Diseases Caused by Fungi and Fungus-Like Organisms

First Report of Leaf Spot Caused by *Xylaria arbuscula* on Flue-Cured Tobacco in China

Honglian Xie^{1,2}, Hancheng Wang^{2,†}, Liuti Cai², Zhenni Guo^{2,3}, Tingting Liu^{1,2}, Meili Sun^{1,2}, and Caihua Shi^{1,†}

¹ College of Agriculture, Yangtze University, Jingzhou 434025, Hubei, P.R. China

² Key Laboratory of Molecular Genetics, Guizhou Academy of Tobacco Science, Guiyang 550081, Guizhou, P.R. China

³ College of Life Science, Yangtze University, Jingzhou 434025, Hubei, P.R. China

Funding: Funding was provided by China National Tobacco Corporation (110202001035[LS-04]), National Natural Science Foundation of China (31960550), Guizhou Excellent Young Talent Platform ([2017]5619), and Guizhou Tobacco Company (201914, 2020XM22, and 2020XM03). Plant Dis. 105:1858, 2021; published online as <https://doi.org/10.1094/PDIS-11-20-2436-PDN>. Accepted for publication 7 January 2021.

Flue-cured tobacco (*Nicotiana tabacum* L.) is a leafy, annual, solanaceous plant grown commercially for its leaves in China. Around 70% of tobacco production in China occurs in southwest China. In summer of 2019, leaf spot symptoms were observed on 10 to 20% of tobacco plants in a 2-ha commercial field in Bijie (27.32° N, 105.29° E), Guizhou province, China. The leaf spots were white with dark-brown edges, irregularly round and oval, and diseased tissue dropped out, leaving the leaves ragged in appearance. One diseased leaf from each of five plants was sampled. From five leaves, a total of 15 small (5 × 5 mm) pieces of leaf tissue were cut from the edge of the lesions after surface sterilization and placed on potato dextrose agar (PDA) medium. Five fungal colonies that were similar in appearance were isolated; one (BEZ22) was purified and was selected arbitrarily for identification. Mycelium of the pathogen was initially white and dense, and then black carbonized mycelia appeared from the center of the colony after 7 days of incubation. Mycelium was white, sparse, and radiated when incubated on oatmeal agar. Genomic DNA of the isolate was extracted. The internal transcribed spacers (ITS) with primers ITS1/ITS4 (White et al. 1990), actin (*ACT*) gene with pri-

mers ACT-512F/ACT-738R (Hsieh et al. 2005), beta-tubulin (*TUB2*) with primers T1/T22 (O'Donnell and Cigelnik 1997), and RNA polymerase II second largest subunit gene (*RPB2*) with primers fRPB2-5F/fRPB2-7cR (Liu et al. 1999) were amplified and sequenced, respectively. The generated sequences were deposited in GenBank with accession numbers MT804353 (ITS), MT809582 (*ACT*), MT799790 (*TUB2*), and MT799789 (*RPB2*). Using BLASTN searches, the sequences of each gene above were aligned with the voucher specimen *Xylaria arbuscula* 89041211. The number of nucleotides that were similar for ITS (GU300090) was 550/551 (99%); for *ACT* (GQ421286), 266/266 bp (100%); for *TUB2* (GQ478226), 1,501/1,501 bp (100%); and for *RPB2* (GQ844805), 1,135/1,135 bp (100%), respectively. A phylogenetic tree was constructed based on these four sequences with a final alignment of 3,456 characters (ITS, 551; *ACT*, 266; *TUB2*, 1,501; and *RPB2*, 1,138). Thus, based on morphological and phylogenetic analyses, the isolate BEZ22 was identified as *X. arbuscula*. To verify pathogenicity, six tobacco plants at seedling stage (five to six leaves) without visible disease were inoculated using mycelial plugs (5 mm in diameter). Leaves inoculated with PDA-only plugs served as controls. After inoculation, all tobacco plants were maintained in a greenhouse with 85% relative humidity at 25°C under a 12-h/12-h light/dark cycle. Five days after inoculation, typical early symptoms were observed on the inoculated leaves and not on the control leaves. Koch's postulates were fulfilled by reisolation of the pathogen from diseased leaves. *X. arbuscula* has also been reported as a pathogen of macadamia in Hawaii (Wenhsung 2009) and sugarcane in Indonesia (Maryono et al. 2020). However, to our best knowledge, this is the first report of *X. arbuscula* causing leaf spot on tobacco in China. This leaf spot has the potential to cause serious damage to tobacco in this region that could result in reduced production; consequently, disease management of this pathogen should be considered.

References:

- Hsieh, H. M., et al. 2005. Mycologia. 97:844.
- Liu, Y. J., et al. 1999. Mol. Biol. Evol. 16:1799.
- Maryono, T., et al. 2020. Sugar Tech. 22:105.
- O'Donnell, K., and Cigelnik, E. 1997. Mol. Phylogenet. Evol. 7:103.
- Wenhsung, K. 2009. Bot. Stud. (Taipei, Taiwan). 50:1.
- White, T. J., et al. 1990. Page 315 in: PCR Protocols: A Guide to Methods and Applications. Academic Press, San Diego, CA.

The author(s) declare no conflict of interest.

e-Xtra

Keywords: fungi, field crops, pathogen detection

[†]Indicates the corresponding authors.

H. C. Wang; xiaobaiyang126@hotmail.com, and C. H. Shi; shicaihua1980@126.com