Diseases Caused by Fungi and Fungus-Like Organisms

First Report of Coffee Canker Disease Caused by *Ceratocystis fimbriata* in China

K. C. Xu,¹ R. Q. Zhang,² H. X. Lu,¹ J. L. Zhang,² J. Yang,^{2,†} and Q. Huang^{2,†}

¹ College of Pharmacy, Guizhou University of Traditional Chinese Medicine, Guiyang, Guizhou 550025, China

² State Key Laboratory for Conservation and Utilization of Bio-Resources in Yunnan, Yunnan Agricultural University, Kunming, Yunnan 650201, China

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Coffee (*Coffea arabica* L.) is one of the most important agricultural commodities in the world. As an important cash crop in China, coffee is cultivated mainly in Yunnan and Hainan provinces. During October 2013 and September 2020, coffee trees showing typical dieback and wilt symptoms were found in Nanping town (N 22° 38', E 101° 0'), Pu'er, and Puwen town (N 22° 32', E 101° 4'), Xishuangbanna, in Yunnan province, China. Symptomatic trees initially exhibited yellowing of foliage, expanding in size along the leaf margin, then became blighted and dry, and the internal xylem in the main stem was discolored brown to black. Infected trees eventually developed dieback and wilt. Disease incidence ranged from 10 to 22% and 25 to 40% of crown symptoms in the affected trees. In extreme cases, 50% of 380 trees were affected. The stems of trees with typical symptoms were collected. The diseased tissues were surface disinfected with 75% ethanol for 30 s and 0.1% HgCl₂ for 2 min, rinsed three times with sterile distilled water, plated onto potato dextrose agar (PDA) medium, and incubated at 25°C. After 6 days, fungal mycelium was observed growing from the tissue. Three isolates (C3-1, C3-2, and C3-2-1) were obtained by picking spore masses from the apices of perithecia and transferring them to malt extract agar medium and incubated at 25°C for 10 days to observe the cultural features. In culture, colonies reaching 65 mm within 10 days, mycelium initially white, becoming light blue-green. After 6 days of formation, perithecia were black, globose (123.8 to 173.4 × 138.2 to 180.6 μm), and had a long black neck (414.2 to 650.0 μm). Ascospores with outer cell wall forming a brim, hat-shaped, accumulating in

a mucilaginous mass at the tips of ostiolar hyphae (4.3 × 6.0 μm). Cylindrical endoconidia (14.1 to 45.2 × 3.5 to 5.7 μm) were hyaline. Chains of barrel-shaped conidia (6.6 to 10.2 × 6.8 to 8.8 μm) were found. Aleuroconidia (10.8 to 16.9 × 9.1 to 13.0 μm) were olive-brown, ovoid or obpyriform, and smooth. Morphological characteristics were consistent with the description of *Ceratocystis fimbriata* Ellis & Halst. (Engelbrecht and Harrington 2005). The three isolates were used for molecular identification, and their genomic DNA was extracted using the chelex-100 method (Xu et al. 2020). The internal transcribed spacer (ITS) region of rDNA was sequenced using the procedures of Thorpe et al. (2005). Analysis of the ITS sequence data (GenBank accessions KY580836, KJ511480, and KJ511479) showed that the isolates were 100% homologous to isolates of *C. fimbriata* from *Punica granatum*, *Camellia sinensis*, and *Cucumis sativus* in China (GenBank accessions KY580891, KY580870, and MH535909, respectively) by BLAST analysis. Neighbor-joining phylogenetic analysis was performed using MEGA 6.06 based on the ITS sequences. The three isolates clustered on the same clade with other *C. fimbriata* isolates with a high bootstrap value (90%). Therefore, the fungus was identified as *C. fimbriata* based on both morphological and molecular characteristics. Pathogenicity of the three isolates was tested by inoculating 1-year-old pot-grown seedlings by drenching the soil with 30 ml of spore suspension (1 × 10⁶ spores/ml). Control plants were inoculated with 30 ml of sterile distilled water. The trees were kept in a controlled greenhouse at 25°C and watered weekly. One month after inoculation, all inoculated plants produced typical dieback and wilt symptoms, whereas the control trees showed no symptoms. The same fungus was isolated from the inoculated trees on PDA and identified as *C. fimbriata* according to the methods described above, and no fungal growth was observed in the controls, thus fulfilling Koch's postulates. Coffee canker disease caused by *C. fimbriata* has been reported in Indonesia and Colombia (Marin et al. 2003). To our knowledge, this is the first report of *C. fimbriata* causing canker disease of coffee trees in China.

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[†]Indicates the corresponding authors.

J. Yang; yangjin-18@163.com, and

Q. Huang; huangqiong88hs@163.com