First Report of Leaf Spot of *Campanula medium* Caused by *Alternaria alternata* in Italy

A. Garibaldi, S. Franco Ortega, G. Gilardi, and M. L. Gullino
Centre of Competence for Innovation in the Agro-Environmental Sector (AGROINNOVA), Largo Paolo Braccini 2, Italy; and M. L. Gullino, AGROINNOVA and DISAFA, Largo Paolo Braccini 2, Grugliasco, Italy.

Campanula medium L. (Canterbury bell, also known as bell flower) is an annual or biennial low maintenance flower plant belonging to the Campanulaceae, typically grown in parks and gardens. During summer 2015, extensive necrosis was observed on leaves of plants grown in a private garden near Biella in northern Italy (45.612166° N, 8.056297° E) at an altitude of 850 m. The garden, about 8,000 m^2, was designed a century ago, and it is planted with many other native acid-loving species. The disease affected 30 to 70% of about 100 plants (10 to 20 months old), grown under high relative humidity, at temperatures of 15 to 26°C. The first symptoms were usually brown to black, circular lesions surrounded by a yellow halo, frequently localized on the tips and margins of leaves. Lesions sometimes coalesced and infected leaves eventually turned brown. In some cases, stems were affected, and plants died; no symptoms were observed on flowers since they were not present at the appearance of symptoms. A fungus was consistently isolated from infected leaves on potato dextrose agar (PDA), added with streptomycin sulfate at 25 mg/liter. The monoconidial isolate AltCm42, grown for 7 days with a 12-h photoperiod at 20 to 22°C on PDA, produced a greenish mycelium with dark brown conidia, in chains of 2 to 8 elements, ovoid, elliptical or obclavate in shape, with a beak. Conidia showed 2 to 6 transverse and 0 to 4 longitudinal septa, and measured 17.7 to 48.4 (avg. 24.4) × 6.8 to 8.9 (avg. 7.8) µm. The beaks showed a color generally lighter than the rest of the conidia, 2.2 to 9.4 (average 6.2) µm long. Based on its morphological characteristics, the pathogen was identified as *Alternaria* sp. (Simmons 2007). Genomic DNA was extracted with E.Z.N.A. Plant DNA Kit (Omega Bio-Tek, Norcross, GA) from pure culture. PCR reactions were performed using primers ITS1/ITS4 (White et al. 1990) to amplify the ITS, the intergenic region between 28S and 18S sequences of the ribosomal RNA, including the 5.8S sequence. Amplicon was analyzed by electrophoresis and sequenced by BMR Genomics (Padova, Italy), obtaining 484 bp (GenBank KU512288). BLAST analysis showed 100% identity with *Alternaria* sp. (KP245769). To identify the species, PCR of the beta-tubulin 2 gene (TUB2) between exons 2 and 6 was performed with the primers T1 (O'Donnell and Cigelnik 1997) and βt2b (Glass and Donaldson 1995). The PCR product was purified and sequenced in both directions by BMR Genomics. BLASTn analysis of the 1,005-bp product produced a 100% homology with *Alternaria alternata* (Fr.) Keissl. (KJ396337). The sequence has been deposited to GenBank (KU512287). Pathogenicity tests were performed by inoculating leaves of healthy 7-month-old plants of *C. medium* with a mycelium and spore suspension (10^5 CFU/ml) obtained from cultures of the isolate AltCm42 grown on PDA for 10 days, in light-dark, at 22 ± 1°C. Plants inoculated only with sterilized water served as controls. Three inoculated and noninoculated plants were used in each trial. Plants were covered with plastic bags for 5 days after inoculation and maintained outdoors at 15 to 22°C. First lesions developed on leaves 6 days after inoculation, whereas control plants remained healthy. *A. alternata* was consistently reisolated from the lesions of the inoculated plants only. No fungal colonies were isolated from asymptomatic leaves of noninoculated plants. The pathogenicity test was carried out twice. This is, to our knowledge, the first report of *A. alternata* on *C. medium* in Italy as well as worldwide. The presence and importance of this disease is, at present, limited. *A. alternata* has a wide host range and this report increases the list with a new host.

References

Glass, N. L., and Donaldson, G. C. 1995. Appl. Environ. Microbiol. 61:1323. ISI
O'Donnell, K., and Cigelnik, E. 1997. Mol. Phylogenet. Evol. 7:103. https://doi.org/10.1006/mpev.1996.0376 Crossref, ISI

Simmons, E. G. 2007. Page 775 in: Alternaria. An Identification Manual. CBS, Utrecht, The Netherlands.
White, T. J., et al.. 1990. Page 315 in: PCR Protocols: A Guide to Methods and Applications. Academic Press, San Diego.