Diseases Caused by Fungi and Fungus-Like Organisms

First Report of Leaf Spot Disease Caused by *Stemphylium vesicarium* on *Fagopyrum esculentum* in China

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Common buckwheat (*Fagopyrum esculentum*) is a widely cultivated non-grass cereal. It has a considerable market value with nutritional qualities and ability to treat or prevent cancers, hypertension, and diabetes (Cawoy et al. 2006). In July to August of 2018 and 2019, leaves exhibiting unfamiliar symptoms were observed in Huan County and Huachi County, Gansu, China, with 15% incidence and moderate to severe severity across the field. Initial symptoms consisted of small chlorotic, circular to oval leaf spots. As the disease progressed, the spots enlarged and turned light brown to brown with chlorotic margins. When the disease was severe, the leaf spots coalesced, and the leaves became prematurely chlorotic and senescent. Spots occurred mostly but not exclusively on older foliage. Diseased tissues were surface sterilized with 75% ethanol for 20 s and 0.1% NaClO for 2 min, placed on potato dextrose agar (PDA) medium, and incubated at 20°C for 48 to 72 h. A total 24 isolates were obtained and purified through single-spore cultures, 19 of which were characterized. Colonies on PDA of all 19 isolates were identical, exhibiting a

light gray color, with whitish aerial mycelium that later turned light brown on the reverse of the culture plates, and sporulated sparsely. Conidia were brown colored, cylindrical, and borne singly, often had three main transverse septa, at which points there were conspicuous constrictions, and measured 17 to 37 µm long × 13 to 21 µm wide ($n = 30$) in V8 juice agar, 22 to 38 µm long × 11 to 19 µm wide ($n = 30$) in potato carrot agar (PCA), 21 to 41 µm long × 13 to 20 µm wide ($n = 30$) in Spezieller Nährstoffarmer agar (SNA); the mean length/width ratio was 1.6 to 2.0 in V8 juice agar, 1.6 to 2.5 in PCA, and 1.3 to 2.2 in SNA. Conidiophores were unbranched, 5.1 to 7.9 µm wide. Dictyosporae were produced on well-differentiated conidiophores; the apical cell of the conidiophore was slightly to distinctly swollen. Based on morphological features, the isolates were tentatively identified as a member of the *Stemphylium vesicarium* species complex (Koike et al. 2013; Simmons 1967). Genomic DNA of representative isolate B1 was extracted, and the internal transcribed spacer (*ITS*) region and calmodulin gene (*cmdA*) were amplified using ITS1/4, V9G/ITS4, and CALDF1/CALDR1, respectively. The resulting sequences were deposited in GenBank (accession nos. MT629829, MW406903, and MW417122). Nucleotide BLAST similarity analysis of the sequence fragment of *ITS* and *cmdA* from isolate B1 resulted in higher than 99% (99.32% for ITS1/4, 100% for V9G/ITS4, and 100% for CALDF1/CALDR1) identity with *S. vesicarium* strains (GenBank accession nos. LC512757, MH863402, and MH206181). Based on morphological features and molecular data, the buckwheat isolates were identified as *S. vesicarium*. To verify pathogenicity, the back side of leaves from six asymptomatic plants were inoculated by spraying the spore suspension (10^4 spores/ml) harvested from isolate B1 grown on V8 juice agar for 20 days. Control plants were sprayed with sterile water. Each plant was covered with a black plastic bag for 48 h and then was kept in a greenhouse. *Stemphylium* spot symptoms were observed on all inoculated leaves after 14 days, whereas control leaves were symptomless. The pathogen was reisolated from symptomatic leaf spots; micromorphological features and colony characters of the reisolated fungi were identical to the original isolate. To our knowledge, this is the first documentation of leaf spot of buckwheat caused by *S. vesicarium* in China and the first characterization of a *Stemphylium* foliar pathogen on this crop.

References:

- Cawoy, V., et al. 2006. Physiol. Plant. 128:689.
- Koike, S. T., et al. 2013. Plant Dis. 97:315.
- Simmons, E. G. 1967. Mycologia 59:67.

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