Diseases Caused by Fungi and Fungus-Like Organisms

First Report of Leaf Spot Caused by *Nigrospora aurantiaca* in Tobacco in China

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Tobacco (*Nicotiana tabacum* L.) is one of the most important cash crops in China. In June 2019, tobacco (cv. Yunyan 87) samples with gray spots surrounded by a yellowish ring were collected in Zhengan (107.43° N, 28.55° E), Guizhou province, China. Pieces of leaf tissue (3 × 3 mm) that were cut at the junction of diseased and healthy portions were surface sterilized and plated on potato dextrose agar (PDA). After incubation at 25°C in the dark for 7 days, an isolate (T22) was chosen and used for pathogen identification. The colonies had aerial hyphae that were initially white and then turned gray and produced a soluble red pigment on PDA. The colonies were floccose aerial mycelia, dark gray, with pale brown hyphae, and produced conidia on

oatmeal agar. Conidia were ovoid or ampulliform, black, smooth. Based on morphological characteristics, isolate T22 was identified as *Nigrospora aurantiaca* (Wang et al. 2017). For molecular identification, the large subunit (LSU) and internal transcribed spacer (ITS) of ribosomal RNA, β -tubulin (TUB), and translation elongation factor 1-alpha (TEF1) genes of T22 were amplified by PCR with the primer sets LROR/LR5, ITS1/ITS4, Bt2a/Bt2b, and EF1-728F/EF2 (Suwannarach et al. 2019), and then PCR products were sequenced. Their GenBank accession numbers were MT341787, MT328649, MT348395, and MT348394, respectively. The phylogenetic tree of combined LSU, ITS, TUB, and TEF sequences showed that isolate T22 was assigned to *N. aurantiaca* strain (CGMCC 3.18130 and LC 7034) with 100% bootstrap support. Based on morphological characteristics and multigene molecular analysis, isolate T22 was identified as *N. aurantiaca*. To fulfill Koch's postulates, PDA plugs grown with *N. aurantiaca* were placed on the leaves of four tobacco plants (cv. Yunyan 87) at the 10-leaf stage. Leaves inoculated with PDA only plugs served as the controls. Treated plants were maintained in a greenhouse with temperatures ranging from 18 to 28°C. Five days after inoculation, typical symptoms were observed on inoculated leaves but not on the controls. *N. aurantiaca* was reisolated from the diseased leaves but not from the controls. To our best knowledge, this is the first report of *N. aurantiaca* causing leaf spot on tobacco in China. *N. aurantiaca* has been reported to cause leaf spot on *Castanea mollissima* in China (Luo et al. 2020). Due to potential serious damage caused by the disease in this region, proper disease management practices should be developed and implemented.

References:

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