Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Ramularia coleosporii* Causing Leaf Spot on *Perilla frutescens* in Korea

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Perilla (*Perilla frutescens* var. *japonica*), a member of the family Labiatae, is an annual herbaceous plant native to Asia. Its fresh leaves are directly consumed, and its seeds are used for cooking oil. In July 2018, leaf spot symptoms were observed in an experimental field at Gangneung-Wonju National University, Gangneung, Gangwon province, Korea. Approximately 30% of the perilla plants growing in an area of about 0.1 ha were affected. Small, circular to oval, necrotic spots with yellow borders were scattered across upper leaves. Masses of white spores were observed on the leaf underside. Ten small pieces of tissue were removed from the margins of the lesions, surface disinfected with NaOCl (1% v/v) for 30 s, and then rinsed three times with distilled water for 60 s. The tissue pieces were then placed on potato dextrose agar (PDA) and incubated at 25°C for 7 days. Five single-spore isolates were obtained and cultured on PDA. The fungus was slow-growing and produced 30- to 50-mm-diameter, whitish colonies on PDA when incubated at 25°C for 15 days. Conidia ($n = 50$) ranged from 5.5 to 21.3 $\times$ 3.5 to 5.8 μ m, were catenate, in simple or branched chains, ellipsoid-ovoid, fusiform, and old conidia sometimes had one to three conspicuous hila. Conidiophores ($n = 10$) were 21.3 to 125.8 $\times$ 1.3 to 3.6 μ m in size, unbranched, straight or flexuous, and hyaline. The morphological characteristics of five isolates were similar. Morphological characteristics were consistent with those described for *Ramularia coleosporii* (Braun 1998). Two representative isolates (PLS 001 and PLS003) were deposited in the Korean Agricultural Culture Collection (KACC48670 and KACC48671). For molecular identification, a multilocus sequence analysis was conducted. The internal transcribed

spacer (ITS) regions of the rDNA, partial actin (ACT) gene, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene were amplified using primer sets ITS1/4, ACT-512F/ACT-783R, and gpd1/gpd2, respectively (Videira et al. 2016). Sequences obtained from each of the three loci for isolates PLS001 and PLS003 were deposited in GenBank with accession numbers MH974744 and MW470869 (ITS), MW470867 and MW470870 (ACT), and MW470868 and MW470871 (GAPDH), respectively. Sequences for all three genes exhibited 100% identity with *R. coleosporii*, GenBank accession numbers GU214692 (ITS), KX287643 (ACT), and 288200 (GAPDH) for both isolates. A multilocus phylogenetic tree, constructed by the neighbor-joining method with closely related reference sequences downloaded from the GenBank database, and these two isolates demonstrated alignment with *R. coleosporii*. To confirm pathogenicity, 150 ml of a conidial suspension (2×10^5 spores/ml) was sprayed on five 45-day-old perilla plants. An additional five plants, to serve as controls, were sprayed with sterile water. All plants were placed in a humidity chamber (>90% relative humidity) at 25°C for 48 h after inoculation and then placed in a greenhouse at 22/28°C (night/day). After 15 days, leaf spot symptoms, similar to the original symptoms, developed on the leaves of the inoculated plants, whereas the control plants remained symptomless. The pathogenicity test was repeated twice with similar results. A fungus was re-isolated from the leaf lesions on the inoculated plants, which exhibited the same morphological characteristics as the original isolates, fulfilling Koch's postulates. *R. coleosporii* has been reported as a hyperparasite on the rust fungus *Coleosporium plumeriae* in India and Thailand and also as a pathogen infecting leaves of *Campanula rapunculoides* in Armenia, *Clematis gouriana* in Taiwan, *Ipomoea batatas* in Puerto Rico, and *Perilla frutescens* var. *acuta* in China (Baiswar et al. 2015; Farr and Rossman 2021). To the best of our knowledge, this is the first report of *R. coleosporii* causing leaf spot on *P. frutescens* var. *japonica* in Korea. This disease poses a threat to production, and management strategies to minimize leaf spot should be developed.

References:

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