Diseases Caused by Fungi and Fungus-Like Organisms

First Report of Leaf Spot in *Farfugium japonicum* Caused by *Alternaria alternata* in China

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Farfugium japonicum (L.) Kitam (with the common name leopard plant) is known as a garden and medicinal herb, and it belongs to the family Asteraceae. In May 2019, a leaf spot disease was observed on the upper leaf surface of *F. japonicum* in Changsha city, Hunan province, China. More than 98% of the *F. japonicum* plants were infected in a garden of Donghu district (28°13' N; 112°56' E). Leaf symptoms included small (1 to 10 mm in diameter), brown spots that were circular, tan to gray in the center, and had distinct brownish-yellow margins. Severely affected leaves were blighted, and plants were dying. For isolation, symptomatic leaf tissue was surface sterilized in 75% ethanol, rinsed in sterile distilled water, and plated on potato dextrose agar amended with 50 µg/ml of streptomycin sulfate followed by incubation at 25°C in darkness. By a single-spore isolation technique, pure fungal cultures were obtained and displayed gray-brown and gray-white aerial mycelia after 5 days of incubation. One representative isolate (HnAa-1) was selected for further studies. Conidia of HnAa-1 were olive-brown, obpyriform, either branched or unbranched, with a short beak, one to five transverse septa, and zero to three longitudinal or oblique septa. The conidia were 10 to 35 µm long and 2 to 12 µm wide. HnAa-1 was identified as an *Alternaria* sp. based on the morphological characterization by Simmons (2007). Further identification to species level was made by molecular analyses. DNA of

HnAa-1 was extracted from the following regions: internal transcribed spacer (ITS), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), and partial Alt a 1 major allergen (ALT) gene. Amplification and sequencing were carried out with the method described by Woudenberg et al. (2015). BLASTn searches showed that the ITS, GAPDH, and ALT sequences had the highest similarity with *Alternaria alternata* strains, with 100% (548/548) identity for ITS (GQ169728), 100% (567/567) identity for GAPDH (MK903028), and 99.36% (466/469) identity for ALT (MN184998). Moreover, the ITS, GAPDH, and ALT sequences had more than 99% identities with the epitype CBS 916.96 of *A. alternata* (ITS, AF347031; GAPDH, AY278808; ALT, AY563301). The ITS, GAPDH, and ALT sequences of HnAa-1 were submitted to GenBank (accession nos. MT767170, MW115639, and MW316727). Pathogenicity tests were conducted by spraying a 10-ml conidial suspension (1.0×10^5 conidia/ml) on surfaces of leaves of three healthy plants (8 weeks old). Leaves of three healthy plants were sprayed with sterile distilled water as a control treatment. All inoculated plants were maintained in a growth chamber at 25°C with a 12-h photoperiod. The pathogenicity test was repeated twice. After 5 days of inoculation, typical brown spots and necrotic lesions similar to those observed in the field had developed on all inoculated plants but not on water-treated control plants. *A. alternata* was reisolated from the symptomatic tissue of inoculated plants but not from the control plants and was reidentified with morphological and molecular methods, which fulfilled Koch's postulates. This host-pathogen association has been reported in Korea (Lee et al. 2013), but this is the first report of *A. alternata* causing leaf spots on *F. japonicum* in China. Because *A. alternata* is a ubiquitous and very important plant pathogen causing leaf spot diseases in over 100 species of plant, the occurrence of this disease is a serious threat to *F. japonicum* and might lead to economic losses. Therefore, appropriate prevention strategies for *F. japonicum* should be adopted.

References:

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