Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Colletotrichum aenigma* Causing Walnut Anthracnose in China

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Funding: This research was supported by the Fundamental Research Funds for the Central Non-profit Research Institution of Chinese Academy of Forestry (no. CAFYBB2016QA013) and National Natural Science Foundation of China (no. 31600514). Plant Dis. 105:225, 2021; published online as <https://doi.org/10.1094/PDIS-07-20-1430-PDN>. Accepted for publication 26 July 2020.

English/Persian walnut (*Juglans regia* L.) is grown as an economically valuable crop in temperate and subtropical regions. In August 2018, serious fruit anthracnose, with brown to black circular or subcircular or irregular sunken lesions, occurred on walnut trees ('Xiangling' and 'Lvling') in 33-, 23-, and 20-ha orchards in Lincheng and Neiqiu county, in Xingtai, Hebei, China. Diseased fruits were observed on 41% (about 7,790 trees), 31% (about 4,123 trees), and 34% (about 3,876 trees) of walnut trees. Diseased leaves, with circular or irregular brown to gray sunken lesions, were observed on 2% (about 380 trees), 2% (about 266 trees), and 1% (about 114 trees) of walnut trees. From each orchard, 25 diseased fruits and leaves were collected, respectively. Twenty-one single-spore isolates were obtained from fruits of three orchards and none from leaves as described by Cai et al. (2009). Six representative isolates 1811-1, 1811-4, 1811-7, 1811-8, 1811-11, and 1811-18, two from each orchard, were selected for further study. Colonies on PDA grew 11.8 mm/day at 25°C under a 12-h/12-h light/dark cycle for 7 days. The upper side of colonies was milky, and the reverse side was dark brown to brownish yellow. A few acervuli were observed on colonies. Conidiogenous cells were cylindrical to clavate, 10.6 to 29.7 × 3.1 to 5.3 µm (mean = 21.3 × 4.0 µm, *n* = 30). Setae were not observed. Conidia were smooth-walled, aseptate, straight or slightly

distorted, cylindrical with one end slightly acute or broadly rounded ends, and 16.6 to 21.6 × 6.0 to 7.5 µm (mean = 19.2 × 6.7 µm, *n* = 30). Appressoria were mostly irregular in outline, deeply lobed or lightly lobed, gray brown to dark brown, 8.3 to 16.6 × 7.1 to 14.5 µm (mean = 12.5 × 9.7 µm, *n* = 30). Microscopic features were similar to the description of *C. aenigma* (Weir et al. 2012). To further identify isolates, the ribosomal internal transcribed spacers (ITS), β-tubulin 2 (TUB2), calmodulin (CAL), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), glutamine synthetase (GS), and chitin synthase (CHS-1) loci of representative isolates were amplified using ITS4/ITS5, Bt2a/Bt2b, CL1/CL2, GDF1/GDR1, GSF1/GSR1, and CHS-79F/CHS-345R primers (Carbone and Kohn 1999; Prihastuti et al. 2009). Sequences of representative isolate 1811-1 were submitted to GenBank (ITS, MN893316; TUB, MN893317; CAL, MN893312; GAPDH, MN893314; GS, MN893315; CHS-1, MN893313). Maximum likelihood analysis of sequences of representative isolates and reference sequences of *Colletotrichum* spp. from GenBank revealed that six isolates clustered together with *Colletotrichum aenigma* ex-type culture ICMP18608, and the bootstrap value was 100%. Pathogenicity tests were conducted on walnut fruit as described by Wang et al. (2017, 2018) and Cai et al. (2009). Ten wounded and 10 nonwounded fruits (Xiangling, 35-mm diameter) were inoculated with isolates 1811-1, 1811-7, and 1811-11 conidial suspension (10⁶ spore/ml) obtained from 10-day-old colonies grown on PDA at 25°C, respectively. Ten wounded and 10 nonwounded fruits were inoculated with sterile water. Inoculated and control fruits were incubated in containers at 25°C in a 12-h/12-h light/dark cycle. After 10 days, necrotic lesions were observed in all inoculated fruits. The pathogen *C. aenigma* was reisolated from all inoculated fruits but not from control fruits. To our knowledge, this is the first report of *C. aenigma* causing walnut anthracnose in China. It is urgent to control walnut anthracnose caused by different species of *Colletotrichum*.

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The author(s) declare no conflict of interest.

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Keywords: walnut anthracnose, *Colletotrichum aenigma*, pathogenicity test, *Colletotrichum*

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