Diseases Caused by Fungi and Fungus-Like Organisms

First Report of Leaf Spot Caused by *Ramularia sphaeroidea* on *Vicia villosa* var. *glabrescens* in China

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Hairy vetch (*Vicia villosa*) is widely grown in southwestern China for green manure and forage. In December 2019, a leaf disease occurred on 80% plants of *V. villosa* var. *glabrescens* in an area representing greater than 6 ha (total area planted was 8 ha) located in Qujing City (N 25°28'12", E 103°36'22"), Yunnan Province, China. Its symptoms were irregular, brown to dark brown leaf spots with white mold. Twenty diseased leaves from five plants (four leaves per plant) were randomly collected from the field. Its pathogen was observed under a microscope and isolated from diseased leaves sterilized with 75% ethanol for 30 s and 1% NaClO for 1 min 15 s, rinsed three times with sterile distilled water, dried between sterile filter paper, and placed onto potato dextrose agar (PDA). The isolates obtained were purified by transferring 1- to 2-mm emerging hyphal tips onto fresh PDA plates and grew slowly at a rate of 0.7 mm/day at 20°C for 4 weeks. A diseased plant specimen and three isolates were deposited at the Mycological Herbarium of Lanzhou University (MHLZU), under the accessions MHLZU19326 and YN1931401, YN1931402, and YN1931403, respectively. Conidia of this fungus cultured on PDA were hyaline, spherical, smooth, aseptate, and 2.13 to 3.67 × 4.56 to 5.77 μm ($n = 50$), and conidiophores were hyaline, smooth, and straight. DNA of purified isolates was extracted, and the ITS, *tefl-α*, *his3*, and *gapdh* genes were amplified and sequenced with primers ITS1/ITS4

(White et al. 1990), EF1-728F/EF2 (Carbone and Kohn 1999; O'Donnell et al. 1998), CylH3F/CylH3R (Crous et al. 2004), and *gpd1/gpd2* (Berbee et al. 1999), respectively. DNA sequences of the strains YN1931401, YN1931402, and YN1931403 were deposited in GenBank for the ITS (GenBank accession nos. MW092181, MW332205, and MW332206), *tefl-α* (MW448172 to MW448174), *his3* (MW448175 to MW448177), and *gapdh* (MW448178 to MW448180). These regions showed similarities with other sequences of *Ramularia sphaeroidea* in GenBank of 99% (514/516, 515/517, and 514/517 base pairs) for the ITS, 99% (402/403, 403/405, and 405/405 base pairs) for *tefl-α*, 99% (377/378, 378/378, and 376/378 base pairs) for *his3*, and 100% (558/557, 557/559, and 561/565 base pairs) for *gapdh*. These sequences were used for constructing a phylogenetic tree. The fungus was morphologically and molecularly identified as *R. sphaeroidea*. Infection experiments were carried out with 50 plants of this plant species in 10 pots (five seedlings per pot). A conidial suspension from YN1931401 was prepared by adding sterile distilled water and scraping the cultures with a sterile scalpel. The leaves of 25 healthy plants were sprayed with a conidial suspension of 1.0×10^6 conidia/ml with 0.01% Tween 80; control plants consisted of 25 plants that received a sterile water spray. All plants were covered with clear polyethylene bags for 3 days to maintain high humidity and kept in a greenhouse at day (18°C, 18-h light) and night (22°C, 6-h dark). Ten days postinoculation, the inoculated plants exhibited brown lesions similar to symptoms in the fields, whereas no symptoms appeared on the control plants. The same fungus was reisolated and identified as outlined above. To our knowledge, *R. sphaeroidea* has also occurred on *V. fabae* and *V. sativa* in Ethiopia and Israel (Braun 1998), and it is also reported on *V. cracca* in China (Zhang et al. 2006), but not on *V. villosa* in China yet. Therefore, this is the first report of *R. sphaeroidea* causing leaf spot on hairy vetch (*V. villosa*) in China.

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