Diseases Caused by Fungi and Fungus-Like Organisms

First Report of Round Leaf Spot on *Sophora tonkinensis* Caused by *Didymella glomerata* in China

Lisha Song, Ni Jiang,[†] Guiyu Tan, Qianping Chen, Shixin Feng, and Zhanjiang Zhang

Guangxi Botanical Garden of Medicinal Plants, Nanning, Guangxi 530023, China

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The root of *Sophora tonkinensis* Gagnep. is an important medicinal material in China. An unknown foliar disease, first observed in July 2018, occurred over 240 ha of *S. tonkinensis* (total cultured 600 ha) in Guangxi, China, in December 2019. The initial symptoms on leaf were seen as small, tawny spots (0.5 to 1.5 mm in diameter). As the disease progressed, the lesions enlarged into grayish and dark brown concentric rings (5 to 10.0 mm in diameter), resulting in black protuberances in the center of the spots. Severe infections would adversely affect plant growth and cut the production by 30 to 40%. Symptomatic leaves were sampled, and the surface was sterilized with 75% ethanol for 30 s and then soaked in 0.1% HgCl₂ for 2 min. After three washes with sterile distilled water, the samples were dried, placed aseptically onto potato dextrose agar (PDA) plates, and incubated at 28°C. Three days later, the isolates were placed on new PDA medium for subsequent purification and sporulation. The fungus, SDG-1, was recovered from 85% of the total 40 isolates. Its colonies were whitish initially and then became olive green 7 days after incubation at 28°C. The pycnidia were globose to subglobose, initially brown and darkened at

maturity, 85 to 300 × 70 to 280 µm in size. The conidia were colorless, single-celled, rounded to ellipsoidal, and 3.5 to 6.6 × 1.5 to 3.8 µm. The chlamydospores were multicellular and brick trellised, usually forming branched or unbranched chains, light to dark brown in color, and measured 28.5 to 44.5 × 8.2 to 16.5 µm. The morphological characteristics were consistent with *Didymella glomerata*. The rDNA-ITS, β-tubulin, and actin of strain SDG-1 were PCR amplified, and the DNA sequencing results were almost 100% identical to those of *D. glomerata*, respectively (GenBank accession nos. MN 435377, MN447333, and MN447334, respectively). Multilocus phylogenetic analysis was carried out with the obtained sequences, which revealed that the isolates clustered within *D. glomerata* with similarity of 100%. Therefore, based on the morphology and phylogenetic tree, strain SDG-1 was identified as *D. glomerata*. For pathogenicity tests, the *S. tonkinensis* was cultured for 2 years, and the conidial suspension of SDG-1 (1 × 10⁶ conidia/ml) was prepared by harvesting conidia from a 10-day-old culture on PDA. Conidia were sprayed onto healthy leaves of *S. tonkinensis* for coculture, whereas the control group was sprayed with sterile distilled water. Each experiment was performed three times. All plants were covered with plastic bags for 3 days in order to maintain high humidity and cultured in a greenhouse at 28°C with a 12-h/12-h light/dark cycle. Symptoms appeared 7 days after the leaves were inoculated with spores, which were identical to those observed in the field. The pathogenic fungus was reisolated from the infected leaves and identified as previously described. The control leaves remained symptomless during the pathogenicity tests. According to the previous literature, *D. glomerata* is a plant-pathogenic fungus with a wide host range, which can damage up to 100 species of woody and herbaceous plants (Aghapour et al. 2009; Lahoz et al. 2007). To our knowledge, this is the first report of *D. glomerata* causing round leaf spot on *S. tonkinensis* in China.

References:

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[†]Indicates the corresponding author.
E-mail: N. Jiang; jiangni292@126.com