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First Report of *Alternaria alternata* on *Campanula rapunculoides* in Italy

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Creeping bellflower (*Campanula rapunculoides* L.) is a popular flowering plant commonly used in parks and gardens, belonging to Campanulaceae. An unknown leaf spot first appeared in May 2018 on *C. rapunculoides* plants grown in soil and pots in a garden located in Campiglia Cervo near Biella (northern Italy). The first symptoms were small, light-brown, circular spots, becoming irregular and dark brown. Lesions progressively enlarged to all the leaf surface, and severely affected plants were partially defoliated. Symptoms were widespread during the rainy months from May to July, when temperatures ranged from 15 to 25°C, on 80 to 90 plants grown in the garden. Small fragments of symptomatic tissues were surface sterilized in sodium hypochlorite (1% for 1 min), rinsed in sterile water, and plated on potato dextrose agar amended with 25 mg/liter of streptomycin sulfate. Olivaceous fungal colonies were consistently observed after 7 days at 22°C under 12-h light and dark. The 7- to 10-day-old monoconidial culture of three isolates grown on plate agar medium produced conidia that were dark brown, obclavate, obpyriform, ovoid or ellipsoid, with three to five transverse and zero to two longitudinal septa, and measured 11.4 to 30.9 (average 21.3) × 4.9 to 11.5 (average 8.0) µm. Sometimes a pale light bright to brown beak, 2.2 to 6.5 (average 3.7) µm, was observed. Conidiophores measured from 24.7 to 30.2 µm. Under a stereo microscope (Leica M165C), conidia were single or in chains of 9 to 13

elements, exhibiting a sporulation pattern with more than three branches of conidia per chain. On the basis of morphological characteristics, the pathogen was identified as *Alternaria* sp. (Simmons 2007). Genomic DNA of the representative isolate IT-22 was extracted from a pure culture with an E.Z.N.A. Fungal DNA Mini Kit (Omega Bio-Tek, Darmstadt, Germany). A polymerase chain reaction (PCR) was carried out using primers ITS1/ITS4, resulting in a 501-bp sequence (GenBank accession no. MH560609). A BLASTn analysis showed 100% homology with *Alternaria* sp. (MH399363). To identify the species, PCR was carried out using primers for sequencing the β-tubulin region subsequently: T1 (5′-AACATGCGTGAGATTGTAAGT-3′) (O'Donnell and Cigelnik 1997) and β-tub-2 (5′-ATCATGTTCTTGGGGTCGAA-3′) (Peever et al. 2004). A BLASTn analysis of the 999-bp β-tubulin sequence (GenBank accession no. MH560610) showed 100% identity with *Alternaria alternata* (Fr.) Keissl (KU512287). Pathogenicity tests were performed by spraying leaves of 3-month-old healthy plants of *C. rapunculoides* grown in 3-liter pots (one per pot) with an aqueous suspension at 10^5 conidia/ml obtained from IT-22 grown on PDA for 15 days. Plants sprayed only with water served as a control. Five pots were used for each treatment during two repeated tests. Plants were covered with plastic bags for 5 days and maintained in a greenhouse at a temperature between 20 and 24°C. Seven days after inoculation, a leaf spot similar to that previously described was observed in both tests, whereas control plants remained healthy. *A. alternata* was consistently reisolated from the lesions. A leaf spot caused by *A. alternata* on *C. medium* was previously reported in Italy (Garibaldi et al. 2016). Because the pathogen can cause severe defoliation of creeping bellflower, the presence of this disease is critical on this popular ornamental plant. This is, to our knowledge, the first report of *A. alternata* on *C. rapunculoides* in Italy as well as elsewhere.

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