

First Report of Leaf Spot of Peppermint (*Mentha × piperita*) Caused by *Alternaria alternata* in Italy

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plant disease

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DISEASE NOTES

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Peppermint (*Mentha × piperita* L.) is a popular herb belonging to the Lamiaceae, extensively grown in Italy for the oil used in foods as flavor, in cosmetics, and in the pharmaceutical industry. During summer 2017, extensive necrosis was observed on leaves of peppermint grown in soil and in plastic pots in a private garden in Biella province, Northern Italy

(45°36'00"N, 8°03'00"E). About 30% of the 60 plants, 3 to 4 months old, grown mainly in partial shadow at temperatures from 20 to 28°C, were affected. The first symptoms were usually brown lesions 1 to 30 mm in diameter, which progressively turned black. Lesions usually started at the margins and tips on the upper side of older leaves and showed a yellow halo. Severely affected plants were defoliated. Several isolations were carried out during the summer from infected leaves on potato dextrose agar medium amended with 25 mg/liter of streptomycin sulfate. Eight dark olive colonies of a fungus with similar morphological characteristics were consistently obtained. The conidia of IT61 isolate, used as a reference, produced 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 measured 20.9 to 42.4 (average 36.8) µm by 6.3 to 13.1 (average 12.8) µm. Conidia were produced in chains (7 to 10 elements) and sometimes presented a conical or cylindrical beak, 3.5 to 7.4 µm, pale light brown to brown. On the basis of its morphological characteristics the fungus was identified as *Alternaria* sp. (Simmons 2007). DNA was extracted from one selected monoconidial isolate (IT61) by using the E.Z.N.A. Fungal DNA Mini Kit (Omega Bio-Tek, Darmstadt, Germany). The internal transcribed spacer (ITS) region of rDNA of this isolate was amplified with the primers ITS1/ITS4 and sequenced at the BMR Genomics Centre (Padua, Italy). A BLASTn analysis of the 432-bp fragment from IT61 showed a 100% identity to the rDNA ITS region of *Alternaria alternata* strain SRLS-1 (GenBank accession no. KX894536). The sequence was deposited to GenBank under accession number MF997592. The ITS sequence obtained by ITS1/ITS4 primers is not conclusive in differentiation between *A. alternata* and *A. tenuissima* (Zheng et al. 2015). Therefore, the portion of the histone 3 gene of isolate IT61 was amplified using the primers H31a (5'-ACTAAGCAGACCGCCCGCAGG-3') and H31b (5'-GCGGGCGAGCTGGATGTCCTT-3') (Glass and Donaldson 1995) and sequenced as described above. The obtained sequence (417 bp) was deposited (GenBank accession no. MF997593), and it was 100% identical to that of *A. alternata* isolate JT-LJT-NWG-A1 (GenBank accession no. KF997067). Furthermore, the phylogenetic analysis based on the histone 3 gene sequences resulted in a clear separation of *A. alternata* isolates (including the IT61 isolate) from the isolates of *A. tenuissima* and *A. solani*, which gathered into different groups. Pathogenicity tests were performed by spraying leaves of 2-month-old apparently healthy peppermint plants with an aqueous 10⁵ CFU/ml spore and mycelial suspension obtained from IT61 cultures produced on PCA. Plants sprayed only with water served as a control. Three pots (3 plants/pot) were used for each treatment. Plants were covered with plastic bags for 4 days after inoculation and maintained in the same garden at an average temperature of 24°C. Lesions developed on leaves 7 to 10 days after the artificial inoculation, fulfilling Koch's postulates, whereas control plants remained healthy. *A. alternata* was consistently reisolated from these lesions. The pathogenicity tests were carried out twice. The presence of *A. alternata* on peppermint was reported in Poland and recently in Iran (Zarandi et al. 2014). This is, to our knowledge, the first report of *A. alternata* on *Mentha × piperita* in Italy. Due to the importance of peppermint in many Mediterranean countries, the potential impact of this disease is high.

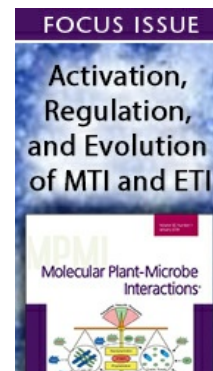
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