Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Colletotrichum nymphaeae* Causing Leaf Spot of *Lithocarpus litseifolius* in China

Yongxi Du,¹ Chengcai Zhang,¹ Wei Guan,² Chuanzhi Kang,¹ Jiahui Sun,¹ Yang Ge,¹ Binbin Yan,¹ Tielin Wang,^{1,†} Lanping Guo,¹ and Luqi Huang¹

¹National Resource Center for Chinese Materia Medica, China Academy of Chinese Medical Sciences, State Key Laboratory Breeding Base of Dao-di Herbs, Beijing, 100700, P.R. China

²State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, P.R. China

Funding: Funding was provided by Scientific and technological innovation project of China Academy of Chinese Medical Sciences ORG (CI2021A03905) and Key Project at Central Government Level: The ability for establishment of sustainable use for valuable Chinese medicine resource (2060302). Plant Dis. 106:1529, 2022; published online as <https://doi.org/10.1094/PDIS-09-21-1964-PDN>. Accepted for publication 24 October 2021.

Lithocarpus litseifolius (Hance) Chun has been used as tea for 1,500 years in China since the Southern and Northern Dynasties (423 CE). In mid-May 2021, a leaf spot disease of *L. litseifolius* was observed in Dexing County in Jiangxi Province, China (28°56'54.1"N 117°45'08.0"E). The disease incidence was estimated to be above 30%. Symptoms on plant leaves were early necrotic lesions, with a grayish-white center and a brown halo (<5 mm). Later, the lesion expanded to the edge of the leaf, with a yellowish-brown center and brown surrounding. Samples were collected from the field, and small tissues around the lesion were cut off and sterilized in 75% ethanol for 3 min, then washed with sterile water three times. The sterilized tissues were placed on PDA plates and incubated at 28°C in darkness. After 3 days of incubation, the hyphal tips from the edges of growing colonies were transferred to fresh PDA plates for further purification. Finally, five isolates showing the same morphology were obtained, and one pure single-colony fungal strain JM7 was selected for further analysis. After 7 days of incubation on PDA at 28°C, the fungal colonies showed white aerial mycelium and gelatinous orange conidial masses. The conidia were unicellular, smooth-

walled, hyaline, cylindrical with obtuse to rounded ends, and 8.44 to 15.48 × 2.95 to 3.42 μm (mean = 10.33 × 3.14 μm, *n* = 60). These morphological characteristics correspond to those of *Colletotrichum nymphaeae*. For molecular identification, the internal transcribed spacer, partial actin, β-tubulin 2, glyceraldehyde-3-phosphate dehydrogenase, chitin synthase, and histone H3 regions of JM7 were amplified by PCR using primer pairs ITS1/ITS4, ACT-512F/ACT-783R, T1/Bt2b, GDF/GDR, CHS-79F/CHS-354R, and CYLH3F/CYLH3R, respectively (Crous et al. 2004; Weir et al. 2012). The sequences of the amplified fragments were deposited in GenBank (MZ496772, MZ561462 to MZ561466), and showed 98 to 100% identities with the corresponding sequences of *C. nymphaeae*. A phylogenetic tree based on the above six genes of type specimens or ex-type of *Colletotrichum* (Damm et al. 2012) was generated using the maximum likelihood method by MEGA X, in which the strain JM7 and other *C. nymphaeae* strains clustered in the same branch. Thus, strain JM7 was identified as *C. nymphaeae* based on its morphological and molecular characteristics. Pathogenicity tests were conducted on leaves of *L. litseifolius*. Six leaflets were superficially disinfected with 75% ethanol and wound-inoculated with 6-mm plugs of PDA medium containing *C. nymphaeae* acervuli and conidia, while the control treatments received only PDA discs. The inoculated leaves were wrapped with a plastic bag containing moistened cotton and incubated in a greenhouse at 26°C and 60% humidity with a photoperiod of 12 h. Symptoms similar to the original observations were observed on leaflets inoculated with *C. nymphaeae* after 5 days. No symptoms were observed in the control treatments. The pathogen was reisolated from symptomatic tissues, and was identified as *C. nymphaeae*, which fulfilled Koch's postulates. To our knowledge, this is the first report of *C. nymphaeae* infection on *L. litseifolius* in China. These findings will help develop better preventive measures in accordance with the emergence of this new pathogen.

References:

- Crous, P. W., et al. 2004. Stud. Mycol. 50:415.
- Damm, U., et al. 2012. Stud. Mycol. 73:37.
- Weir, B. S., et al. 2012. Stud. Mycol. 73:115.

The author(s) declare no conflict of interest.

e-Xtra

Keywords: *Colletotrichum nymphaeae*, leaf spot, *Lithocarpus litseifolius*

[†]Indicates the corresponding author.
T. Wang; WTL82@163.com