First Report of Rhizoctonia Crown Rot on Carpathian Bellflower (*Campanula carpatica*) in Northern Italy

A.Garibaldi, G. Gilardi, D. Bertetti, S. Franco Ortega, 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.

Carpathian bellflower (*Campanula carpatica* Jacq.) is a low-maintenance flowering plant commonly grown in gardens. During the summer of 2017, symptoms of a crown rot were observed on plants of Carpathian bellflower cultivated in a private garden located in Biella province (Northern Italy). A high incidence of crown rot was observed on 90-day-old plants grown in 10 pots (5 plants/pot). As the disease progressed, lesions developed on the stems, causing blight symptoms. Stem fragments of the diseased tissues taken from five plants were disinfected for 10 s in 1% NaOCl, rinsed with sterilized water, and plated on potato dextrose agar (PDA) with 25 mg/liter of streptomycin sulfate. A fungus with the morphological characteristics of *Rhizoctonia solani* was consistently isolated (Sneh et al. 1991). A pure culture of one of the isolates, named IT10, was grown on PDA for about 30 days. The colony showed a light brown, coarse, flattened mycelium, with hyphae developing radially and a few small, dark brown, rounded sclerotia with a crusty surface. The isolate IT10 was paired with *R. solani* isolates AG 1, AG 2, AG 4, AG 7, and AG 11 and then observed microscopically for anastomosis (Carling 1996). Three pairings were made for each tested group. IT10 formed anastomoses only with *R. solani* isolate AG 4 (fusion frequency < 30%), showing typical morphological characteristics reported for this group (Sneh et al. 1991). The genomic DNA of IT10 was extracted with an E.Z.N.A. Plant DNA Kit (Omega Bio-Tek) from a pure culture. Polymerase chain reaction (PCR) was carried out using primers ITS1/ITS4 to amplify the internal transcribed spacer region between the 18s and 28s ribosomal RNA sequences that includes the 5.8s rRNA sequence. The PCR product was purified and sequenced by BMR Genomics Sequencing Services (Padova, Italy). The 468-bp sequence was analyzed using BLASTn (Altschul et al. 1997) and showed a 100% homology with *R. solani* AG 4 HG-I (GenBank accession nos. KX468085.1, KX631341.1, KX631297.1, KX631291.1, and KX631249.1). The sequence was deposited to GenBank with accession number MG384802. The isolate IT10 was tested for its pathogenicity by placing mycelial plugs of a 7-day-old culture grown on PDA close to the crown of 10 healthy plants of *C. carpatica* (30 days old), grown in 2-liter pots filled with a peat substrate previously steamed at 70°C for 90 min. The same number of plants, inoculated with pure PDA plugs, served as controls. Plants were maintained in a growth chamber at 25 ± 1°C with 12-h light/dark. The first symptoms, similar to those observed in the garden, developed 5 days after inoculation, and 10 days later all the inoculated plants were dead. Colonies with morphological characteristics typical of *R. solani* AG 4 were reisolated from infected leaves of inoculated plants. No symptoms developed on control plants. The pathogenicity test was conducted twice with similar results. To our knowledge, this is the first report of crown rot of *C. carpatica* caused by *R. solani* AG 4 in Italy, as well as worldwide. The wide host range of *R. solani* AG 4 is well known, and this report extends it to a new host (Farr and Rossman 2017).

References

Altschul, S. F., et al.. 1997. Nucleic Acids Res. 25:3389. https://doi.org/10.1093/nar/25.17.3389
Carling, D. E. 1996. Page 37 in: Rhizoctonia Species: Taxonomy, Molecular Biology, Ecology, Pathology and Disease Control. Kluwer Academic, Netherlands.
Farr, D. F., and Rossman, A. Y. 2017. Fungal Databases, Syst. Mycol. Microbiol. Lab. ARS, USDA. Online publication. Retrieved 28 December 2017 from https://nt.ars-grin.gov/fungaldatabases/
Sneh, B., et al.. 1991. Identification of Rhizoctonia Species. APS Press, St Paul, MN.