Diseases Caused by Fungi and Fungus-Like Organisms

First Report of Leaf Spot on Industrial Hemp (*Cannabis sativa*) Caused by *Alternaria alternata* in China

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Industrial hemp is an economically important plant with traditional uses for textiles, paper, building materials, food, and medicine (Li 1973; Russo et al. 2008; Zias et al. 1993). In August 2020, an estimated 80% of the industrial hemp plants with leaf spots were observed in a greenhouse in Minzhu town, Harbin City, Heilongjiang Province, China (45.8554°N, 126.8167°E), resulting in yield losses of 20%. Leaf symptoms began as small spots on the upper surface of leaves and gradually developed into brown spots with light yellow halos. These irregular spots expanded gradually and eventually covered the entire leaf; the center of the spots was easily perforated. To identify the pathogen, 20 diseased leaves were collected, and small sections of (3 to 5 mm) were taken from the margins of lesions of infected leaves. The pieces were sterilized with 75% alcohol for 30 s, a 0.1% mercuric chloride solution for 1 min, and then rinsed three times with sterile water. Samples were then cultured on potato dextrose agar at 28°C in darkness for 4 days. A single-spore culture was obtained by monospore isolation. Conidiophores were simple or branched, straight or flexuous, brown, and measured 22 to 61 µm long × 4 to 5 µm wide ($n = 50$). Conidia were solitary or in chains, brown or dark brown, obclavate, obpyriform or ellipsoid. Conidia ranged from 23 to 55 µm long × 10 to 15 µm wide ($n = 50$) with one to eight transverse and several longitudinal septa. For molecular identification (Jayawardena et al. 2019), genomic DNA of pathogenic isolate MZ1287 was

extracted by a cetyltrimethylammonium bromide protocol. Four gene regions including the rDNA internal transcribed spacer (ITS), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), translation elongation factor 1- α (TEF1), and RNA polymerase II beta subunit (RPB2) were amplified with primers ITS1/ITS4, GDF1/GDR1, EF1-728F/EF1-986R, and RPB2-5F/RPB2-7cR, respectively (White et al. 1990). Resulting sequences were deposited in GenBank with accession numbers MW272539.1, MW303956.1, MW415414.1 and MW415413.1, respectively. A BLASTn analysis showed 100% homology with *Alternaria alternata* (GenBank accession nos. MN615420.1, MH926018.1, MN615423.1, and KP124770.1), respectively. A neighbor-joining phylogenetic tree was constructed by combining all sequenced loci in MEGA7. The isolate MZ1287 clustered in the *A. alternata* clade with 100% bootstrap support. Thus, based on morphological (Simmons 2007) and molecular characteristics, the pathogen was identified as *A. alternata*. To test pathogenicity, leaves of 10 healthy, 2-month-old potted industrial hemp plants were sprayed using a conidial suspension (1×10^6 spores/ml). Control plants were sprayed with sterile water. All plants were incubated in a greenhouse at 25°C for a 16-h light and 8-h dark period at 90% relative humidity. The experiment was repeated three times. After 2 weeks, leaf spots of industrial hemp developed on the inoculated leaves, whereas the control plants remained asymptomatic. The *A. alternata* pathogen was reisolated from the diseased leaves on inoculated plants, fulfilling Koch's postulates. Based on morphology, sequencing, and pathogenicity testing, the pathogen was identified as *A. alternata*. To our knowledge, this is the first report of *A. alternata* causing leaf spot disease of industrial hemp (*Cannabis sativa* L.) in China and is worthy of our attention for the harm it may cause to industrial hemp production.

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