Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Nigrospora osmanthi* Causing Leaf Spot on Tartary Buckwheat in China

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Buckwheat (*Fagopyrum tataricum*) is a traditional short-season pseudocereal crop originating in southwest China and is cultivated around the world. Antioxidative substances in buckwheat have been shown to provide many potential cardiovascular health benefits. Between August and November in 2019, a leaf spot was found in several Tartary buckwheat cultivar Pinku1 fields in Xiangxiang County, Hunan Province, China. The disease occurred throughout the growth cycle of buckwheat after leaves emerged, and disease incidence was approximately 50 to 60%. Initially, infected leaves developed a few round lesions, light yellow to light brown spots. Several days later, lesions began to enlarge with reddish brown borders and eventually withered and fell off. Thirty lesions (2 × 2 mm) collected from three locations with 10 leaves in each location were sterilized in 70% ethanol for 10 s, in 2% sodium hypochlorite for 30 s, rinsed in sterile water three times, dried on sterilized filter paper, placed on potato dextrose agar (PDA) with lactic acid (3 ml/liter), and incubated at 28°C in the dark for 3 to 5 days. Fungal colonies were initially white and later turned black with the onset of sporulation. Conidia were single-celled, black, smooth, spherical to subspherical, and measured 9.2 to 15.6 µm long and 7.1 to 11.6 µm wide ($n = 30$). Each conidium was terminal and borne on a hyaline vesicle at the tip of conidiophores. Morphologically, the fungus was identified as *Nigrospora osmanthi* (Wang et al. 2017). Identification was confirmed by amplifying and sequencing the internal

transcribed spacer (ITS) region and the translation elongation factor 1- α (TEF1- α) and partial beta-tubulin (TUB2) genes using primers ITS1/ITS4 (Mills et al. 1992), EF1-728F/EF-2 (Carbone and Kohn 1999; O'Donnell et al. 1998), and Bt-2a/Bt-2b (Glass and Donaldson 1995), respectively. BLAST searches in GenBank indicated the ITS (MT860338), TUB2 (MT882054), and TEF1- α (MT882055) sequences had 99.80, 99, and 100% similarity to sequences KX986010.1, KY019461.1, and KY019421.1 of *N. osmanthi* ex-type strain CGMCC 3.18126, respectively. A neighbor-joining phylogenetic tree constructed using MEGA7.0 with 1,000 bootstraps based on the concatenated nucleotide sequences of the three genes indicated that our isolate was closely related to *N. osmanthi*. A pathogenicity test was performed using leaves of healthy *F. tataricum* plants. The conidial suspension (1×10^5 conidia/ml) collected from PDA cultures with 0.05% Tween 20 buffer was used for inoculation by spraying leaves of potted 20-day-old Tartary buckwheat cultivar Pinku1. Five leaves of each plant were inoculated with spore suspensions (1 ml per leaf). An equal number of control leaves were sprayed with sterile water to serve as a control. The treated plants were kept in a greenhouse at 28°C and 80% relative humidity for 24 h and then transferred to natural conditions with temperature ranging from 22 to 30°C and relative humidity ranging from 50 to 60%. Five days later, all *N. osmanthi*-inoculated leaves developed leaf spot symptoms similar to those observed in the field, whereas control leaves remained healthy. *N. osmanthi* was reisolated from 12 infected leaves with frequency of 100%, fulfilling Koch's postulates. The genus *Nigrospora* has been regarded by many scholars as plant pathogens (Fukushima et al. 1998), and *N. osmanthi* is a known leaf blight pathogen for *Stenotaphrum secundatum* (Mei et al. 2019) and *Ficus pandurata* (Liu et al. 2019) but has not been reported on *F. tataricum*. *Nigrospora sphaerica* was also detected in vegetative buds of healthy *Fagopyrum esculentum* Moench (Jain et al. 2012). To our knowledge, this is the first report of *N. osmanthi* causing leaf spot on *F. tataricum* in China and worldwide. Appropriate strategies should be developed to manage this disease.

References:

- Carbone, I., and Kohn, L. M. 1999. Mycologia 91:553.
- Fukushima, T., et al. 1998. Phytochemistry 48:625.
- Glass, N. L., and Donaldson, G. C. 1995. Appl. Environ. Microbiol. 61:1323.
- Jain, D. K., et al. 2012. J. Plant Dev. Sci. 4:659.
- Liu, J., et al. 2019. Plant Dis. 103:2685.
- Mei, S. S., et al. 2019. Plant Dis. 103:1783.
- Mills, P. R., et al. 1992. FEMS Microbiol. Lett. 98:137.
- O'Donnell, K., and Kistler, H. C., et al. 1998. Proc. Natl. Acad. Sci. U.S.A. 95:2044.
- Wang, M., et al. 2017. Persoonia 39:118.

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