Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Stagonosporopsis vannaccii* Causing Leaf Spot on *Crassocephalum crepidioides* in China

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Crassocephalum crepidioides (Benth.) S. Moore, native to tropical Africa, is an important invasive weed in many countries, seriously threatening the agricultural ecosystem. In December 2018, 100% of *C. crepidioides* plants exhibited leaf spots in the kudzu (*Pueraria lobata*) garden in Tianlin County, Baise City, Guangxi, China (24°40'20.42"N, 106°11'33.51"E), but kudzu was not affected. Spots were small and brown, surrounded by a yellow-green halo initially, enlarged to subrotund or irregular in shape, slightly sunken, developed as a dark brown to dark spot with gray-white necrotic center, and exuded an orange droplet under humid conditions. Symptomatic leaf tissues were cut in 5 × 5-mm pieces from the junction of necrotic and healthy tissues, disinfected in 75% ethanol for 30 s and 0.1% HgCl₂ for 30 s, and rinsed with sterile water three times. Tissues were plated on PDA and incubated at 28°C under natural sunlight conditions. Four isolates with similar morphology were obtained after purification. Colonies exhibited creme-orange margins, and aerial mycelium was sparse. Colonies formed concentric circles on the surface that were fusco-black, violet-slate, and vinaceous-gray (from center to edge), fusco-black on the reverse after 7 days, and then pycnidia and conidia produced for ~30 days. Pycnidia of representative isolate YTH-12 were black, subglobose, and unilocular, 95.60 to 168.27 µm (avg. 128.32 µm) (*n* = 40) in diameter. The ostiole was single and central, slightly papillate to papillate, and occasionally rostrate. Conidia were hyaline, oval to elliptical, aseptate, 2.30 to 5.83 × 1.42 to 3.50 µm (avg. 4.36 × 2.03 µm) (*n* = 50). Morphology is consistent with *Stagonosporopsis vannaccii* (Crous et al. 2019). Then, the rDNA ITS region, 28S LSU rRNA, *RPB2*, and *TUB2* genes were amplified by PCR with primer pairs ITS1/ITS4 (White et al. 1990), LR0R (Rehner and Samuels 1994)/LR7 (Vilgalys and Hester 1990), Btub2Fd/Btub4Rd (Woudenberg et al. 2009), and RPB2-5F2 (Sung et al. 2007)/RPB2-7cR (Liu et al. 1999), respectively. PCR products were

purified and sequenced by Sangon Biotech (China). Sequences were deposited in GenBank (MN892355, MN893911, MN905510, MN905511). ITS (522 bp), LSU (1,313 bp), *TUB2* (380 bp), and *RPB2* (1193 bp) nucleotide sequences showed 100% identity to *S. vannaccii* strain LFNO148 (MK519453, MK519452, MK519454, MN534891). Phylogenetic analysis based on the multilocus sequences of ITS, LSU, *RPB2*, and *TUB2* was performed in MEGA 6.0 (Chen et al. 2015). Relative stability of the branches was evaluated by bootstrapping with 1,000 replications. YTH-12 was placed in the same clade as *S. vannaccii* with 100% bootstrap support. Based on morphology and molecular analyses, it was identified as *S. vannaccii*. To satisfy Koch's postulates, YTH-12 was inoculated on *C. crepidioides* leaves. Twenty punctured leaves and 20 unwounded leaves were inoculated with a 5-mm-diameter mycelial disc, respectively. Leaves inoculated with sterile PDA discs were blank controls. Plants were maintained in a growth chamber (25 to 28°C, 80 to 90% RH). Brown spots were observed on inoculated leaves (both punctured and unwounded) ~30 h after inoculation (AI); typical symptoms appeared ~55 h AI and black pycnidia and orange droplets 10 days AI. All inoculated leaves developed symptoms similar to naturally infected plants in the garden, and incidence reached 100%; control leaves remained symptomless. The same fungus was reisolated from inoculated leaves. This is the first report of *S. vannaccii* causing leaf spot on *C. crepidioides* in China. So far, *S. vannaccii* has been reported as a plant pathogenic fungus only in Brazil, causing anthracnose on soybean pods (Crous et al. 2019). *C. crepidioides* is a widely distributed weed. If *S. vannaccii* has strong host specificity, it could be used as a biocontrol fungus. Conversely, if *C. crepidioides* has a wider host range, it may act as a bridge to spread the pathogen. This study deepens the understanding of *S. vannaccii* and its associated plant diseases.

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