Diseases Caused by Fungi and Fungus-Like Organisms

First Report of Anthracnose Disease on *Bletilla striata* Caused by *Colletotrichum orchidophilum* in Yunnan, China

K. Yang,^{1,2} H. L. Wang,^{1,2} K. H. Ye,^{1,2} C. Luo,^{1,2} Z. X. Wei,^{1,2} H. P. Huang,^{1,2} S. S. Zhu,^{1,2} L. W. Guo,^{1,2,†} and X. H. He^{1,2,3,†}

¹ State Key Laboratory for Conservation and Utilization of Bio-Resources in Yunnan, Yunnan Agricultural University, Kunming, Yunnan 650201, China

² Key Laboratory of Agro-Biodiversity and Pest Management of Education Ministry of China, Yunnan Agricultural University, Kunming, Yunnan 650201, China

³ Southwest Forestry University, Kunming, Yunnan 650224, China

K. Yang and H. L. Wang contributed equally.

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Bletilla striata (Thunb.) Reichb. f. has high ornamental and medicinal value. *B. striata* has been used in Chinese medicine for thousands of years to treat various diseases, such as peptic ulcers, gastrointestinal diseases, lung diseases, and traumatic bleeding (Jiang et al. 2021). In September 2020, about 30% of *B. striata* plants in a plantation in Lancang County (Puer City, Yunnan Province, 22°48'17" N, 99°46'58" E) were found with symptoms resembling *Bletilla* anthracnose disease. In May 2021, symptomatic leaves were observed again with a disease incidence rate of 54.05% and collected. The initial symptoms were the appearance of one or more round light brown spots on the leaves. Subsequently, the lesions became larger, forming oval brown-black spots. On severely affected leaves, several spots coalesced, causing withering and death of the leaves. To isolate the pathogen, 10 symptomatic tissues (5 mm²) of 10 infected leaves were disinfected in 75% ethanol for 30 s followed by 2% sodium hypochlorite for 3 min, rinsed with sterile water three times, and incubated on potato dextrose agar (PDA) plates at 25°C for 5 to 7 days with a 12 h light/dark cycle. Finally, 10 pathogen strains with similar morphology were isolated and pure cultures were obtained by single spore isolation. The morphology of fungal colonies was round, carpet-like, flat and neat, and gray black. The conidiophores were colorless to brown and

oval or stick-shaped. The conidia were cylindrical or oblong, consisted of a single cell with blunt round ends, and were on average 12.92 (8.13 to 21.21) $\mu\text{m} \times 3.96$ (2.55 to 5.90) μm ($n = 200$). Based on the morphology and conidial characteristics, the pathogen was identified as *Colletotrichum* sp. (Damm et al. 2012). The total genomic DNA of representative isolates (LCTJ-02, LCTJ-03, LCTJ-04, LCTJ-05, and LCTJ-06) was extracted and the ITS region, *GAPDH*, *ACT*, and *HIS3* gene regions were amplified and sequenced using the primer pairs ITS1/ITS4, GDF1/GDR1, 512F/783R, and CylH3F/CylH3R, respectively (Cai et al. 2009). The sequences were deposited in GenBank (MZ433189 to MZ433193 for ITS, MZ436430 to MZ436434 for *GAPDH*, MZ436435 to MZ436439 for *HIS3*, and MZ448474 to MZ448478 for *ACT*). BLAST search revealed that all sequences showed 98 to 100% homology with the corresponding sequences of *Colletotrichum orchidophilum* ex-type (CBS 632.80). Phylogenetic trees were constructed using neighbor-joining and maximum likelihood methods for the combined data set of the ITS, *GAPDH*, *ACT*, and *HIS3* genes by MEGA-X (Kumar et al. 2018). In general, LCTJ-02, LCTJ-03, LCTJ-04, LCTJ-05, LCTJ-06, and *C. orchidophilum* type strains clustered on the same clade. To confirm pathogenicity, the five isolates of *C. orchidophilum* were inoculated in healthy leaves of potted *B. striata*, as described by Cai et al. (2009), with slight modifications. Each leaf was inoculated with three drops (10 μL drop⁻¹) of conidia suspension (10⁶ spores.mL⁻¹). The control group was mock inoculated with sterile water the same way. All samples were covered with plastic bags and maintained at 70 to 80% relative humidity for 7 days. After this period, all inoculated leaves showed similar lesions, and the symptoms were identical to those observed in the field. The control plants remained healthy. The pathogens were reisolated from two leaves of each treatment and reidentified as *C. orchidophilum*. To our knowledge, this is the first report of *C. orchidophilum* causing anthracnose on *B. striata* in China. In the future, the occurrence and transmission of this pathogen should be further studied in order to develop reasonable control measures.

References:

- Cai, L., et al. 2009. Fungal Divers. 39:183. <https://www.fungaldiversity.org/fdp/sfdp/FD39-8.pdf>
- Damm, U., et al. 2012. Stud. Mycol. 73:37.
- Jiang, S., et al. 2021. J. Ethnopharmacol. 280:114263.
- Kumar, S., et al. 2018. Mol. Biol. Evol. 35:1547.

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[†]Indicates the corresponding authors.

X. H. He; hexiahong@hotmail.com, and L. W. Guo; g87112w12@163.com