Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Colletotrichum boninense* Causing Anthracnose on *Rosa chinensis* in China

Hai-Xia Ding,^{1,2} Wan-Peng Dong,³ Wei-Di Mo,¹ Li-Juan Peng,^{4,†} and Zuo-Yi Liu^{2,†}

¹ College of Agriculture, Guizhou University, Guiyang, Guizhou 550025, China

² Guizhou Key Laboratory of Agricultural Biotechnology, Guizhou Academy of Agricultural Sciences, Guiyang, Guizhou 550006, China

³ Guizhou Botanical Garden, Guiyang, Guizhou 550001, China

⁴ College of Tobacco Science, Guizhou University, Guiyang, Guizhou 550025, China

Funding: Department of Education of Guizhou Province (QianJiaoHe KY[2018]101), Guizhou Science and Technology Department (QianKeHeFuQi [2019]4016), Forestry Scientific Research in Guizhou Province (QianLinKeHe J[2018]08), China Postdoctoral Science Foundation (2020M683658XB). Plant Dis. 105:2717, 2021; published online as <https://doi.org/10.1094/PDIS-01-21-0102-PDN>. Accepted for publication 6 April 2021.

Chinese rose (*Rosa chinensis* Jacq.) is cultivated for edible flowers in southwestern China (Zhang et al. 2014). In March 2020, a leaf spot disease was observed on about 3 to 5% leaves of Chinese rose cultivar 'Mohong' in Guizhou Botanical Garden (26°37'45"N, 106°43'10"E), Guiyang, Guizhou Province, China. The symptomatic plants displayed circular, dark brown lesions with black conidiomata in gray centers on leaves, and leaf samples were collected. After surface sterilization (0.5 min in 75% ethanol and 2 min in 3% NaOCl, washed three times with sterilized distilled water) (Fang 2007), small pieces of symptomatic leaf tissue (0.3 × 0.3 cm) were plated on potato dextrose agar (PDA) and incubated at 28°C for about 7 days. Two single-spore isolates, GZUMH01 and GZUMH02, were obtained, which were identical in morphology and molecular analysis. Therefore, the representative isolate GZUMH01 was used for further study. The pathogenicity of GZUMH01 was tested through a pot assay. Ten healthy plants were scratched with a sterilized needle on the leaves. Plants were inoculated by spraying a spore suspension (10⁶ spores ml⁻¹) of GZUMH01 onto leaves until runoff, and the control leaves sprayed with sterile water. The plants were maintained at

25°C with high relative humidity (90 to 95%) in a growth chamber. The pathogenicity test was carried out three times (Fang 2007). The symptoms developed on all inoculated leaves but not on the control leaves. The lesions were first visible 48 h after inoculation, and typical lesions similar to those observed on field plants appeared after 7 days. The same fungus was reisolated from the infected leaves but not from the noninoculated leaves, fulfilling Koch's postulates. Fungal colonies on PDA were villiform and greyish. The conidia were abundant, oval-ellipsoid, aseptate, and 15.8 (13.7 to 18.8) × 5.7 (4.3 to 6.8) μm. The fungal colonies, hyphae, and conidia were consistent with the descriptions of *Colletotrichum boninense* Moriawaki, Toy. Sato & Tsukib. (Damm et al. 2012; Moriawaki et al. 2003). The pathogen was confirmed to be *C. boninense* by amplification and sequencing of the internal transcribed spacer region (ITS), the glyceraldehyde-3-phosphate dehydrogenase (GADPH), actin (ACT), and chitin synthase 1 (CHS-1) genes using primers ITS1/ITS4, GDF1/GDR1, ACT512F/ACT783R, and CHS-79F/CHS-345R, respectively (Damm et al. 2012; Moriawaki et al. 2003). The sequences of the PCR products were deposited in GenBank with accession numbers MT845879 (ITS), MT861006 (GADPH), MT861007 (ACT), and MT861008 (CHS-1). BLAST searches of the obtained sequences of the ITS, GADPH, ACT, and CHS-1 genes revealed 100% (554/554 nucleotides), 100% (245/245 nucleotides), 97.43% (265/272 nucleotides), and 99.64% (279/280 nucleotides) homology with those of *C. boninense* in GenBank (JQ005160, JQ005247, JQ005508, and JQ005334, respectively). Phylogenetic analysis (MEGA 6.0) using the maximum likelihood method placed the isolate GZUMH01 in a well-supported cluster with *C. boninense*. The pathogen was thus identified as *C. boninense* based on its morphological and molecular characteristics. To our knowledge, this is the first report of the anthracnose disease on *R. chinensis* caused by *C. boninense* in the world.

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The author(s) declare no conflict of interest.

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Keywords: *Colletotrichum boninense*, anthracnose, *Rosa chinensis*

[†]Indicates the corresponding authors.

L. J. Peng; 296430006@qq.com, and Z. Y. Liu; gzliuzuoyi@163.com