Diseases Caused by Fungi and Fungus-Like Organisms

Brown Leaf Spot of *Cycas debaoensis* Caused by *Colletotrichum siamense* in Sichuan, China

Shan Han,¹ Jimin Ma,² Yanyue Li,¹ Shujiang Li,¹ Yinggao Liu,¹ Tianmin Qiao,¹ Tiantian Lin,¹ Chunlin Yang,¹ Tingting Luo,¹ Lei Xiang,¹ and Tianhui Zhu^{1,†}

¹ College of Forestry, Sichuan Agricultural University, Chengdu, Sichuan Province, 611130, Sichuan, P.R. China

² College of Agriculture and Horticulture, Chengdu Agricultural College, Chengdu, Sichuan Province, 611130, Sichuan, P.R. China

S. Han, J. M. Ma, and Y. Y. Li contributed equally to this work.

Funding: Funding was provided by the General Program of Science and Technology Department of Sichuan (2019YFN0156). Plant Dis. 105:2019, 2021; published online as <https://doi.org/10.1094/PDIS-10-20-2149-PDN>. Accepted for publication 13 January 2021.

Cycas debaoensis Y. C. Zhong et C. J. Chen is an endemic species in China that is listed among China's national key preserved wild plants (class I) (Xie et al. 2005). It is mainly distributed in south China (Guangxi, Guizhou, and other regions). In April 2017, a new leaf disease of *C. debaoensis* was found in Chengdu (30°35'32"N; 104°05'11"E) in China with an incidence of over 40%. Symptoms on *C. debaoensis* initially appeared as brown necrotic lesions on the margin or in the center of leaves. The lesions then enlarged gradually and developed into brown spots, necrotic lesions with dark brown margins. Many small and black dots were observed on necrotic lesions. Eventually, the diseased leaves withered and died. Ten samples were collected and surface sterilized by 3% NaClO and 75% ethanol, respectively, for 60 and 90 s, rinsed with autoclaved distilled water, and then blot-dried with autoclaved paper towels. Five isolates from diseased leaves with similar morphology were isolated from single spores. Morphological characteristics were recorded from pure cultures grown on potato dextrose agar (PDA) incubated at 25°C for 3 to 9 days. Initially, the colonies grown on PDA were white and then became pale gray with concentric zones and greenish black beneath. Conidia were single-celled, smooth-walled, straight, colorless, cylindrical with both ends bluntly rounded, 13.0 to 16.5 × 4.7 to 5.8 µm in size ($n = 100$ spores). For molecular identification, the genomic DNA of the isolates was extracted using a DNeasy Plant Mini Kit (Qiagen). The

internal transcribed spacer (ITS) (ITS1/ITS4, White et al. 1990), β -tubulin (TUB2) (BT2A/BT2B, O'Donnell and Cigelnik 1997), actin (ACT) (ACT512F/ACT, Carbone and Kohn 1999), calmodulin (CAL) (CL1C/CL2C, Weir et al. 2012), mating type protein and chitin synthase (CHS-1) (CHS-9 79F/CHS-345R, Carbone and Kohn 1999) were amplified. BLAST results indicated that the ITS, TUB2, ACT, CAL, and CHS-1 sequences (GenBank MN305712, MN605072, MT478663, MT465591, and MT478664) showed 99 to 100% identity with *Colletotrichum siamense* sequences at NCBI (GenBank JF710564, MK341542, MK855094, MH351155, and MK471373). The phylogenetic tree inferred from the combined datasets (TEF, TUB, and ACT) showed that the isolate belongs to *C. siamense* clade with a credibility value of 99%. Two-year-old potted plants of *C. debaoensis* (10 plants) were used for the pathogenicity test. On each plant, five leaves were sprayed with a conidial suspension (1×10^6 conidia/ml) on both sides of the leaves. Autoclaved distilled water was used as a negative control (10 plants). Plants were kept in the greenhouse at 25°C under 16-h/8-h photoperiod and 70 to 75% relative humidity. The symptoms observed on the inoculated plants were similar to those observed in the field, whereas the controls remained asymptomatic. *C. siamense* was reisolated from all diseased inoculated plants, and the culture and fungus characteristics were the same as the original isolate. The morphological characteristics and molecular analyses of the isolate matched the description of *C. siamense* (Prihastuti et al. 2009). *C. siamense* was previously reported infecting *Citrus reticulata* (Cheng et al. 2013), but this is the first report of brown leaf spot on *C. debaoensis* caused by *C. siamense* in China. This finding provides an important basis for further research on the control of the disease.

References:

- Carbone, I., and Kohn, L. M. 1999. Mycologia 91:553.
- Cheng, B. P., et al. 2013. Plant Dis. 97:1508.
- O'Donnell, K., and Cigelnik, E. 1997. Mol. Phylogenet. Evol. 7:103.
- Prihastuti, H., et al. 2009. Fungal Divers. 39:89.
- Weir, B. S., et al. 2012. Stud. Mycol. 73:115.
- White, T. J., et al. 1990. Page 315 in: PCR Protocols: A Guide to Methods and Applications. Academic Press, San Diego, CA.
- Xie, J. G., et al. 2005. Aust. J. Bot. 53:141.

The author(s) declare no conflict of interest.

e-Xtra

Keywords: *Cycas debaoensis*, *Colletotrichum siamense*, brown leaf spot

[†]Indicates the corresponding author.
T. H. Zhu; zhutianhui63@163.com