Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Diaporthe cercidis* Causing Leaf Blotch of *Acer pictum* subsp. *mono* in China

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Acer pictum subsp. *mono* (Maxim.) H. Ohashi is a common deciduous tree widely distributed in Northeast and Northern China, including the Yangtze River Basin (Liu et al. 2014). A foliar disease with ~90% incidence (19/21 trees) occurred on *A. pictum* subsp. *mono* in a community park in Nanjing, Jiangsu, China in July 2019. On average, ~80% of the leaves per tree were infected. Symptoms initially appeared as brown, necrotic lesions at leaf tips; half the leaf would become dark brown and, finally, almost all of leaves were infected. Pieces of leaf tissue (3 to 5 mm²) cut from lesion margins were surface sterilized in 75% ethanol for 30 s and 1% NaClO for 90 s, rinsed with sterile H₂O three times, and placed on PDA at 25°C in the dark. The same fungus was isolated from 92% of samples. Pure cultures were obtained by single-spore isolation. Three representative isolates (WJF1, -3, and -4) were obtained; WJF1 was deposited in China's Forestry Culture Collection Center (CFCC 54806), and WJF3 and -4 were deposited at the Nanjing Forestry University (NFU 083 and NFU 084). The culture on PDA was white, with white vigorous aerial mycelia at the edge. Black pycnidia developed on alfalfa stems at 25°C under a 14/10 h light/dark cycle for 20 days. Conidiophores were hyaline, branched, septate, straight, 16.4 to 34.7 × 1.5 to 3.0 µm (*n* = 30). Conidiogenous cells were 9.0 to 24.6 × 1.3 to 2.3 µm (*n* = 30). α-Conidia were 7.0 ± 0.6 × 2.2 ± 0.2 µm (*n* = 30), fusiform, hyaline, smooth, and multiguttulate. β-Conidia were 25.5 ± 4.3 × 1.3 ± 0.1 µm (*n* = 30), hyaline, smooth, and hamate. Morphological characteristics of all three isolates matched *Diaporthe* spp. (Gomes et al. 2013). DNA of three isolates was extracted, and the ITS and partial sequences of *EF1-α*, *CAL*, *β-tub*, and histone H3 (*HIS*) genes were amplified with primers ITS1/ITS4 (White et al. 1990), EF1-728F/EF1-986R, CAL228F/CAL737R (Carbone et al. 1999),

βt2a/βt2b, and CYLH3F/H3-1b (Crous et al. 2004; Glass and Donaldson 1995), respectively. Sequences of WJF1, -3, and -4 were deposited in GenBank (WJF1: MW301339 for ITS, MW363932 to MW363935 for *EF1-α*, *β-tub*, *HIS*, and *CAL*; WJF3: MW453062 and MW561566 to MW561569; WJF4: MW453063 and MW561570 to MW561573). BLAST results showed that the ITS, *EF1-α*, *β-tub*, *HIS*, and *CAL* sequences of WJF1 were similar to *Phomopsis liquidambari* C. Q. Chang, Z. D. Jiang & P. K. Chi JQ676191 (540/540; 100%); *Diaporthe huangshanensis* H. Zhou & C. L. Hou MN224671 (291/292; 99%); *D. peticola* Dissan., J. Y. Yan, Xing H. Li & K. D. Hyde MK691230 (438/438; 100%); *D. spinosa* Y. S. Guo & G. P. Wang MK726170 (437/438; 99%); and *D. cercidis* C. M. Tian & Qin Yang MK691114 (452/452; 100%), respectively. BLAST results are in the supplementary materials. Maximum-likelihood and Bayesian posterior probability analyses using IQtree v. 1.6.8 and Mr. Bayes v. 3.2.6 with the concatenated sequences placed WJF1, -3, and -4 in the clade of *D. cercidis*. Based on the five-locus phylogeny and morphology, they were identified as *D. cercidis*. Pathogenicity was tested on potted 3-year-old seedlings. Healthy leaves were wounded with a sterile needle and inoculated with 10 µl of 10⁶ conidia/ml suspension and control leaves with sterilized H₂O. In total, 12 seedlings were used (3 per treatment) and five leaves were inoculated per seedling. Plants were covered with plastic bags, and sterilized H₂O was sprayed into the bags twice a day; they were kept in a greenhouse at 25 ± 2°C/16 ± 2°C (day/night). In 5 days, all inoculated leaves had lesions similar to those in the field. *D. cercidis* was reisolated from inoculated leaves and was confirmed based on morphological characteristics and ITS sequence analysis. No symptoms were observed on control leaves and no fungus was isolated. *D. cercidis* was previously reported on twigs of *Cercis chinensis* (Yang et al. 2018) and causing pear shoot canker (Guo et al. 2020). This is the first report of *D. cercidis* causing leaf blotch on *A. pictum* subsp. *mono*. Pathogen identification is imperative for diagnosing and controlling this potentially high-risk disease on *A. pictum* subsp. *mono* and for future studies.

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The author(s) declare no conflict of interest.

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