Diseases Caused by Fungi and Fungus-Like Organisms

Leaf Spot Disease of *Orychophragmus violaceus* Caused by *Alternaria tenuissima* in China

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Orychophragmus violaceus (L.) O. E. Schulz (OV, Brassicaceae), called February orchid or Chinese violet cress, is widely cultivated as a green manure and garden plant in China. In a rainy period in August 2020, leaf spot was observed in the garden of Northeast Agricultural University, Harbin, Heilongjiang province. One week after the rains, the disease intensified; incidence reached ~80%. Symptoms began as small brown spots on leaves and expanded to irregular or circular spots. Spots became withered with grayish-white centers and dark brown margins. The centers collapsed into holes. Sometimes, spots coalesced into large necrotic areas and caused defoliation. No conidiophores or hyphae were present, and symptoms were not observed on other tissues. Ten leaves with typical symptoms were collected from different plants. Leaf pieces (5 × 5 mm) were excised from the junction of diseased and healthy tissues, disinfected in 75% ethanol for 1 min, rinsed in sterile distilled water, and placed on PDA in Petri dishes (9 cm). After 3 days at 25°C in darkness, mycelia were transferred to fresh PDA and purified by single-spore isolation. Nine isolates (NEAU-1 to 9) with similar morphology were obtained; no other fungi were isolated. The isolation frequency was ~90%. On PDA, colonies were gray-white with loose, cottony aerial hyphae; they turned olive-green and eventually brown with gray-white margins. The fungus formed pale brown conidiophores with sparsely branched chains on potato carrot agar (PCA) plates after incubation at 25°C in darkness for 7 days. Conidia were ellipsoidal or ovoid, light brown, and 18.4 to 59.1 × 9.2 to 22.3 µm, with zero to two longitudinal and one to five transverse septa and with a cylindrical light brown beak ($n = 50$). Cultural and morphological characteristics indicated *Alternaria tenuissima* (Simmons 2007). Genomic DNA was extracted from mycelia of NEAU-1 to 5. The *ITS* region was amplified and sequenced using ITS1/ITS4 (White et al. 1990). BLAST analysis showed the isolates had the same *ITS* sequence, and that of NEAU-5 (GenBank MW139354) showed 100% identity with the type strains of *A. alternata*

CBS916.96 and *A. tenuissima* CBS918.96. *TEF*, *RPB2*, and *GPD* of NEAU-5 were amplified and sequenced using EF1-728F/EF1-986R (Carbone and Kohn 1999), RPB2-5F2/RPB2-5R (Sung et al. 2007), and Gpd1/Gpd2 (Berbee et al. 1999), respectively. *RPB2*, *GPD*, and *TEF* sequences were submitted to GenBank as MW401634, MW165223, and MW165221, respectively. Phylogenetic trees based on *ITS*, *RPB2*, *GPD*, and *TEF* were constructed with the neighbor-joining (NJ) and maximum-likelihood (ML) algorithms using MEGA v. 7.0. NEAU-5 formed a robust clade with *A. tenuissima* CBS918.96 (99 and 96% bootstrap values) in the NJ and ML trees. NEAU-5 produced rarely branched conidial chains on PCA, consistent with *A. tenuissima* (Kunze) Wiltshire but distinct from *A. alternata*'s abundant secondary ramification (Simmons 2007). Based on morphology and phylogeny, NEAU-5 is *A. tenuissima*. In pathogenicity tests, five unwounded leaves were inoculated with ~10⁶ conidia/ml suspension of NEAU-5 on five healthy plants in a garden, and five leaves on those plants with sterilized ddH₂O. Leaves were covered with clear plastic bags for 3 days. After 6 days, typical spots were observed on inoculated leaves but not controls. Tests were repeated thrice. Tests were also performed on 2-month-old potted plants in a growth chamber (28°C, 90% RH, 12 h light/12 h dark) with two repetitions. Five healthy plants were inoculated by spraying 20 ml of ~10⁶ conidia/ml suspension of NEAU-5 on unwounded leaves, and five with sterilized ddH₂O. After 7 days, symptoms were observed on inoculated but not control leaves. The pathogen was reisolated from inoculated leaves and identified as *A. tenuissima* by morphological and molecular methods. *A. tenuissima* could successfully infect unwounded leaves, indicating a direct interaction. High humidity and temperatures favor *Alternaria* leaf spot epidemics (Yang et al. 2018), which may explain the severe disease after prolonged rain. *A. brassicicola* and *A. japonica* caused leaf blight and spot disease on OV in Hebei and Jiangsu Provinces, respectively (Guo et al. 2019; Sein et al. 2020). Although they could lead to similar symptoms on OV leaves, it is easy to distinguish them by conidiophore morphology and *ITS* sequences. This is the first report of *A. tenuissima* causing leaf spot of OV in China.

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The author(s) declare no conflict of interest.

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