Diseases Caused by Fungi and Fungus-Like Organisms

First Report of Leaf Blotch of *Aesculus chinensis* Caused by *Colletotrichum gloeosporioides* and *Colletotrichum fruticicola* in China

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Aesculus chinensis Bunge is an important landscaping tree widely planted in China. Since 2019, a foliar disease, with a disease incidence of 100%, occurred on *A. chinensis* at the campus of Nanjing Forestry University. The brown necrotic lesions with a dark margin and exuding orange spore masses under humid conditions mostly appeared at leaf tips and edges. Small leaf tissues (3 to 4 mm²) from lesion margins were surface sterilized with 75% ethanol for 30 s and 1% NaClO for 90 s, rinsed with sterile dH₂O three times, and placed on potato dextrose agar (PDA) at 25°C. Using monospore isolation, six isolates were obtained, and two representative isolates (QYS2-1 and QYS3-1-2) were used for further study. On PDA, the QYS2-1 colony was white and pale orange toward the center on the reverse side. The conidia were one-celled, straight, hyaline, subcylindrical with rounded ends, and measured $16.6 \pm 1.7 \times 5.9 \pm 0.6 \mu\text{m}$ ($n = 50$). Appressoria were one-celled, pale brown, thick-walled, ellipsoidal, and $8.2 \pm 0.6 \times 5.8 \pm 0.5 \mu\text{m}$ ($n = 50$). The QYS3-1-2 colony was grayish-green with white edges and dark green on the reverse side, and the conidia and appressoria were almost identical to those of QYS2-1 with sizes of $16.0 \pm 1.0 \times 5.8 \pm 0.3 \mu\text{m}$ ($n = 50$) and $7.8 \pm 0.6 \times 6.1 \pm 0.4 \mu\text{m}$ ($n = 50$), respectively. The morphological characteristics of QYS2-1 and QYS3-1-2 fitted with the description of the *Colletotrichum gloeosporioides* complex (Weir et al. 2012). The internal transcribed spacer (ITS) region

and the genes encoding glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*), chitin synthase (*CHS*), and actin (*ACT*) were independently amplified with primers ITS1/ITS4 (White et al. 1990), GDF/GDR (Silva et al. 2012), CHS-79F/CHS-345R (Carbone and Kohn 1999), and ACT-512F/ACT-783R (Templeton et al. 1992), respectively. The sequences were deposited in GenBank (accession nos. MN901256 and MT113110 to MT113112 for QYS2-1; MN901251 and MT113107 to MT113109 for QYS3-1-2). The results of BLASTn showed that the ITS, *ACT*, *CHS*, and *GAPDH* consensus sequences of QYS2-1 were 100, 99, 99, and 100% identical to those of *C. gloeosporioides* (JX073027, KF772083, MK514478, and MG561658, respectively). For QYS3-1-2, BLAST analysis with the same loci showed 100, 100, 98, and 99% homology with those of *Colletotrichum fruticicola* (MN295193, MG657349, MH151146, and MH270793, respectively). A maximum likelihood-based phylogenetic analysis performed using MEGA7 with these concatenated sequences placed QYS2-1 and QYS3-1-2 in the clades of *C. gloeosporioides* sensu stricto (s.s.) and *C. fruticicola*, respectively. The pathogenicity of the two species was verified on leaves inoculated with 5-mm mycelial plugs cut from the edge of 5-day-old cultures on PDA and 10 μl of spore suspension (10^6 conidia/ml), each performed with 10 seedlings (five for mycelia, and five for conidia). Controls were treated with PDA plugs or sterile dH₂O. The inocula were placed at a distance of 2 to 3 cm on the leaves, which were wounded with a sterile needle. Inoculated plants were kept in a greenhouse (relative humidity >80%, $25 \pm 2^\circ\text{C}$). Within 5 days, all the inoculated points showed lesions similar to those observed outdoors, whereas controls were asymptomatic. Recovery rates of QYS2-1 and QYS3-1-2 were 100% for each isolate. This is the first report of *C. gloeosporioides* s.s. and *C. fruticicola* causing leaf blotch on *A. chinensis* in China. These data will help develop effective strategies for managing this disease.

References:

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