Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Alternaria alternata* Causing Leaf Blight on *Clematis terniflora* var. *mandshurica* in China

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Clematis terniflora var. *mandshurica* (Rupr.) Ohwi, a traditional Chinese medicinal plant, is widely distributed in northeast China, such as in Heilongjiang, Jilin, and Liaoning Provinces. In August 2019, *C. terniflora* var. *mandshurica* leaf blight was found in Harbin (45.72°N, 126.68°E), Heilongjiang, China. The incidence was up to 90% on the 0.4 ha plantation, and almost every plant leaf was withered. Initial symptoms were chlorosis at the leaf apex or margin, and the leaf apex gradually formed brown lesions. As the infection progressed, the lesions expanded, and leaves withered. Ten symptomatic leaves were randomly collected from 10 plants in a *Clematis* plantation. The leaves (5 × 5 mm) between symptomatic and healthy tissue were cut and disinfected with 75% ethanol for 1 min and 1% NaClO for 2 min, rinsed three times in distilled water, plated on potato dextrose agar (PDA) on 90-mm-diameter Petri dishes, incubated for 7 days at 25°C, and further purified by the single-spore isolation method. The morphology of all 10 isolates was similar. The colonies were round or subround, 60- to 68-mm in diameter, velvety, and brownish green with distinct concentric rings. Conidiophores were solitary or clustered, suberect, brown, 30.3 to 82.4 × 2.5 to 5 µm (*n* = 150), and with a septum. Conidia were solitary or in chains, smooth surfaced, obclavate, ellipsoid, brown, 12.5 to 46.25 × 10 to 16.25 µm (*n* = 150), with two to seven transverse septa, and zero to three longitudinal septa. Conidia were produced continuously and extended in a synaxial manner. The morphological characters

matched those of *Alternaria alternata* (Simmons 2007). Two representative strains, TXL0816 and TXL0916, were used for molecular identification. The internal transcribed spacer (ITS) region, glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*), translation elongation factor 1- α (*tef1*), RNA polymerase second largest subunit (*RPB2*), and *Alternaria* major allergen (*Alt a 1*) were amplified with the primers ITS1/ITS4 (Iturrieta-González et al. 2020), RPB2-5F2/RPB2-7CR (Khodaei and Arzanlou 2013), Alt-for/Alt-rev (Hong et al. 2005), *gpd1/gpd2*, and EF1-728F/EF1-986R (Nishikawa and Nakashima 2020), respectively. The obtained sequences were deposited in GenBank (ITS: MW403865 and MT508666, *GAPDH*: MZ298638 and MZ298639, *tef1*: MZ298640 and MZ298641, *RPB2*: MW432545 and MT510746, *Alt a 1*: MW432544 and MT501719). The phylogenetic tree of combined sequences showed that isolates TXL0816, TXL0916, and *A. alternata* CBS916.96 were clustered together with 100% bootstrap values. To verify pathogenicity, 20 leaves of eight plants were sprayed with spore suspensions (1×10^6 spores/ml) of the 7-day-old isolates TXL0816 and TXL0916 (four plants were used as replicates for each isolate). As a control, four plants were sprayed with sterile distilled water. The plants were incubated at 25°C in a greenhouse. Twenty days after inoculation, brown lesions appeared on the leaf apex. The pathogenicity test was repeated three times. The fungi were reisolated from inoculated leaves with the same morphological and molecular traits as *A. alternata*, fulfilling Koch's postulates. No fungi were isolated from the control group. To our knowledge, this is the first report of *A. alternata* causing leaf blight on *C. terniflora* var. *mandshurica*. This study provides a basis for the pathogenesis of the leaf blight on *C. terniflora* var. *mandshurica* and helps to formulate control measures to reduce future economic losses.

References:

- Hong, S. G., et al. 2005. Fungal Genet. Biol. 42:119.
- Iturrieta-González, I., et al. 2020. Mycoses 63:212.
- Khodaei, S., and Arzanlou, M. 2013. Arch. Phytopathol. Plant Prot. 46:2224.
- Nishikawa, J., and Nakashima, C. 2020. Fungal Syst. Evol. 5:197.
- Simmons, E. G. 2007. *Alternaria: An Identification Manual*. CBS Fungal Biodiversity Centre, Utrecht, The Netherlands.

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