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DISEASE NOTES

First Report of Leaf Spot Caused by *Alternaria alternata* on *Echinacea purpurea* in Italy

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Purple coneflower (*Echinacea purpurea* L.) is a herbaceous ornamental plant of the Asteraceae, widely grown in Italy for its long-blooming elegance and resistance to drought. In summer 2017, a previously unknown leaf spot was observed on 6- to 10-month-old plants grown in gardens in Campiglia Cervo near Biella (northern Italy) at 900 m asl. Affected plants showed black-brown, circular leaf spots, usually 5 to 40 mm in diameter. Lesions usually started on the upper side of older leaves at margins and tips, enlarging to cover the entire surface. Severely affected plants were defoliated. The disease affected 40 to 60% of leaves at temperatures of 18 to 28°C. Fragments of diseased tissues were disinfected for 10 s in 1% NaOCl, rinsed with sterilized water, and plated on PDA with 25 mg/liter of streptomycin sulfate. A fungus was consistently isolated from affected leaves. The conidia produced from two selected monoconidial isolates (IT44 and IT44.2) on potato carrot agar (PCA) were dark brown, obclavate, obpyriform, ovoid or ellipsoid, with 3 to 7 transverse and 0 to 4 longitudinal septa, and 12.5 to 37.8 (avg. 25.5 ± 1.2) × 7.8 to 17.9 (avg. 11.8 ± 0.5) µm. About 39% of conidia showed beaks usually lighter in color than the body, 1.5 to 9.4 (avg. 4.1 ± 0.4) µm. The 10-day-old monoconidial culture of both the isolates observed under a stereomicroscope (Leica M165C) showed conidia singly or in chains of 8 to 15 from conidiophores (17.4 to 56.9 µm). On the basis of its morphological characteristics, the pathogen was identified as *Alternaria* sp. (Simmons 2007). DNA was extracted from IT44 and IT44.2 with the E.Z.N.A. Fungal DNA Mini Kit (Omega Bio-Tek). The internal transcribed spacer (ITS) region of rDNA, endopolygalacturonase (*endoPG*), *Alternaria* major allergen (*Alta1*), and the portion of the histone 3 (*H3*) gene of these two isolates were amplified with primers ITS1/ITS4, PG3/PG2b, Alt-for/Alt-rev, and H31a/H31b, respectively (Glass and Donaldson 1995; Woudenberg et al. 2015). Amplicons were sequenced at the BMR Genomics Centre (Padova, Italy). BLASTn analyses of IT44 and IT44.2 showed 100% identity to the rDNA ITS region of *A. tenuissima* (KP278184) and *A. alternata* (KX783405), respectively. Furthermore, IT44 and IT44.2 showed 100% identity to the *endoPG* region of *Alternaria* sp. (KY969550), 100% identity to the *Alta1* region of *A. alternata* (KX894537), and 100% identity to the *H3* region of *A. alternata* (KY548071). The sequences of IT44 and IT44.2 were deposited to GenBank under accessions MG182428 and MG598794 (ITS), MG598798 and MG598799 (*endoPG*), MG598796 and MG598797 (*Alta1*), and MG182429 and MG598795 (*H3*), respectively. Based on the multigene phylogeny study by Woudenberg et al. (2015), *A. alternata* and *A.*

tenuissima are the same species and have synonymized (with five others) as a single species *A. alternata*. Molecular data of the four studied genes suggest that the identified species belongs to *A. alternata*. Furthermore, phylogenetic analyses based on *H3* gene sequences and the other three genes (data not shown) resulted in a clear grouping of the IT41 and IT44.2 isolates together with reference *A. alternata* isolates. Pathogenicity tests were performed by spraying leaves of healthy 6-month-old purple coneflower plants with an aqueous suspension at 10^5 conidia/ml obtained from IT44 grown on PCA for 15 days, 12-h light-dark, at 23 ± 1°C. Plants sprayed with water served as controls. Three pots were used for each treatment during two repeated tests. Plants were covered with plastic bags for 5 days and maintained in the same garden at 16 to 26°C. Results from both tests showed leaf spots similar to those previously described 10 to 12 days after inoculation, whereas control plants remained healthy. The pathogen was consistently reisolated from the lesions. A leaf spot caused by *A. alternata* was previously reported on purple coneflower in Poland (Kucharski 1997), but this is, to our knowledge, the first report of *A. alternata* on *E. purpurea* in Italy. The use of *Echinacea* spp. has significantly increased in Italian gardens. As a consequence, *A. alternata* could become a problem in many gardens, requiring effective management.

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