Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Colletotrichum fioriniae* Causing Anthracnose on Mu Oil Tree in China

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Mu oil tree (*Vernicia montana*) is an economically important woody oil plant, which is widely distributed in southern China. In mid-May 2020, a leaf spot disease was observed on the leaves of mu oil tree in Taihe County in Jiangxi Province, China (26°55'25.55"N, 114°49'5.85"E). The disease incidence was estimated to be above 40%. Initial symptoms were circular red-brown spots that were 1 to 2 mm in diameter and then enlarged with a red-brown center. In later stages, the spots coalesced and formed large patches, and subsequently red-brown centers of lesions gradually dried and fell out, forming a "shot hole" appearance. To identify the pathogen, diseased leaves were collected from Taihe County. Leaf tissues (5 × 5 mm) were cut from the margins of typical symptomatic lesions, surface sterilized in 75% ethanol for 30 s and 3% sodium hypochlorite for 60 s, and then rinsed with sterile distilled water three times. Leaf pieces were placed on potato dextrose agar (PDA; 1.5%, Difco-BD Diagnostics) and incubated at 25°C in the dark. Pure cultures were obtained from individual conidia by recovering single spores. On PDA, colonies were initially white and cottony. The mycelia then became pinkish to deep-pink with time at the center on the front side and pink on the reverse side. Colonies produced pale orange conidial masses after 9 days. Conidia were fusiform with acute ends, smooth-walled, hyaline, and measured 3.6 to 5.5 × 8.1 to 14.5 μm (4.5 ± 0.5 × 10.6 ± 1.0 μm, *n* = 100). The morphological characteristics of the isolate matched the descriptions of *Colletotrichum acutatum* complex (Damm et al. 2012). For molecular identification,

the internal transcribed spacer (ITS) region, glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*), chitin synthase (*CHS-1*), beta-tubulin 2 (*TUB2*), and actin (*ACT*) were sequenced using the primers ITS1/ITS4, GDF/GDR, CHS-79F/CHS-345R, T1/Bt2b, and ACT-512F/ACT-783R, respectively (Weir et al. 2012). The obtained sequences were deposited in GenBank (accession nos. MW584317 [ITS], MW656269 [*GAPDH*], MW656270 [*TUB2*], MW656268 [*CHS-1*], and MW656267 [*ACT*]). All the sequences showed 94 to 100% similarity with those of *Colletotrichum fioriniae*. A neighbor-joining phylogenetic tree was generated by combining all the sequenced loci using MEGA7.0 (Kumar et al. 2016). The isolate TH-M4 clustered with *C. fioriniae*, having 99% bootstrap support. Based on the morphology and multigene phylogeny, isolate TH-M4 was identified as *C. fioriniae* (Damm et al. 2012). To confirm pathogenicity, 20 healthy leaves of 10 mu oil trees (3 years old) grown outdoors were inoculated with a drop of spore suspension (10⁶ conidia/ml) of the isolate TH-M4 in September 2020. Another 10 plants were inoculated with sterile water as the control. The leaves were wounded with a sterile toothpick. All the inoculated leaves were covered with black plastic bags to maintain humidity for 2 days. The pathogenicity test was repeated twice. The resulting symptoms were similar to those on the original infected plants, whereas the control leaves remained asymptomatic. The same fungus was reisolated from the lesions on the inoculated plant, fulfilling Koch's postulates. *C. fioriniae* has been recorded as an anthracnose pathogen on *Mahonia aquifolium* (Garibaldi et al. 2020), *Paeonia lactiflora* (Park et al. 2020), *Solanum melongena* (Xu et al. 2018), and *Juglans regia* (Varjas et al. 2019). To our knowledge, this is the first report of *C. fioriniae* associated with leaf spot disease on mu oil tree in China. This study provided crucial information for epidemiologic studies and appropriate control strategies for this oil plant disease.

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