrane ATPase gene (ATP), and calmodulin gene (CAL), which were respectively amplified with primers ITS4/ITS5 (Guo et al. 2012), EF1-728F/EF1-986R (Carbone and Kohn 1999), ACT-512F/ACT-783R (Carbone and Kohn 1999), ATPDF1/ATPDR1, and CALDF1/CALDR1 (Lawrence et al. 2013) (GenBank accession nos. MN746334, MT627208, MT627209, MT558864, and MT558865). The species of *Alternaria* could not be confirmed by sequencing the above genes, as described previously (Zheng et al. 2015). Hence, a partial coding sequence of the histone 3 gene (GenBank accession no. MN744235) was amplified using primers H3-1a/H3-1b (Zheng et al. 2015), and it shared 98.09% sequence identity with *Alternaria tenuissima* (KP267543). The ITS sequence (MN746334) was 99.81% similar to the reference sequences of *A. tenuissima* (KT223327) in GenBank. A maximum-likelihood tree was then reconstructed based on the ITS, CAL, and ATP sequences by MEGA7, which showed that YY-1 was most closely related to *A. tenuissima*. Therefore, YY-1 was identified as *A. tenuissima* based on its morphological and molecular characteristics. To perform Koch's postulates, 20 healthy leaves from greenhouse-grown dandelion were inoculated with 5-µl drops of a conidial suspension (1×10^5 conidia/ml) of isolate YY-1. Sterile water was used as a control. The inoculated plants were placed in a growth chamber at 28°C and 80 to 90% relative humidity. After 10 days, similar symptoms were observed on plants inoculated with YY-1, whereas control plants did not produce symptoms. The pathogen was reisolated from the inoculated leaves and identified by morphological and molecular methods as *A. tenuissima*. To our knowledge, this is the first report of *A. tenuissima* causing purple spot on *T. mongolicum* in China.

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