Diseases Caused by Fungi and Fungus-Like Organisms

Fusarium acuminatum Associated with Root Rot of Maidong (*Ophiopogon japonicus*) in China

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Ophiopogon japonicus (Linn. f.) is a perennial evergreen in the Liliaceae family that is cultivated in many provinces of China due to its high medicinal and economic value. In April 2019, an unknown root rot disease was observed on the rhizomes of *O. japonicus* in a commercial production field in Xiangyang City (30.83° N, 112.53° E), Hubei Province. Disease incidence was approximately 10 to 20%. Symptoms included chlorosis, drooping, and rolling of the leaves followed by rapid death of the entire plant. Infected roots appeared to be softened, necrotic, and shriveled with reddish fungal growth. Infected tissues were disinfested on the surface with 75% ethanol for 30 s and 0.1% HgCl₂ for 1 min, rinsed with sterile distilled water, and dried. Small pieces (2 × 2 mm) were then excised from disinfested tissue and incubated on potato dextrose agar (PDA) medium at 25°C in the dark. After 3 days of incubation, six isolates with 75% isolation rate and the same colony morphology were subcultured and purified by hyphal tip isolation. Purified cultures grew rapidly, and media plates (70 × 70 mm) were covered with hyphae after 3 to 4 days. Cultures were initially white and became pink or red over 5 days. Microconidia were not observed. Macroconidia were produced from monophialides on branched conidiophores, which were slender, equilaterally curved, and

measured 32.5 to 53.5 µm in length and 3.5 to 5.1 µm in width, with three to five septa. All strains were preliminarily identified as *Fusarium acuminatum* (Leslie and Summerell 2006) on the basis of morphology. To confirm the identity of the pathogen, molecular identification was performed with strain MD1. Following DNA extraction, PCR was performed using the TSINGKE 2×T5 Direct PCR Mix kit. Target areas of amplification were internal transcribed spacer (ITS), RNA polymerase second largest subunit (RPB2), and beta-tubulin gene (TUB2) regions of rDNA, using ITS1/4 (Yin et al. 2012), RPB2-5f2/7cr (O'Donnell et al. 2010), and Btu-F-F01/Btu-F-R01 primers (Wang et al. 2014), respectively. Nucleotide sequences were deposited in NCBI (GenBank MT525360.1, MW164629, MT588110.1). BLAST analysis of the ITS sequence had 100% similarity to a 517-bp portion of *F. acuminatum* sequence in GenBank (MK764994.1); the RPB2 sequence had 100% similarity to a 687-bp portion of *F. acuminatum* sequence in GenBank (HM068330.1); and the TUB2 sequence had 99% similarity to a 964-bp portion of *F. acuminatum* sequence in GenBank (KT965741.1). A pathogenicity test was performed in the laboratory on *O. japonicus* roots with isolate MD1. Mycelial plugs (5 mm) were excised from the margin of colony cultured for 5 days and were placed on 3-year-old tuberous roots, covered with wet sterile cotton, and kept at 25°C under 80% relative humidity. Controls were inoculated with noncolonized PDA plugs (5 mm). All treatments had three replicate plants. On inoculated plants, white hyphae covered *O. japonicus* roots at 3 days postinoculation (DPI) and became pink, and by 5 DPI roots had rot symptoms. By comparison, the control plants had no symptoms. The pathogen was reisolated from the inoculated roots and exhibited the same morphological characteristics and ITS sequence as those of *F. acuminatum*. *F. acuminatum* was reported to cause fruit rot on postharvest pumpkin and *Vaccinium corymbosum* in China (Li et al. 2019; Wang et al. 2016). To our knowledge, this is the first report of root rot caused by *F. acuminatum* on *O. japonicus* in China.

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