Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Coniella castaneicola* Causing Leaf Blight on Blueberry (*Vaccinium virgatum*) in China

Jiahao Lai, Guihong Xiong, Bing Liu,[†] Weigang Kuang, and Shuilin Song

College of Agriculture in Jiangxi Agricultural University, Nanchang, China, 330045

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Blueberry (*Vaccinium virgatum*), an economically important small-fruit crop, is characterized by its highly nutritive compounds and high content and wide diversity of bioactive compounds (Miller et al. 2019). In September 2020, an unknown leaf blight disease was observed on ‘Rabbiteye’ blueberry at the Agricultural Science and Technology Park of Jiangxi Agricultural University in Nanchang, China (28°45′51″ N, 115°50′52″ E). Disease surveys were conducted at that time; the results showed that disease incidence was 90% from a sampled population of 100 plants in the field, and this disease had not been found at other cultivation fields in Nanchang. Leaf blight disease on blueberry caused the leaves to shrivel and curl, or even fall off, which hindered floral bud development and subsequent yield potential. Symptoms of the disease initially appeared as irregular brown spots (1 to 7 mm in diameter) on the leaves, subsequently coalescing to form large, irregular taupe lesions (4 to 15 mm in diameter), which became curly. As the disease progressed, irregular gray-brown and blighted lesions ran throughout the leaf lamina from leaf tip to the entire leaf sheath and, finally, caused dieback and even shoot blight. To identify the causal agent, 15 small pieces (5 mm²) of symptomatic leaves were excised from the junction of diseased and healthy tissue, surface sterilized in 75% ethanol solution for 30 s and 0.1% mercuric chloride solution for 2 min, rinsed three times with sterile distilled water, then incubated on potato dextrose agar (PDA) at 28°C for 5 to 7 days in darkness. Five fungal isolates showing similar morphological characteristics were obtained as pure cultures by single-spore isolation. All fungal colonies on PDA were white with sparse, creeping hyphae. Pycnidia were spherical, light brown, and produced numerous conidia. Conidia were 10.60 to 20.12 by 1.98 to 3.11 µm (average 15.27 × 2.52 µm, *n* = 100), fusiform, sickle-shaped, and light brown, without septa. Based on morphological characteristics, the

fungal isolates were suspected to be *Coniella castaneicola* (Cui 2015). To further confirm the identity of this putative pathogen, two representative isolates (LGZ2 and LGZ3) were selected for molecular identification. The internal transcribed spacer region (ITS) and large subunit (LSU) were amplified and sequenced using primers ITS1/ITS4 (Peever et al. 2004) and LROR/LR7 (Castlebury and Rossman 2002), respectively. The sequences of the ITS region (GenBank accession nos. MW672530 and MW856809) showed 100% identity with accessions numbers KF564280 (576/576 bp), MW208111 (544/544 bp), and MW208112 (544/544 bp) of *C. castaneicola*. LSU gene sequences (GenBank accession nos. MW856810 and MW856811) was 99.85% (1,324/1,326 and 1,329/1,331 bp, respectively) identical to the sequences of *C. castaneicola* (KY473971, KR232683, KR232684). Pathogenicity was tested on three blueberry varieties (Rabbiteye, ‘Double Peak’, and ‘Pink Lemonade’), and four healthy young leaves of a potted blueberry of each variety with and without injury were inoculated with a 20-µl suspension of prepared spores (10⁶ conidia/ml) derived from 7-day-old cultures of LGZ2. In addition, four leaves of each variety with and without injury were sprayed with sterile distilled water as a control. The experiment was repeated three times, and all plants were incubated in a growth chamber (period of 12 h of light and 12 h of darkness at 25°C and RH > 80%). After 4 days, all of the inoculated leaves started showing disease symptoms (large, irregular, gray-brown lesions) similar to those observed in the field and there were no differences in severity recorded between the blueberry varieties, whereas the control leaves showed no symptoms. The fungus was reisolated from the inoculated leaves and confirmed as *C. castaneicola* by morphological and molecular identification, fulfilling Koch’s postulates. To our knowledge, this is the first report of *C. castaneicola* causing leaf blight on blueberry in China. The discovery of this new disease and the identification of the pathogen will provide useful information for developing effective control strategies, reducing economic losses in blueberry production, and promoting the development of the blueberry industry.

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[†]Indicates the corresponding author.
B. Liu; lbzm0418@126.com