Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Stemphylium eturmiunum* Causing Leaf Spot and Black Spot on Apple in Zhaotong, Yunnan, China

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Apple is one of the largest fruit tree crop in the world, and China is the largest apple-producing country in the world. Zhaotong, Yunnan Province, is a typical cold, mountainous apple-producing area in China. However, apple production is threatened by various diseases throughout the growing season, and among them, apple leaf spot and fruit black spot are severe. In previous reports, the main pathogen causing apple leaf spot and fruit black spot was *Alternaria* spp. (Gur et al. 2017); however, different pathogens were identified. In the current study, seven red ‘Fuji’ apple fruits with typical black spot samples were collected randomly in Dongda company orchard, Sujiayuan town, Zhaotong, Yunnan, on March 25, 2021. The spots on the surface of these apples appeared rounded, the diseased parts turned brown or black, and the flesh became soft and rotten. Tissues of the fruit epidermis at the edge between diseased and healthy parts were cut, soaked in 75% alcohol for 30 s, washed with sterile water three times, and air dried. Five pieces of tissue were placed on PDA medium amended with rifampicin (50 mg/ml) and incubated in the dark at 25°C for 3 to 5 days. After colonies grew, mycelial clumps were picked out from the edges of the colonies, transferred to new PDA plates, and incubated at 25°C for 6 days. The diameter of the colonies reached up to 5.7 cm. A representative isolate was retained for further work and named P6-3-1. The hyphae were white and dense at an early stage, the culture medium on the underside became yellow, and the middle parts of the

colonies were darker. With maturity, hyphae were clumped and became red with other colors interspersed, and the medium became dark red. Light brown spores were produced, with more vertical septa and fewer transverse septa. Two or three transverse septa were generally observed with obvious constriction at the transverse septa. Average spore size was $22.83 \pm 2.04 \times 14.58 \pm 1.97 \mu\text{m}$. DNA was extracted from mycelium, purified, and amplified with two pairs of primers, ITS1/ITS4 (White et al. 1990) and gpdF/gpdR (Câmara et al. 2002). The PCR products were sequenced and deposited in GenBank (accession nos. OK560128 and OK627661). The similarity of ITS sequences between the isolate and MH843733 (*Stemphylium eturmiunum* strain ST14) was 100%, and that of gpd sequences between the isolate and MH843728 (*S. eturmiunum* strain ST20) was 100%. The maximum parsimony method of MEGA7.0 was used and demonstrated that the studied isolate converged to the same branch as *S. eturmiunum*. Koch’s postulates were applied to identify the pathogenicity of this isolate. A disc of P6-3-1 culture on PDA (5 mm in diameter) was placed on apple leaves and fruit wounds. Sterile PDA was used as a control. All plants were kept in a growth chamber at 25 to 30°C. Four days after inoculation, disease spots were observed on the inoculated sites and fruit, and with the extension of incubation time, the diseased spots continued to grow, and the leaf spots were not limited by the veins. The pathogen was reisolated from the inoculated leaves and fruit, satisfying Koch’s postulates. This pathogen can also cause postharvest rot of sweet cherry (Spadoni et al. 2020) and postharvest rot on tomato (Prencipe et al. 2021). This is the first report that *S. eturmiunum* can cause apple leaf spot and fruit black spot in Yunnan Province, China. The apple black spot caused by *S. eturmiunum* was accurately identified. By distinguishing between the two similar diseases mentioned above, resistance to the host and management practices can be determined based on the characteristics of the pathogen, its epidemiological pattern, and the choice of an effective chemical fungicide.

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