Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Colletotrichum aenigma* Causing Apple Glomerella Leaf Spot on the Granny Smith Cultivar in China

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Glomerella leaf spot (GLS) is a devastating fungal disease causing premature defoliation on apple (*Malus domestica*). It was first reported in 1970s and since then has been reported in North America, South America, and Asia. GLS disease is caused by *Colletotrichum* fungi, and the pathogens are genetically diverse, encompassing at least nine species belonging to three species complexes (Velho et al. 2018). In August 2018, a disease with sudden leaf necrosis symptom, typical of GLS symptom appearance, occurred in a ‘Granny Smith’ orchard in Wugong county, China; over 70% of tree leaves bore brown and necrotic lesions. Small leaf tissues (3 to 4 mm²) cut from lesion margins were surface sterilized for 30 s in 3% NaClO and 30 s in 75% ethanol, followed by rinsing three times in sterile water before transferring onto potato dextrose agar (PDA) plates (25 ± 2°C). Seven isolates were obtained, all producing round cottony colonies on PDA, being white to pale on the upper side and dark green on the reverse side. Conidia were single-celled, cylindrical, and transparent ($17.33 \pm 1.29 \times 5.11 \pm 0.77 \mu\text{m}$, $n = 50$). Appressoria were single-celled, thick-walled, dark brown, oval or irregular shaped, sometimes lobed ($9.07 \pm 0.88 \times 6.66 \pm 0.33 \mu\text{m}$, $n = 50$). The morphological and cultural characteristics of the fungal isolates matched the descriptions of *Colletotrichum aenigma* (Weir et al. 2012). To confirm the species identity, genomic DNA was isolated from two representative isolates (QSG1 and QSY1), and the internal transcribed spacer (ITS), actin (ACT), glyceraldehyde-3-phosphate dehydrogenase

(GAPDH), beta-tubulin (TUB2), manganese-superoxide dismutase (SOD2), chitin synthase (CHS), and calmodulin (CAL) regions were amplified by PCR using reported primers (Weir et al. 2012). The sequences were deposited in GenBank (accession nos. MT872061, MT873580, MT873581, MT888183, MT888185, MT888187, and MT888189 for QSG1; and MT834933, MT835166, MT873579, MT888182, MT888184, MT888186, and MT888188 for QSY1). BLASTn search against the GenBank nr database showed that ITS sequences of the two strains showed high nucleotide identity (over 99%) to sequences derived from the *Colletotrichum gloeosporioides* species complex (CGSC). Further concatenated phylogenetic analysis with reported CGSC strains (Weir et al. 2012) placed QSG1 and QSY1 in the clade of *C. aenigma*. To fulfill Koch’s postulates, a field pathogenicity test was performed. The experiment was performed in an orchard located in Yangling in September 2020, with the daily average temperature ranging between 15 and 20°C. Healthy Granny Smith leaves were surface sterilized with 70% alcohol and inoculated with conidial suspension (10⁶ conidia/ml) using cotton swabs. For each isolate, 10 leaf inoculations were performed. Inoculation with distilled water served as a negative control. Inoculated leaves were covered with plastic bags to maintain high humidity, and the bags were removed at 48 h postinoculation. Conidium-inoculated leaves started to exhibit GLS-resembling necrotic lesions from 5 days postinoculation (dpi) onward. The lesion extent, however, varied among inoculated leaves, ranging from blurry, small-sized lesions to blight of the entire leaf. In contrast with conidium inoculations, water-inoculated leaves remained asymptomatic until 14 dpi. Reisolated fungi from the symptomatic leaf tissues were identical to *C. aenigma* in morphological appearance. Taken together, this is the first report of GLS on Granny Smith apple and the first report of *C. aenigma* causing apple GLS in China. This information should provide an important guideline for developing field control practices for GLS.

References:

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