Diseases Caused by Fungi and Fungus-Like Organisms

First Report of Leaf Spot Caused by *Colletotrichum fructicola* on *Callerya speciosa* (*Millettia speciosa*) in Guangxi, China

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Callerya speciosa Champ is a climbing shrub in the Fabaceae. It is widely grown in Guangxi, Guangdong, and Hainan provinces in China and so on, and it is widely cultivated for its tubers, which are edible and are reported to have antibacterial and antioxidant effects (Yin et al. 2010). In September 2014, leaf spot symptoms were observed on foliage of *C. speciosa* in a plantation in Nanning, Guangxi, China. In a 1,300-m² cultivated area in Guangxi Agricultural Vocational College, there was a more than 50% incidence of leaf spots in the 700 two-year-old plants in this field. The disease severity varied between plants; some leaves showed small spots, and some young leaves were entirely blighted. Initial symptoms appeared as greenish yellow foliar spots, which turned brown and developed into circular, diamond, or elliptical shaped lesions, surrounded by yellow halos. Numerous acervuli were observed in the lesions at later stages. Acervuli were mainly epiphyllous, with two to three dark brown septate acicular setae. Diseased 2-mm-long leaf segments were surface sterilized in 75% ethanol for 30 s, in 0.1% HgCl₂ for 30 s, rinsed with sterile distilled water, and plated on potato dextrose agar (PDA). A

single *Colletotrichum* species morphotype was consistently recovered, with an 85% isolation rate from among 20 leaf pieces. On PDA, the colonies were white to light gray and black brown with abundant aerial hyphae. A gelatinous layer of conidiogenous cells not delimited in obvious sporodochia and lacking setae was observed developing on portions of the colonies forming on PDA plates. Conidia were elliptical, aseptate, and hyaline, $13.8 \pm 0.2 \times 6.5 \pm 0.1 \mu\text{m}$ ($n = 100$). A single-spore isolate, NDL-3, was selected for molecular identification. The rDNA internal transcribed spacer region (ITS), actin (ACT), Apn2-Mat1-2 intergenic spacer and partial mating type (Mat1-2) (ApMat), calmodulin (CAL), chitin synthase (CHS-1), and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) genes (Weir et al. 2012) were sequenced (deposited in GenBank as accession nos. MH721408, MH719084, MK610437, MK636569, MK592616, and MK592617, respectively). Phylogenetic analysis of NDL-3 by MrBayes (version 3.2.6) based on a concatenation of multiple genes sequences (ITS, ACT, CAL, CHS-1, and GAPDH) and the ApMat gene locus plus related sequences obtained from GenBank revealed that the isolate was *C. fructicola* (Weir et al. 2012). Pathogenicity tests were conducted on 30 attached artificially wounded leaves of three young healthy 2-year-old plants with 20- μl droplets of conidial suspension (10^6 spores/ml) per wound site. Wounded leaves were also mock inoculated with 20- μl droplets of water as controls. Inoculated plants were sprayed with water and throughout the incubation were always covered with plastic bags. After 2 weeks of incubation at 28°C, typical symptoms were seen on inoculated leaves but none on control leaves. Koch's postulates were fulfilled by reisolation of *C. fructicola* from diseased leaves. Although *C. fructicola* is known to occur on many different hosts, this is the first report of *C. fructicola* infecting *C. speciosa* in China. This research may accelerate the development of future epidemiological studies and management strategies for anthracnose caused by *C. fructicola* on *C. speciosa*.

References:

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