Disease Note

Diseases Caused by Fungi and Fungus-Like Organisms

First Report of Necrotic Leaf Spot Caused by *Plectosphaerella cucumerina* on *Aquilegia flabellata* in Italy

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Fan columbine (*Aquilegia flabellata* Siebold & Zucc.), Ranunculaceae family, is a perennial herbaceous plant producing blue-purple flowers that is used in gardens and for cut flower production. During June 2020, brown spots (0.5 to 1.0 mm in diameter) appeared on 20% of leaves of 115 plants of *A. flabellata* growing in a private garden located in Biella province (northern Italy). Progressively, spots enlarged to 5 to 6 mm and formed irregular necrosis surrounded by chlorotic halos that sometimes coalesced. Several affected leaves were disinfected in sodium hypochlorite (1%) for 30 s and then rinsed in sterile water. Fragments were excised from the margins of necrosis and plated on potato dextrose agar (PDA) amended with streptomycin sulfate (25 mg/liter). Plates were maintained in a 17-h photoperiod at 25 ± 1°C for 9 days before subculturing onto PDA medium. The fungal morphology was pale orange with hyaline mycelium that formed hyphal coils. Conidia were ellipsoidal with rounded apices, not septate, guttulate, and measured 4.2 to 8.8 × 1.8 to 3.2 (average 6.5 × 2.4) µm (length/width ratio 2.7; n = 50). Conidia were supported by straight, sometimes sinuous, tapered phialides. Phialides were 5.1 to 22.6 (average 11.2) µm (n = 30) and sometimes with a septum near the base. These morphological characteristics resembled those of the fungi *Plectosphaerella* (Carlucci et al. 2012). The DNA was extracted with an E.Z.N.A. Plant DNA Kit (Omega Bio-Tek) from pure cultures of three isolates belonging to the collection of Agroinnova. PCR was carried out with primers ITS1/ITS4 (White et al. 1990), and the PCR products were purified and sequenced, obtaining 481-bp-long sequences (GenBank accession nos. MW205832, MW205833, MW205834). BLASTn analysis (Altschul et al. 1997) of these sequences showed 99.6% nucleotide identity with the sequence of *Plectosphaerella cucumerina* (Lindf.) W.Gams (ex-type CBS 131.739; GenBank accession no. LR026809). Pathogenicity testing was performed inoculating the isolates 20/14-1, 20/14-2, and 20/14-3 on 5-month-old healthy plants of *A. flabellata* (nine plants per each isolate). Inoculum was prepared from colonies grown on PDA at 21 ± 1°C for 18 days. A conidial suspension was obtained for each isolate to the final concentration of 5×10^5 conidia/ml and sprayed on leaves (5 ml per plant). Nine control plants were sprayed with sterile water. Plants were maintained in moistened plastic bags for 7 days, at temperatures ranging from 15 to 25°C. About 15 days after inoculation, the first spots appeared on inoculated leaves, which later expanded and became necrotic, whereas control plants remained healthy. *P. cucumerina* has been reported in several countries, on many hosts (Farr and Rossman 2020); however, this is the first report of this pathogen on *A. flabellata*. The incidence of *P. cucumerina* on fan columbine constitutes a problem in Italy due to the widespread use of *A. flabellata* in public gardens and the difficulty of managing this disease.

References:

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