First Report of *Alternaria alternata* Causing Leaf Spot on *Bellis perennis* in China

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Bellis perennis L., commonly known as the daisy or sun chrysanthemum, belonging to the family *Asteraceae*, is a perennial herb and is usually used as an ornamental plant worldwide for its vibrant flowers. Simultaneously, *B. perennis* has been proved to have therapeutic effects used on common colds, wound healing, anti-tumor, anxiolytic and antioxidant (Karakas et al. 2017). In July 2021, typical leaf spot was observed on *B. perennis* with about 50% disease incidence in Ruyue lake wetland park of Zibo (36.71°N, 118.01°E), Shandong Province, China. We surveyed more than 1000 square meters of planting area, and the diseased leaves were mostly concentrated in the lower location of plants,

where the humidity was higher under the forest. Symptoms on the initially diseased leaves appeared as light yellow, round or oval lesions with light or brown borders. With the development of the disease, the area of the lesion gradually expands, the color deepens, and the shape is becoming irregular. To identify the causal pathogen, small pieces of 15 tissues collected from the infected leaves were sterilized with 75% ethanol for 30 s and then 2% sodium hypochlorite (NaClO) for 60 s, finally rinsed with sterile water three times. All the tissues were placed on potato dextrose agar (PDA) and incubated at 25 ℃ in the dark for 5 days (Zhu et al. 2013). A total of 13 isolates were obtained from the above diseased leaves. The cultures were initially grayish white, then a light green halo appeared in the middle of the medium after 5 days, with numerous gray aerial hyphae. For molecular identification, the RNA polymerase II beta subunit (PRB2), Tsr ribosome biogenesis protein, partial coding sequences of chitin synthase, glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and major allergen Alt a 1 were amplified from genomic DNA extracted from four representative single isolates using the primers PRB2DF/PRB2DR, Tsr1F/Tsr1R, CHSDF1/CHSDR1, GDF1/GDR1, and AltF/AltR (Damn et al. 2019; Lawrence et al. 2013), respectively, and sequenced (GenBank accession nos. OL416000, OL416001, OL416002, OL416003, and OL416004). These genes had more than 99.9% nucleotide identity with the corresponding sequences

(KY131957.1, KY131958.1, KY996470.1, MN657411.1, and KY923227.1) of the reference strains of *Alternaria alternata* in GenBank. For pathogenicity tests, five healthy *B. perennis* plants each with three living leaves were inoculated with mycelial plugs of *A. alternata* from a 5-day-old culture grown on PDA. After inoculation, the plants were placed in a greenhouse with 85% relative humidity at 25 ℃ and monitored daily for symptom development. After 3 days, all inoculated leaves with mycelial plugs of *A. alternata* appeared symptoms similar to those observed in the field previously, while no symptoms appeared on negative controls which were inoculated with sterile PDA plugs. Cultures re-isolated from diseased leaves had the same morphological and molecular results as those isolated in the field, confirming Koch's postulates. The causal agent on *B. perennis* was confirmed as *A. alternata* on the basis of morphological and molecular results (Simmons 2007). To our knowledge, this is the first report on the presence of *A. alternata* affecting *B. perennis* plants in China. The discovery of this new disease is beneficial to the application and protection of *B. perennis*, which is a popular landscape and medicinal plant.

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Figure Leaf spot and colony of *Alternaria alternata*. A: Initial outdoor symptoms of

the disease. B: Late disease symptoms. C: Indoor infection of *Bellis perennis* artificially inoculated with mycelial plugs. D: The colony of *A. alternata* on PDA.