Diseases Caused by Fungi and Fungus-Like Organisms

First Report of Leaf Spot Disease Caused by *Colletotrichum gloeosporioides* on *Chaenomeles sinensis* in China

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Chaenomeles sinensis is a shrub or small arbor of the genus *Chaenomeles* in Rosaceae, which is widely planted in China. It is a kind of garden ornamental plant and has high economic value. Since 2020, a leaf disease occurred on the foliage of *C. sinensis* at the campus of Nanjing Forestry University, Nanjing, China (32°04'34.53"N, 118°48'42.06"E). After investigating, *C. sinensis* was found with leaf spot disease at a 100% infection rate, which caused gigantic ornamental loss. Leaf spots are round to irregular, distributed on the leaves; in addition, the color of spots is brown. There are yellow halos on the edge of the lesion. Small leaf tissues (3 to 4 mm²) from lesion margins were surface sterilized with 75% ethanol for 30 s and then rinsed with sterile dH₂O three times. Afterward, they were placed on potato dextrose agar (PDA) at 25°C. Pure cultures were obtained by monospore isolation, and a representative isolate (NJTI.1) was obtained. When cultured on PDA, the colony of NJTI.1 was white and cottony. On the reverse side, the color of the colony nearly light yellow. The colony was placed in the liquid carboxymethyl cellulose medium. After culturing for 24 h in a shaker at 25°C and 150 rpm, the spore liquid was

taken by us. The conidia were one-celled, straight, hyaline, subcylindrical with rounded ends, and measured 15.1 to 23.6 × 5.4 to 7.9 μm (*n* = 30). Appressoria were one-celled, brown, thick-walled, ellipsoidal, and measured 7.7 to 13.8 × 6.4 to 10.3 μm (*n* = 30). The morphological characteristics of NJTI.1 fitted with the description of the *Colletotrichum gloeosporioides* complex (Weir et al. 2012). For accurate identification, the internal transcribed spacer (ITS) and the genes encoding glyceraldehyde-3-phosphate dehydrogenase (GAPDH), actin (ACT), and chitin synthase (CHS-1) were amplified with primers ITS1/ITS4, GDF/GDR, ACT-512F/ACT-783R, and CHS-79F/CHS-345R (Zhu et al. 2019), respectively. The sequences were deposited in GenBank (accession nos. MT984264, MW030495, MW030496, and MW030497 for NJTI.1). A BLAST search of GenBank showed that ITS, GAPDH, ACT, and CHS-1 sequences of NJTI.1 were 99, 99, 100, and 100% identical to those of *C. gloeosporioides* (MH571757.1, KY995355.1, MN058143.1, and MN313581.1). A neighbor-joining phylogenetic tree was generated by combining all sequenced loci in MEGA7. The isolate NJTI.1 clustered in the *C. gloeosporioides* clade with 99% bootstrap support. The pathogenicity of NJTI.1 was verified both on detached and living leaves. The detached leaves were inoculated with 5-mm mycelial plugs cut from the edge of 6-day-old cultures on PDA and 20 μl of spore suspension (10⁶ conidia/ml), and each treatment had five replicates. Controls were treated with sterile dH₂O. The inocula were placed at a distance of 2 to 3 cm on the leaves, which were wounded with a sterile needle. All of them were placed in 20-cm dishes on wet filter paper at 25°C. After 5 days, all the inoculated points showed lesions that were similar to those observed outdoors, whereas controls were asymptomatic. At the same time, the plugs of *C. gloeosporioides* were inoculated on living leaves that were wounded. After 7 days, the leaves that were inoculated also showed lesions. This is the first report of *C. gloeosporioides* causing leaf blotch on *C. sinensis* in China. These data will help develop effective strategies for managing this disease.

References:

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