Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Colletotrichum fruticola* Causing Anthracnose on Water Hyacinth in China

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Water hyacinth (*Eichhornia crassipes*), a highly invasive aquatic weed, has caused widespread problems for the water bodies and water resources, particularly in China. Plant pathogens are a promising alternative as biocontrol agents (Dagno et al. 2011), but success in this strategy will require the selection of some highly virulent pathogen strains. In September 2020, irregular necrotic lesions on leaves and stems, as well as crown and petiole rot symptoms, occurred on water hyacinth, in Minjiang and Xiyuanjiang watershed, in Fuzhou, China. Fragments from symptomatic leaf tissue (5 × 5 mm) were superficially disinfected in 0.1% HgCl₂ solution for 30 s, followed by rinsing three times in sterile water, placed on potato dextrose agar, and then incubated in darkness at 28°C for 5 days. Two fungal isolates (F3 and F11) were recovered; pure cultures were obtained from the affected leaves and deposited in the Institute of Oceanography, Minjiang University. The colonies were stale, with felted, dense, pale gray aerial mycelium, scattered dark based acervuli with orange conidial masses near the center; in reverse side pinkish orange with patches of gray pigment near the center. The hyphae were septate, branched, and 2 to 6 µm in width. Appressoria were not observed. Conidiogenous cells were 20 to 24 × 3.5 to 4.5 µm, cylindric to flask-shaped, toward margin the conidiophores with a much looser structure, conidiogenous loci at apex and often also at septa. Asci were 60 to 80 × 15 to 20 µm, cylindric to subfusoid, eight-spored. Ascospores were 17 to 23 × 4 to 6 µm, gently curved, tapering to quite narrow, rounded ends. Perithecia mature after about 15 days, and were dark brown, subglobose, and 50 to 150 µm in diameter, and with scattered, dark brown setae about 50 to 80 µm long. Conidia were 15 to 25 × 4.5 to 6 µm, unicellular, colorless, and cylindrical to fusiform. Genomic DNA from two isolates

was extracted with a modified DNA Midi Kit (Tiangen, Beijing, China) and amplified using ITS4/ITS1F, CL1/CL2A, CHS-79F/CHS-345R, T1/T2, and GDF/GDR primers by PCR (Weir et al. 2012; White et al. 1990). Sequences of F3 and F11 were submitted to GenBank (accession nos.: ITS, MW307302, MW307303; CAL, MW303427, MW303429; CHS-1, MW303428, MW303430; TUB, MW531006, MW531007; and GAPDH, MW531008, MW531009). A phylogenetic tree using the maximum likelihood methods and including ITS-CHS-CAL-TUB-GAPDH concatenated sequences from *Colletotrichum gloeosporioides* complex was obtained (Cai et al. 2009; Damm et al. 2018; Weir et al. 2012). Phylogenetic analyses revealed that isolates F3 and F11 were grouped into the clade *C. fruticola*. To test Koch's postulates, conidial suspensions (10⁷ CFU/ml) of the isolates F3 and F11 were microinjected into 20 water hyacinth seedlings per isolate. Another 20 seedlings were injected with water without conidia as a control. Inoculated plants were kept in 50-liter plastic tanks and maintained in a greenhouse at room temperature (19 to 24°C) for 2 weeks. Koch's postulates were conducted twice. After 10 days, typical anthracnose symptoms similar to the field appeared on the inoculated leaves, whereas the control leaves remained asymptomatic. *C. fruticola* was reisolated and identified by microscopy, PCR, and sequencing, but not from noninoculated controls. Anthracnose disease caused by *C. fruticola* has been reported affecting numerous plants worldwide, including cotton, coffee, grape, citrus, and so on (Guarnaccia et al. 2017). However, to our knowledge, this is the first report of *C. fruticola* causing anthracnose on water hyacinth in China. Further studies for the efficacy of *C. fruticola* and/or of the genus *Colletotrichum* as biocontrol agent for water hyacinth or another aquatic plant are required (Dagno et al. 2012; Ding et al. 2007).

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