Diseases Caused by Fungi and Fungus-Like Organisms

First Report of Anthracnose on *Rubus rosaefolius* Caused by *Colletotrichum boninense* in China

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Raspberry (*Rubus rosaefolius* Smith), also called march bubble or milk bubble, is widely distributed and economically important in China. Raspberries are rich in nutrients such as essential amino acids, vitamin C, dietary fiber, superoxide dismutase, and minerals (Yang et al. 2019). In May 2019, a leaf spot disease was observed on raspberry in Enshi (N29°07'10", E108°23'12"), Hubei province of China. The symptoms were small dark-brown spots on over 90% of observed plants. To isolate the pathogen, leaf sections (5 × 3 mm) from the border of the symptomatic tissue were cut and sterilized with 75% ethanol for 30 s, followed by 2% sodium hypochlorite (NaClO) for 2 min, and then rinsed three times with sterile water. Leaf sections were placed on potato dextrose agar (PDA) medium amended with 25 µg/ml of ampicillin and incubated at 25°C in the dark for 3 days. Isolated colonies were subcultured on PDA by hyphal-tip transfer. Eight fungal isolates with similar morphology, abundant white aerial hyphae, were collected. Colonies on PDA grew up to 80 mm in diameter by 7 days at 25°C. The center of each colony became black. Conidia were unicellular, oval, and hyaline. Conidia ranged in size from 14.5 to 19.75 × 5.80 to 10.20 µm ($n = 50$) in 20% (v/v) V8 vegetable juice medium. No appressoria were observed. Morphological characteristics are similar to those of *Colletotrichum* spp. (Moriwaki et al. 2003). Total genomic DNA of representative isolate S1 was extracted with a CTAB method (Stenglein and Balatti 2006). Internal transcribed spacer (*ITS*) region of rDNA, actin (*ACT*), beta-tubulin (*TUB2*), and glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) genes were amplified and sequenced with the primer pairs of ITS4/ITS5, ACT512F/ACT783R, Bt-2a/Bt-2b, and GDF1/GDR1, respectively (Weir et al. 2012). BLAST

results showed that *ITS*, *ACT*, *TUB2*, and *GAPDH* gene sequences (GenBank accession nos. MN498030, MT780498, MT780496, and MT780497, respectively) were 99% identical to those of *Colletotrichum boninense* Moriwaki, Sato & Tsukiboshi (GenBank accession nos. MF076598, JX009583, JQ005588, and JX009905, respectively). Concatenated sequences of the four genes were used to conduct a phylogenetic analysis using neighbor-joining method in MEGA7 (Schappe et al. 2019). The isolate S1 clustered with the above *C. boninense* strains retrieved from the NCBI database. Therefore, the present isolate S1 was identified as *C. boninense*. Pathogenicity tests were performed using 1-month-old raspberry plants, 24 controls and 30 inoculated. The plants were sprayed with conidial suspension (10⁶ conidia/ml) cultured on 20% (v/v) V8 vegetable juice medium for 15 days. The control plants were sprayed with sterile distilled water. All plants were covered with plastic bags for 24 h to maintain the relative humidity in the field. Fifteen days after inoculation, typical symptoms of brown spots were observed on leaves similar to the disease on field plants, whereas the leaves from the control group remained asymptomatic. *C. boninense* was reisolated and identified from inoculated symptomatic leaves. Anthracnose on raspberry caused by *Colletotrichum gloeosporioides* (Dai et al. 2013) and *C. fioriniae* (Schoeneberg et al. 2020) has previously been reported. However, to the best of our knowledge, this is the first report of *C. boninense* causing leaf spot on raspberry in China. If more reports of this pathogen are found on raspberries, then it may be necessary to develop effective management strategies for controlling this disease.

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The author(s) declare no conflict of interest.

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