Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Phyllosticta capitalensis* Causing Black Freckle Disease on *Rubus chingii* in China

Wenhao Zhang, Dan Su,[†] and Rui Sun

College of Life Science and Engineering, Shenyang University, 110044, China

Funding: This work was supported by Open Fund of Key Laboratory of Biotechnology and Bioresources Utilization (Dalian Minzu University), Ministry of Education (no. KF2021002), China, Natural Science Foundation Guidance Plan of Liaoning Provincial 2019-ZD-0549, Liaoning Education Department Youth Seedling Project of Education (2020JYT02), and the Program for Science and Technology Youth Talents in Department of Education in Guizhou Province 2017330. Plant Dis. 106:1517, 2022; published online as <https://doi.org/10.1094/PDIS-05-21-1031-PDN>. Accepted for publication 11 November 2021.

Rubus chingii, an important traditional Chinese medicine, belongs to the family Rosaceae. The fruit has multiple pharmacological activities, including as an antioxidant and anti-inflammatory and for improving cognitive impairment (Han et al. 2012). In June 2019, a new fungal infection was observed on the leaves of *R. chingii* in Qiandongnan Miao and Dong Autonomous Prefecture, Guizhou Province, China, forming small lesions with reddish-brown edges along leaf veins. Over 500 plants were surveyed, and nearly 20% of the plants were symptomatic. The diseased plants grew poorly and appeared stunted, and severely affected plants died. Five symptomatic leaves were randomly collected from the field and washed with tap water and distilled water successively. The edges of infected leaf tissue were cut into small pieces (4 to 5 mm²), surface sterilized with 70% ethanol for 30 s and 0.1% HgCl₂ for 1 min, and then rinsed three times in sterile distilled water (Chen et al. 2016). The same fungus was isolated from 41 pieces. The hyphae of a representative isolate were gray, the colony surface was granular, the edges were uneven and white, and the culture turned black over time with black spherical conidia. Conidia were nearly elliptical, unicellular, and each with a hyaline, unstable apical appendage, 3 to 10 µm long. The size of conidia was 10 to 18 µm in length and 4 to 8 µm in width. These morphological characteristics

are consistent with those described for the fungus *Phyllosticta capitalensis* (Wikee et al. 2013). For an accurate identification, genomic DNA of a representative isolate of the pathogen was extracted to amplify the internal transcribed spacer (ITS) region, the transcription elongation factor (*tefa-1*), and actin (*ACT*) genes with ITS1/ITS4, EF1-728F/EF1-986R, and ACT-512F/ACT-783R (Cheng et al. 2019), respectively. The ITS, *tefa-1*, and actin gene sequences were deposited in GenBank and assigned accession numbers MW308365, MW714380, and MW714381, respectively. BLAST search analysis of GenBank (NCBI) showed that the sequences had 100% similarity with those of *Phyllosticta capitalensis* (GenBank accession nos. ITS, MN548091; *tefa-1*, MN958711; and ACT, MN565575). The pathogenicity of *P. capitalensis* was verified using six healthy detached leaves from healthy *R. chingii* plants around 40 cm tall. A total of nine plants were used, and three leaves from each plant were artificially inoculated. Each wound was inoculated with conidial suspension (10⁶ ml⁻¹), while the control leaves were coated with sterile water. All the treated plants were covered with plastic bags for 2 days and incubated at 28°C and 85% relative humidity, with a 12-h photoperiod. Fifteen days following inoculation, the injured leaves showed similar symptoms to the above-mentioned lesions, while the control and uninjured leaves were still healthy. *P. capitalensis* was reisolated from inoculated leaves, fulfilling Koch's postulates. *P. capitalensis* is an endophyte, widely distributed in various host plants in China (Lu et al. 2016). To the best of our knowledge, this is the first report of black freckle disease caused by *P. capitalensis* on *R. chingii* in China. *P. capitalensis* is a destructive plant pathogen with an unusually broad host range and our findings will be useful for its management and for further research.

References:

- Cheng, L. L., et al. 2019. Plant Dis. 103:2964.
- Chen, Y. J., et al. 2016. Plant Dis. 100:227.
- Han, N., et al. 2012. Food Chem, 132:181.
- Lu, J., et al. 2016. J. Plant Pathol. 98:175.
- Wikee, S., et al. 2013. Fungal Divers. 60:91.

The author(s) declare no conflict of interest.

e-Xtra

Keywords: black freckle disease, *Phyllosticta capitalensis*, *Rubus chingii*

[†]Indicates the corresponding author.
D. Su; sdsyau@163.com