Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Exserohilum rostratum* Causing Leaf Spot on Maize (*Zea mays*) in Henan, China

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In August and September of 2019 and 2020, a leaf spot was observed on maize at growth stages R1 through R5 in Henan. Leaf samples ($n = 63$) were collected from 152 fields from 50 counties. Symptomatic plants had light-yellow to brown elliptic lesions (0.6 to 5.0×0.2 to 0.8 cm) with gray. Incidence rates were 1 to 15% in individual fields. Symptomatic leaf samples (3 to 5 cm²) were surface sterilized (75% ethanol for 30 s and 3% NaClO for 2 min), rinsed three times in sterile distilled water, and allowed to dry. Small sections from the samples were placed on potato dextrose agar (PDA) medium and incubated at 25°C for 4 days. Observed colonies were circular, taupe, pewter in color with light gray edging and many cottony aerial mycelia. Single spores ($n = 45$) were inoculated on water agar medium with wheat straw. Conidiophores on wheat straw were single, erect or slightly curved, in clusters, septate, sympodial, geniculate and 5 to 7 μ m thick. The basal cell was slightly swollen and dark brown. The basal septum was darker and thicker than other septa, with a distinctly protruding truncate hilum. The conidia belonged to two types (Lin et al. 2011). Type A were fusiform, cylindrical, pyriform to oval, dark brown, 2 to 7 septate, 16.5 to 70.5 μ m $\times$ 10.2 to 24.7 μ m ($n = 50$). Type C were straight or slightly bent, ellipsoidal or long ellipsoidal, light brown, 6 to 13 septate, 36.3 to 106.8 $\times$ 10.5 to 16.0 μ m ($n = 50$). All single spore isolates were identified morphologically as *Exserohilum rostratum* (Drechs.) Leonard & Suggs (Ahmadpour et al. 2013; Sivanesan et al. 1987). Four isolates (ML154-1, ML300-2, ML598-4, and ML626-1) were arbitrarily selected for molecular identification and inoculation testing. The internal transcribed spacer (ITS) and glyceraldehyde-3-phosphate dehydrogenase-like (*gpd*) gene were amplified and sequenced using primer

pairs ITS1/ITS4 (White et al. 1990) and Gpd1/Gpd2 (Berbee et al. 1999), respectively. Sequences MW440488, MW440489, MW440491, and MW440492 of ITS and MW448214, MW448215, MW448217, and MW448218 of *gpd* were submitted to GenBank. BLASTn analysis revealed a high match (ITS: 598/600 bp; *gpd*: 527/527 bp) to *E. rostratum* isolate BRIP11422 (ITS: LT837464; *gpd*: LT883541). A phylogenetic tree was constructed based on concatenated sequences of ITS and *gpd* gene in MEGA-X. All four isolates formed a single clade with *E. rostratum* reference isolates CBS732.96, BRIP11422, and BRIP11416. To test pathogenicity in the greenhouse, a conidial suspension of each of the four strains (1×10^4 conidia/ml) was sprayed on the leaves of the common maize hybrids Zhengdan 958 and Xianyu 335 at the V6 or V7 growth stage. Control plants were sprayed with sterile water. Plants were incubated in plastic bags to retain high relative humidity for 24 h at 25 to 27°C. Three days after inoculation, the leaves began to show symptoms, and 7 days later, symptoms of the leaves were identical to those observed in the field. Control plants developed no symptoms. The pathogen was reisolated from the infected leaves and was confirmed to be *E. rostratum*. The first *E. rostratum* isolate was isolated from the leaves of *Eragrostis* in China (Jiang et al. 1959). *E. rostratum* has been reported as the causal agent of leaf spot in *Ananas comosus* (Luo et al. 2012), *Musa paradisica* (Lin et al. 2011), *Hevea brasiliensis* (Liu et al. 2016), and many-gramineous grass weeds (Zhang et al. 2016) in China. To our knowledge, this is the first report of *E. rostratum* causing leaf spot disease on maize in Henan, China. Since this is an economically important pathogen of maize throughout the world, disease management strategies are likely to be needed.

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