Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Alternaria alternata* Causing Leaf Spot on *Kadsura coccinea* in China

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Kadsura coccinea is a traditional Chinese medicinal plant that grows in southwest China. In June 2019, leaf spot on *K. coccinea* was observed in a greenhouse in Jingzhou, Hubei Province, China. Over 300 plants were surveyed, and nearly 20% of the plants were infected. The lesions were dark brown, irregular-shaped, and measured 6 to 10 mm in diameter. Symptomatic leaves were randomly collected for pathogen isolation. Diseased tissues were cut into small fragments, surface sterilized with 75% ethanol for 15 s and 1% NaClO for 2 min, and then rinsed in sterilized distilled water twice. After being dried on sterilized filter papers, the tissues were placed on potato dextrose agar (PDA) and incubated at 25°C in the dark for 5 days. Fungal colonies cultured on PDA were almost the same as each other, and a representative strain, YZU191238, was used for further identification. The strain was deposited in the Fungi Herbarium of Yangtze University, Jingzhou, Hubei, China. Colonies of the strain on PDA were white at the edge and olivaceous buff in the center and were 55 to 56 mm in diameter after 7 days. The conidial morphology of the strain was studied on potato carrot agar at 7 days of incubation at 22°C under an 8-h light/16-h dark cycle. Conidiophores were septate, straight, and measured 20 to 60 × 3 to 4 µm in size. Conidia were normally in chains, obclavate, ovoid or ellipsoid, 20 to 55 × 8 to 18 µm in size, with three to seven transverse septa ($n = 50$). Based on morphological characteristics, strain YZU191238 was

confirmed as *Alternaria* sp. (Simmons 2007). Genomic DNA of the fungus was extracted from 3-day-old culture grown on PDA. Four gene regions including the internal transcribed spacer (ITS) region of rDNA, translation elongation factor 1 alpha (TEF1), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), and the RNA polymerase second largest subunit (RPB2) were amplified with primer pairs ITS5/ITS4, EF1-728F/EF1-986R, gpd1/gpd2, and RPB2-5F/RPB2-7cR, respectively (Woudenberg et al. 2015). The resulting sequences were deposited in GenBank with accession numbers MN615420, MN615423, MN615421, and MN615422, respectively. Maximum likelihood analysis based on the dataset of the combined four gene sequences showed that strain YZU191238 was clustered with *Alternaria alternata* (CBS 479.90 and CBS 102600), supported by a 100% bootstrap value. In addition, all four sequences of the strain were 100% identical with those of CBS 479.90 (accession nos. KP124319, KP125095, KP124174, and KP124787). Pathogenicity testing was conducted on living leaves of *K. coccinea*. Mycelium plugs (3 mm in diameter) taken from 3-day-old culture on PDA were inoculated on surface-sterilized leaves (sprayed with 75% ethanol). PDA plugs with no mycelium were inoculated as the control. Treated leaves were covered with plastic bags and incubated in a greenhouse with a temperature of 25°C. The pathogenicity test was repeated twice. Small light-colored necrotic spots were observed on the inoculated leaves after 1 day of inoculation. The spots gradually turned to dark brown and enlarged to 8 to 9 mm in diameter after 3 days, similar to the symptoms observed in the field. The control plants remained symptomless. The fungus was recovered from infected leaves and confirmed as *A. alternata* based on conidial characteristics and RPB2 gene sequence. To our knowledge, this is the first report of *A. alternata* causing leaf spot on *K. coccinea* in China. The identification of this pathogen will be helpful to prevent and control this disease in the future.

References:

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