Diseases Caused by Fungi and Fungus-Like Organisms

First Report That *Colletotrichum aenigma* Causes Leaf Spots on *Aquilaria sinensis* in China

C. Li, J. A. Liu, and G. Y. Zhou[†]

Key Laboratory of National Forestry and Grassland Administration on Control of Artificial Forest Diseases and Pests in South China, Hunan Provincial Key Laboratory for Control of Forest Diseases and Pests, Key Laboratory for Non-wood Forest Cultivation and Conservation of Ministry of Education, Central South University of Forestry and Technology, Changsha, 410004, China

Funding: Funding was provided by the National Key Research and Development Program of China (2018YFD0600202). Plant Dis. 105:3302, 2021; published online as <https://doi.org/10.1094/PDIS-11-20-2381-PDN>. Accepted for publication 24 March 2021.

Aquilaria sinensis (Lour.) Spreng, also known as eaglewood, belongs to the Thymelaeaceae family and has a considerably high medicinal value. It has been listed as a class II national key protective plant. In June 2019, about 15% of *A. sinensis* treelets in a forest area of China's Hainan province were observed to have anthracnose symptoms. The diseased spots on leaves of *A. sinensis* treelets were usually round or irregular with pale yellow edges. The color of the center of the lesion was first light brown and then black or yellowish-brown. Small pieces of tissue from the edge of the leaf spots were surface sterilized in 75% alcohol for 60 s, washed twice with sterile distilled water, and then cultivated at 28°C in darkness on potato dextrose agar (PDA) medium. One fungus was systematically isolated to get pure cultures. The culturing of the three isolates was carried out in PDA medium at 28°C for 1 week. The average diameter of the collateral colony was 6.80 ± 0.60 cm. Initially, the fungal colonies were white aerial mycelium, and the central area of the colonies slowly turned jacinth. After 7 days, the central mycelium turned grayish-green and the colonies' undersurfaces were gray to white. The colonies' surfaces were fluffy and round with smooth edges. Conidia were cylindrical, smooth, and transparent, with a slight indentation in the middle and uneven distribution of small particles inside, 12.5 to 20.6×3.5 to 6.8 μm (average = $15.9 \pm 1.40 \times 5.18 \pm 1.07$ μm , $n = 50$). Appressoria were typically elliptic or irregular and brown to dark brown. The isolates were

characterized as *Colletotrichum gloeosporioides* species complex on the basis of the conidial morphology and culture representation (Deng et al. 2017; Weir et al. 2012). To further verify the identification of the species, CX-0301, the isolated representative strains were extracted for genomic DNA. Mating type 1-2-1 (Mat-1-2-1) ApMat, actin (ACT) gene, chitin synthase (CHS), and beta-tubulin (TUB2) gene were amplified using the primer pairs VcaMat-5F/VcaMat-5R, ACT-512F/ACT-783R, CHS-1-79F/CHS-1-354R, and TUB2-T1/Bt2b, respectively (Damm et al. 2012; Du et al. 2005). The homologous sequences of MN310694, MN310693, MN310692, and MN310691 were submitted to GenBank. These genes have a $\geq 97\%$ sequence similarity to the genes of *Colletotrichum aenigma* (MG717319.1, MG717317.1, MH476565.1, and MH853679.1, respectively) in GenBank. These morphological and molecular characteristics identified that the pathogen is *C. aenigma* (Weir et al. 2012). To further verify the isolated pathogen, a pathogenicity test was performed on uninfected healthy 2-year-old eaglewood seedlings. The conidial suspension (1×10^6 conidia/ml) of 5 ml was sprayed on both surfaces of 10 leaves of plants of the same age and height, and the controls were treated solely with distilled water (Deng et al. 2017). Upon completion of inoculation, plants were kept under greenhouse conditions with an assigned temperature of $28 \pm 2^\circ\text{C}$ while keeping relative humidity to 90% on a 12-h fluorescent light/dark regime. Anthracnose-like symptoms were observed 6 days postinoculation. The control plant tissues remained healthy. Follow-up reisolation of *C. aenigma* culture was obtained in PDA plates from leaf infected lesions, and the morphological features were found to be consistent with those of CX-0301 isolate, satisfying Koch's postulates. Based on the characterized information, this is the first report of *C. aenigma* responsible for causing leaf spots on *A. sinensis* in China. Thereby, this provides a theoretical reference for the research and control of anthracnose on *A. sinensis*.

References:

- Damm, U., et al. 2012. Stud. Mycol. 73:37.
- Deng, L. C., et al. 2017. Plant Dis. 101:1032.
- Du, M., et al. 2005. Mycologia 97:641.
- Weir, B. S., et al. 2012. Stud. Mycol. 73:115.

The author(s) declare no conflict of interest.

e-Xtra

Keywords: fungi, epidemiology, disease development and spread, trees, forest

[†]Indicates the corresponding author.
G. Y. Zhou; gyzhou2118@163.com