Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Epicoccum tobaicum* Associated with Leaf Spot on Flowering Cherry in South Korea

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Flowering cherry (FC, *Prunus × yedoensis* Matsumura; Somei-yoshino cherry) is an ornamental tree, planted across South Korea and producing stunning flowers in spring. The seasonal blooms are annually celebrated during cherry blossom festivals in many locations across the country. Leaf spot disease is among the most common and important diseases affecting FC trees every year, resulting in premature defoliation and reduced flowering of cherry blossoms in the following year. In May 2018, brown spots (2 to 5 mm), circular to irregular and with dark borders, were observed on FC leaves in Hadong, Gyeongsangnamdo, South Korea (35°07'48.9"N, 127°46'53.8"E), with a disease incidence of 55%. Single lesions often coalesced and were sometimes perforated, leaving shot holes. Sampled leaves were surface sterilized with 1% NaOCl for 1 min followed by 70% ethanol for 30 s and then rinsed twice with sterile distilled water. About 2-mm-long infected leaf pieces from the margins of lesions were put onto water agar (1.5% agar) plates and incubated at 25°C for 72 h. Mycelia grown from symptomatic tissue were transferred to potato dextrose agar (PDA) plates, and five similar fungal isolates were obtained from hyphal tips. They produced a strong reddish-orange diffusible pigment on PDA after 5 days and exudates after 8 days. Conidia were globular to pear-shaped, dark, verrucose, multicellular, and 14.8 to 23.5 µm in diameter (average = 18.7 µm, *n* = 30) for isolate JCK-CSHF10. These morphological characteristics were consistent with the *Epicoccum* genus. Three loci (ITS, *tub2*, and *rpb2*) from three isolates (JCK-CSHF8, JCK-CSHF9, and JCK-CSHF10) were amplified using the primer pairs ITS1F/LR5 (Gardes and Bruns 1993;

Vilgalys and Hester 1990), Btub2Fd/Btub4Rd (Woudenberg et al. 2009), and RPB2-5F2/RPB2-7cR (Liu et al. 1999; Sung et al. 2007), respectively. The ITS, *tub2*, and *rpb2* sequences of the three isolates were deposited in GenBank (MW368668 to MW368670, MW392083 to MW392085, and MW392086 to MW392088, respectively), showing 99.6 to 100% identity to *Epicoccum layuense* (E33), a later synonym for *E. tobaicum* (Hou et al. 2020). The phylogenetic tree using concatenated sequences of the three loci placed the three isolates in a cluster of *E. tobaicum* (CBS 232.59, CGMCC 3.18362, and CBS 384.36; Hou et al. 2020). Taken together, the three isolates were identified as *E. tobaicum*. The pathogenicity of JCK-CSHF10 was tested on 15 healthy leaves on three FC trees (cv. Somei-yoshino, 1.2 m in height) kept in a greenhouse. Five-millimeter-diameter plugs from 7-day-old fungal cultures grown on PDA or mycelia-free PDA plugs as controls were placed on the abaxial side of a leaf at three points, previously wounded by a sterile needle (Zlatković et al. 2016). Inoculation sites were covered with moist cotton plugs. Trees were then covered with a clear plastic bag and maintained in high humidity at 25°C in darkness for 24 h, followed by a 12-h photoperiod. Brown spots appeared on inoculated leaves after 7 days, identical to those observed in the field, whereas control leaves remained symptomless. This experiment was repeated three times. A fungus with the same morphology as JCK-CSHF10 was recovered from lesions, thus confirming Koch's postulates. *E. layuense* (syn. *E. tobaicum*) has been reported as a leaf spot-causing agent on *Perilla* sp. (Chen et al. 2017) and *Camellia sinensis* (Chen et al. 2020). To date, there is no report on the occurrence of *E. tobaicum* from leaf spots on FC. To our knowledge, this is the first report of *E. tobaicum* causing leaf spot on FC in South Korea.

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