Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Botrytis cinerea* Causing Gray Mold on *Astragalus membranaceus* in China

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Plant Dis. 106:1520, 2022; published online as <https://doi.org/10.1094/PDIS-06-21-1330-PDN>. Accepted for publication 20 October 2021.

Astragalus membranaceus, known as Huang-qi, is a perennial herbal medicine plant that grows 50 to 150 cm high, with 13 to 31 small leaves (Fu et al. 2014). In September 2019, a disease investigation was conducted in Weiyuan, Gansu province, China, during cool, rainy weather. In 50% of the investigated fields ($n = 10$, the average size of fields was 0.14 ha), visible gray mold was observed on stems of *A. membranaceus*. In those fields, ~30 to 50% of the stems were withered and necrotic, with abundant dark brown mold from the presence of conidiophores. To determine the causal agent of disease, 12 symptomatic stem samples were collected from four fields (three samples per field). The samples were disinfected with 10% sodium hypochlorite for 1 min, cut into pieces (2 to 3 × 10 mm, $n = 12$), rinsed three times with sterile water, and dried on sterile tissue. Samples were placed on potato dextrose agar (PDA), three pieces per plate, and were incubated at 25°C in the dark. After 3 days, hyphal tips growing from the disinfected tissues were individually transferred to new PDA plates and incubated at 25°C in the dark. From the 10 isolates obtained, GWT6-2 was chosen as a representative isolate for further study. Colonies of GWT6-2 had gray aerial mycelia with margins that eventually turned taupe. In addition, some black, hard sclerotia (1.93 to 12.21 × 1.29 to 11.57 mm, $n = 20$) with a round or irregular shape developed after ~10 days of incubation at 25°C in the dark. Hyaline and round or elliptical conidia (7.86 to 14.53 × 6.81 to 11.79 μm, $n = 50$) were attached on top of brown conidiophores. Based on morphological characteristics, the isolate was initially identified as *Botrytis* sp. (Ellis 1971). For pathogenicity tests, 5 mm agar plugs from 1-week-old cultures of GWT6-2 were placed on four stabbed stems of *A. membranaceus* planted in 230 × 170 mm flowerpots. A similar number of plants inoculated with PDA plugs

(5 mm) were used as a control, and the assay was replicated three times. The treatments were kept in plastic buckets with a temperature of 20 to 25°C and relative humidity of 80 to 90%. After 48 h, the agar plugs were removed, and the plants were kept in the buckets for 3 to 4 days. Waterlogged spots developed on the stems of *A. membranaceus* 3 days after inoculation, which elongated to 3 to 5 cm after 4 to 5 days of inoculation, and the gray mold that appeared on the diseased lesions resembled that observed in the fields. The pathogen was reisolated from diseased tissues, and the cultural (colonies) and microscopic characteristics (conidia and conidiophores) were similar to those of the original isolate. Controls were asymptomatic. To further identify the species, GWT6-2 genomic DNA was extracted, and the internal transcribed spacer (*ITS*), heat shock protein (*HSP60*), and glyceraldehyde-3-phosphate dehydrogenase (*G3PDH*) genes were amplified with the primers ITS1/ITS4 (White et al. 1990), *HSP60*-F/*HSP60*-R, and *G3PDH*-F/*G3PDH*-R (Staats et al. 2005), respectively. The PCR amplicons were sequenced and compared with other sequences in GenBank using BLAST. The *ITS*, *HSP60*, and *G3PDH* sequences of GWT6-2 (MT225784.1, MT230538.1, MT263015.1) were 99.08, 100, and 99.25% identical to sequences of *Botrytis cinerea* strain GZFYQ-1 (MH454037.1, MH479931.1, MH479930.1) and *B. cinerea* isolate 5-3 (MH718836.1, MH796663.1, MH796662.1), respectively. A multilocus phylogenetic tree was constructed with the *ITS*, *HSP60*, and *G3PDH* reference sequences, and the grouping of strain GWT6-2 was supported by 99% bootstrap value. Based on the morphological characteristics and molecular identification, the strain GWT6-2 was identified as *B. cinerea*. To our knowledge, this is the first report of *B. cinerea* causing gray mold on the stems of *A. membranaceus* in China. *B. cinerea* may affect the aerial growth of *A. membranaceus*, causing economic losses of this important medicinal plant. Therefore, further investigations of the impact of this pathogen on *A. membranaceus* growth and management options are needed.

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The author(s) declare no conflict of interest.

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Keywords: *Astragalus membranaceus*, *Botrytis cinerea*, gray mold

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