Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Diaporthe fusicola* Causing Leaf Blotch of *Osmanthus fragrans* in China

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Osmanthus fragrans Lour. is widely distributed in China, Japan, Thailand, and India (Zang et al. 2003) and one of the top 10 most well-known flowering plants in China. Since February 2017, a foliar disease, with a disease incidence of ~60%, occurred on *O. fragrans* in a community park in Luzhai, Guangxi, China. Symptoms began as round or irregular small yellow spots and became pale brown to gray-brown with time. Small leaf tissues (3 to 4 mm²) cut from lesion margins were surface sterilized in 75% ethanol for 30 s and 1% NaClO for 90 s before they were rinsed in ddH₂O and dried on sterilized filter paper. After drying, the sterilized tissues were plated on potato dextrose agar (PDA) and incubated at 25°C in the dark for 5 days. Five single-spore isolates were obtained, and a representative isolate (GH3) was selected and deposited in the China Forestry Culture Collection Center. The colony on PDA was white with concentric zonation and white aerial mycelia, but the reverse was yellow. Black pycnidia developed on alfalfa extract + Czapek medium at 25°C with a 14-h/10-h light/dark cycle after 17 days. Conidiophores were hyaline, branched, septate, straight to sinuous, 12.4 to 24 × 1.9 to 2.5 µm (*n* = 20). The conidia were fusoid, hyaline, smooth, mostly two guttules, and measured 7.2 ± 0.7 × 2.3 ± 0.2 µm (*n* = 50). The morphological characters of pycnidia, conidiophores, and conidia of all five isolates matched those of *Diaporthe* spp. (Gomes et al. 2013). DNA of isolates GH3, GH7, and GH8 was extracted, and the internal transcribed spacer region (ITS) and partial sequences of elongation factor 1-α (*EF1-α*), calmodulin (*CAL*), beta-tubulin (*β-tub*), and histone H3 (*HIS*) genes were amplified with primers ITS1/ITS4 (White

et al. 1990), *EF1-728F/EF1-986R*, *CAL228F/CAL737R* (Carbone et al. 1999), *β2a/β2b*, and *CYLH3F/H3-1b* (Crous et al. 2004; Glass and Donaldson 1995), respectively. The sequences of GH3, GH7, and GH8 were deposited in GenBank (GH3: accession nos. MT499213 for ITS, MT506473 to MT506476 for *EF1-α*, *β-tub*, *HIS*, and *CAL*; GH7: MT856374 and MT860397 to MT860400; GH8: MT856375 and MT860401 to MT860404). BLAST results showed that the ITS, *EF1-α*, *β-tub*, *HIS*, and *CAL* sequences of GH3 were highly similar to sequences of *Phomopsis* sp. (LC168784 [ITS], identity = 506/506 [100%]), *Diaporthe fusicola* (MK654863 [*EF1-α*], identity = 274/275 [99%]), *D. amygdali* (MK570513 [*β-tub*], identity = 461/461 [100%]), *D. fusicola* (MK726253 [*HIS*], identity = 403/403 [100%]), and *D. amygdali* (KC343263 [*CAL*], identity = 428/428 [100%]), respectively. Maximum likelihood and Bayesian posterior probability analyses using IQ-Tree version 1.6.8 and Mr. Bayes version 3.2.6 with the concatenated sequences placed isolates GH3, GH7, and GH8 in the *D. fusicola* cluster and separated them from *D. eres* and *D. osmanthi*, which were previously reported from *Osmanthus* spp. (Gomes et al. 2013; Long et al. 2019). Based on the multigene phylogeny and morphology, all three isolates were identified as *D. fusicola*. The pathogenicity of GH3 was tested on 1-year-old seedlings of *O. fragrans*. Healthy leaves were wounded with a sterile needle and then inoculated with either 5-mm mycelial plugs cut from the edge of a 5-day-old culture of GH3 or 10 µl of conidial suspension (10⁶ conidia/ml). Control leaves were treated with PDA plugs or ddH₂O. Three plants were used for each treatment. The plants were covered with a plastic bag after inoculation, and sterilized H₂O was sprayed into the bags twice per day to maintain humidity; they were kept in a greenhouse at day/night temperatures of 25 ± 2°C/16 ± 2°C. Lesions appeared 3 days later. No lesions were observed on control leaves. The same fungus was reisolated from lesions. This is the first report of *D. fusicola* causing leaf blotch on *O. fragrans*. These results form the basis for developing effective strategies for monitoring and managing this potential high-risk disease.

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