Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Phoma herbarum* Causing Leaf Spot on *Rhapis humilis* in China

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Rhapis humilis Blume is an ornamental plant for landscaping that is widely distributed in China. In February 2020, a leaf spot disease was observed on *R. humilis* in a nursery shed in Zhanjiang (21.17°N, 110.18°E), Guangdong, China. The disease incidence was more than 90%. The early symptom was small water-soaked lesions, which then turned into black necrotic spots. Eventually, the individual lesions coalesced into larger ones, leading to the death of diseased leaves. Ten diseased leaves were collected from the nursery. The diseased tissues were cut into 2 × 2-mm pieces, surface disinfected with 75% ethanol for 30 s and 2% sodium hypochlorite for 60 s, and then rinsed three times with sterile water before pathogen isolation. The tissues were plated on potato dextrose agar (PDA) medium and incubated at 28°C in the dark for 4 days. Pure cultures were produced by transferring hyphal tips to new PDA plates. Three isolates (RHPH-1, RHPH-2, and RHPH-3) were obtained. The colonies of the isolates were approximately 5 cm in diameter after 7 days. They were initially whitish and later became grayish white. The NaOH testing on MEA cultures was negative. No sporulation was detected after 30 days. The fertile structures of the specimens collected in the nursery were examined. Pycnidia were globose, measured 68 to 265 × 72 to 360 μm ($n = 20$), and mostly embedded. Conidia were aseptate, hyaline, and ellipsoid, measuring 3.6 to 6.5 × 2.2 to 2.7 μm ($n = 30$). Based on the morphological characteristics, the fungus was identified as in genus *Phoma* (Boerema et al. 2004). For molecular identification, the colony PCR method with MightyAmp DNA Polymerase (Takara-Bio, Dalian, China) (Lu et al. 2012) was used to amplify the internal transcribed spacer (ITS), partial RNA polymerase II largest subunit (RPB2), and beta-tubulin (β-tub) loci of three isolates using

primer pairs ITS4/ITS5, RPB2-6F/RPB2-7R, and BT2a/BT2b, respectively (Chen et al. 2015; White et al. 1990). The sequences were deposited in GenBank (ITS, MZ419364 to MZ419366; RPB2, MZ562293 to MZ562295; and β-tub, MZ562296 to MZ562298). Based on BLAST analysis, the sequences of the ITS, RPB2, and β-tub all showed 100% similarity to *Phoma herbarum* Westend. (CBS 377.92; accession nos. KT389536 for ITS, KT389663 for RPB2, and KT389837 for β-tub). Pathogenicity testing was performed in a greenhouse with 80% relative humidity at 25 to 30°C. Ten healthy plants of *R. humilis* were grown in pots, with one plant in each pot. The leaves were pinpricked with sterile needles before inoculation. They were inoculated with mycelial plugs of the isolates or sterile agar plugs (as control), with four plugs for each leaf. Five plants were used in each treatment. Disease symptoms similar to those in the nursery were observed on the inoculated plants 2 weeks after inoculation, whereas the control plants remained healthy. The fungus was reisolated from the symptomatic leaves and confirmed as *P. herbarum* by morphology and ITS analysis. *P. herbarum* was reported to cause leaf spot on *Atractylodes lancea*, *Camellia sinensis*, *Elaeis guineensis*, *Lilium brownii*, and *Vetiveria zizanioides* in China; *Bituminaria bituminosa*, *Glycine max*, *Medicago sativa*, and *Pisum sativum* in Australia; and *Salvia nemorosa* in Italy (Li et al. 2011, 2012; Thangaraj et al. 2018). To the authors' knowledge, the present study was the first to report *P. herbarum* causing leaf spot on *R. humilis* in China. *P. herbarum* seriously affected the supply of seedlings in *R. humilis*, and its epidemiology on *R. humilis* should be further studied.

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