Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Colletotrichum fruticicola* Causing Anthracnose on *Phoebe sheareri* in China

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Phoebe sheareri (Hemsl.) Gamble is a high-value timber tree species with a wide range of uses, including for landscape afforestation because of its beautiful shape and luxuriant branches. Previous studies found that *Neofusicum parvum* can cause ulceration and necrosis on the main stems of *P. sheareri* (Chen et al. 2019) and branch wilt of *P. zhennan* (Zhu et al. 2019). In September 2019, anthracnose was observed on *P. sheareri* leaves in Lishui, Zhejiang province, China. The diseased leaves are characterized by dark brown lesions. The infection usually starts from the leaf tip or edge, and then the infected leaves turn yellow, wither, and finally fall off. The infection sometimes occurs on small twigs, causing the whole branch to wither. Plants from 15 plantations were surveyed, and the disease incidence was about 30%. Fifteen freshly infected leaves were collected and cut into small pieces (5 × 5 mm), sterilized in 75% ethanol solution for 30 s, and washed with sterile water three times. They were further sanitized in 2% sodium hypochlorite solution for 2 to 3 min and dried in sterile filter paper after being washed with sterile water another three times. Leaf pieces were transferred to potato dextrose agar (PDA) plates and incubated at 25°C in the dark for 3 days. The fungal cultures were purified and a typical isolate (TJ01) was selected for further morphological characterization and DNA sequence comparison. The strain was initially grayish white, villous, and posteriorly grayish green, and it produced orange red spore clumps. Spores were single celled after maturation, oblong, and colorless, and 8.5 to 19.5 × 4.5 to 5.5 µm. Morphological characteristics

were consistent with those in the genus *Colletotrichum*. DNA of the isolate was extracted, and sequence data were obtained and compared with those downloaded from GenBank. The internal transcribed spacer (ITS), intronic glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*), actin (*ACT*), beta-tubulin (*TUB2*), and calmodulin (*CAL*) genes were partially amplified and sequenced using the primer pairs ITS-1 + ITS-4, GDF + GDR, ACT-512F + ACT-783R, T1 + Bt2b, and CL1C + CL2C, respectively (Fu et al. 2019). The resulting nucleotide sequences were individually subjected to BLAST searches in GenBank. The nucleotide sequences of ITS (MZ088144), *GAPDH* (MZ133607), *ACT* (MZ133608), *TUB2* (MZ133609), and *CAL* (MZ133610) of the isolate showed 99.83% similarity to the ITS sequence (MN829453), 98.24% similarity to the *GAPDH* sequence (MN525875), 98.94% similarity to the *ACT* sequence (MK341539), 100% similarity to the *TUB2* sequence (MG657352), and 100% similarity to the *CAL* sequence (MN525839). A phylogenetic tree was constructed using MEGA 7 based on the tandem of five genes (ITS-*GAPDH*-*ACT*-*TUB2*-*CAL*). It revealed that the isolate resides in the clade of *C. fruticicola* species. Inoculation was done on leaves of 10 *P. sheareri* plants in the field to verify its pathogenicity. Three healthy leaves of each plant were surface sterilized with 75% ethanol and dried. The leaves were punctured using a sterilized needle, inoculated with 5-mm-diameter PDA plugs excised from 7-day-old cultures, and wrapped with Parafilm. Nine pieces of healthy leaves inoculated with sterilized PDA plugs served as controls. Disease symptoms developed on all the *C. fruticicola*-inoculated leaves 5 days after inoculation, and a yellow brown lesion became apparent 16 days later, whereas the control leaves remained asymptomatic. *C. fruticicola* was reisolated from the lesions, but not from the control leaves, fulfilling Koch's postulates. This fungus is a well-known pathogen and has led to anthracnose on many plant species globally. However, our study is the first report of *C. fruticicola* causing anthracnose on *P. sheareri* worldwide, and its potential threat should be evaluated.

References:

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