Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Botryosphaeria dothidea* Causing Gray Mold on Tartary Buckwheat in Southwest China

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Tartary buckwheat (*Fagopyrum tataricum*, Polygonaceae) is an annual plant originating in Southwest China. It has a short growth cycle, barren soil tolerance, and strong stress resistance (Zhang et al. 2021). Because of its high content of proteins, starch, trace elements, phenols, and dietary fiber, Tartary buckwheat is beneficial to the human body and hence has received widespread attention (Joshi et al. 2019, 2020). In the period from September to November 2020, a diseased plant infected with gray mold was found among M₂ generation plants treated using ethyl methanesulfonate (EMS) in a location with potted Tartary buckwheat plants in Huaxi District, Guiyang City, Guizhou Province, China. The diseased plant started to show symptoms during the initial flowering stage; water-soaked spots appeared at first, and then the spots increased in size and turned into light brown patches, with the leaf edges scorched brown. In severe cases, the leaves turned yellow, the diseased spots became dry, and finally the leaves became necrotic. Among the leaves that showed disease symptoms, severely susceptible leaves were selected; a piece of tissue (2 × 2 mm) was removed at the junction of the diseased and healthy tissues. The tissue was then soaked in 75% ethanol for 2 to 3 s, transferred to 1% sodium hypochlorite solution and soaked for 3 min, rinsed three times with sterile water, and placed on sterilized filter paper to dry. Sterile tweezers were used to transfer the tissue blocks to potato dextrose agar containing a *Streptomyces-Penicillium* mixture (100 µg/ml), where they were incubated for 7 to 10 days at 25°C and 70% RH under 16 h light/8 h dark conditions. The colonies were white at the early stages, with developed aerial hyphae; they gradually turned gray-green. In later stages, the back of the colony was black and piles of conidia could be seen. The conidia were scattered,

colorless and transparent, spindle or fusiform, and 8.02 to 11.13 × 2.06 to 3.22 µm (avg. 9.51 × 2.69 µm, *n* = 50). These cultural and morphological characteristics were consistent with descriptions of *B. dothidea* (Fan et al. 2021). The ITS1/ITS4 (Mills et al. 1992), Bt-2a/Bt-2b primers (Glass and Donaldson 1995), and EF1-728F/EF1-986R (Slippers et al. 2004) were amplified and sequenced to analyze the ITS region, β-tubulin gene translation elongation factor 1-α (TEF1-α), and translation elongation factor 1-α (TEF1-α), respectively. According to BLAST search in GenBank, the sequences of ITS (MZ326853), TUB2 (MZ399162), and TEF1-α (MZ399163) had 99.40, 100, and 100% similarity to sequences NR111146.1, AY236927.1, and AY236898.1, respectively, of *B. dothidea* ex-type strain CMW8000. The three nucleotide sequences were concatenated together, and the neighbor-joining method of MEGA-X with 1,000 bootstraps was used to construct a phylogenetic tree. The results showed that our isolate was closely related to *B. dothidea*. Healthy Tartary buckwheat from the M₂ generation was used for the pathogenicity test. Leaves were disinfected with 75% alcohol and sprayed with spore suspension (1 × 10⁵ ml⁻¹). Each treatment included three plants and was repeated three times with sterile water as control. The treatments were kept in a greenhouse at 25°C for 24 h, then transferred to the natural environment of 22 to 28°C, and sterile water was sprayed every morning and evening to keep the leaves moist. After 10 days, the symptoms seen in the field appeared on treated plants, but control plants did not show any symptoms. The diseased parts of the leaves were isolated and cultured again, and the isolates were consistent with the original inoculum, thus confirming Koch's postulates. *B. dothidea* is a fungus with no host preference in the genus *Botryosphaeria*. It can cause canker, leaf spots, trunk diseases, fruit rot, and dieback of many important wood plants all over the world (Marsberg et al. 2017). Recently, it was reported that *B. dothidea* caused soybean canker in China (Chen et al. 2021), but there have been no reports of *B. dothidea* causing Tartary buckwheat gray mold. To our knowledge, this is the first report of *B. dothidea* causing gray mold on Tartary buckwheat. This finding will provide a basis for the prevention and treatment of Tartary buckwheat gray mold.

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