Diseases Caused by Fungi and Fungus-Like Organisms

First Report of a New Disease Caused by *Fusarium tricinctum* on Apple Tree in China

Shuwu Zhang,¹ Jinhuan Chen,¹ Lijun Ma,¹ Enchen Li,¹ Baoli Ji,¹ Chenxi Sun,¹ Jing-Jiang Zhou,^{1,2} and Bingliang Xu^{1,†}

¹ College of Plant Protection, Gansu Agricultural University/Biocontrol Engineering Laboratory of Crop Diseases and Pests of Gansu Province/ Gansu Provincial Key Laboratory of Arid Land Crop Science, Gansu Agricultural University, Lanzhou 730070, China

² State Key Laboratory of Green Pesticide and Agricultural Bioengineering, Ministry of Education, Guizhou University, Huaxi District, Guiyang 550025, China

Funding: This work was supported by the Research Program Sponsored by Scientific Research Start-up Funds for openly recruited Doctors (project 2017RCZX-07); Fuxi Outstanding Talent Cultivation Program, Gansu Agricultural University (Project Gafux-03J03); Longyuan Youth Innovation and Entrepreneurship Talent Project (2021LQTD27); and Program of Introducing Talents to Chinese Universities (111 Program no. D20023) to J.-J. Zhou. Plant Dis. 106:1524, 2022; published online as <https://doi.org/10.1094/PDIS-08-21-1789-PDN>. Accepted for publication 11 November 2021.

Wilting of branches and leaves was observed on 4- to 5-year-old apple trees (cvs. Delicious and Fuji) in orchards in Wushan, Gansu Province, China, in April 2018. Subsequently, stem vascular tissue and woody xylem became discolored and necrotic. Stem dieback expanded rapidly to the entire branch vasculature. Finally, the epidermis of stem bases split and was covered in light pink mold. For pathogen isolation, 25 symptomatic stems were collected from 25 trees in three orchards. Fragments (~0.5 × 0.5 cm) of stems were surface sterilized, individually transferred to Petri dishes containing PDA, and incubated for 4 days at 25°C. Five types of isolates with distinct morphological characteristics (PJ1 to PJ5) were obtained from the stems after single-spore inoculation and subculture. The isolation frequency of PJ1 to PJ5 types was 11, 8, 100, 4, and 13%, respectively, in the 25 stems. A spore suspension of PJ1 to PJ5 types was prepared by adding 5 ml of sterile distilled water to the 14-day-old culture colonies and filtering through 0.22-mm Millipore membranes; the concentration was adjusted to 10⁸/ml for inoculation. To evaluate the pathogenicity of PJ1 (7 isolates), PJ2 (5), PJ3 (32), PJ4 (2), and PJ5 (9), detached healthy apple stems (15 cm long) were surface disinfested and dipped in sterilized tubs containing a spore suspension (10⁸/ml) of each isolate. Stems dipped in sterile distilled water served as the control. Each control and treatment was repeated three times. At day 15 and 35, stems infected with PJ3 developed symptoms similar to those observed in orchards. The

other four types exhibited either no or different symptoms (control, no symptoms). The colony of PJ3 was reisolated from stem bases 35 days postinoculation. PJ3 isolates with the same morphological characteristics as the original ones were examined and identified. After 4 days of incubation on PDA, the colonies of PJ3 developed velvety aerial mycelia, white or light pink (top)/orange-red (reverse). After 14 days, the colony center became yellow-green (top)/carmine red (reverse). Microconidia, macroconidia, and chlamydospores were observed after 14 days of incubation. Microconidia were abundant on aerial mycelia and limoniform, oval, or pyriform with zero to one septa. They were 1.94 to 8.05 × 1.48 to 3.62 μm. Macroconidia were falciform and curved, mostly with three to five septa, and 13.52 to 22.43 × 2.31 to 3.87 μm. Chlamydospores were spherical, intercalary, and formed in chains on PDA plates. Morphological characteristics indicated PJ3 could be *Fusarium tricinctum* (Aktaruzzaman et al. 2018; Cheng et al. 2019). Sequences of the *ITS*, *TEF-1α*, and ribosomal *LSU* of representative isolate PJ3-3 were sequenced and used for molecular identification (Abd-El Salam et al. 2003; Laurence et al. 2011; Miller et al. 1996). The *ITS*, *TEF-1α*, and *LSU* sequences of PJ3-3 were deposited in the NCBI database (MZ799356, MZ820045, and MZ820044, respectively). In BLAST analyses, the PJ3-3 sequences showed 99.47, 100, and 99.01% identity to the corresponding region of *F. tricinctum ITS* (JX179207.1: 3-566, isolate Fyx 1), *TEF-1α* (MK032320.1, isolate ZD3), and *LSU* (KC311496.1, isolate L12), respectively. Phylogenetic analysis clustered PJ3-3 sequences within the same clade as *ITS*, *TEF-1α*, and *LSU* sequences of *F. tricinctum*. PJ3-3 was identified as *F. tricinctum* based on pathogenicity tests, morphological characteristics, and molecular analyses. Previously, symptoms of xylem browning and dieback were observed in twigs of wild apples in Xinjiang, China, and *F. avenaceum*, *F. solani*, *F. tricinctum*, *F. proliferatum*, and *F. sporotrichioides* were isolated (Cheng et al. 2019). We found that *F. tricinctum* can cause stem vascular and woody xylem browning, wilting, and dieback in Delicious and Fuji. These symptoms differ from previously reported ones (Cheng et al. 2019). This is the first report of *F. tricinctum* causing a new disease on apple trees in China following Koch's postulates. Our findings are important for the management of apple disease and protection of trees in the future.

References:

- Abd-El Salam, K. A., et al. 2003. Afr. J. Biotechnol. 2:82.
- Aktaruzzaman, Y., et al. 2018. J. Gen. Plant Pathol. 84:407.
- Cheng, Y., et al. 2019. For. Pathol. 49:e12542.
- Laurence, M. H., et al. 2011. Fungal Divers. 49:101.
- Miller, P. E., et al. 1996. J. Phycol. 32:646.

The author(s) declare no conflict of interest.

Keywords: apple disease, *Fusarium tricinctum*, stem vascular and xylem browning and dieback

[†]Indicates the corresponding author.
B. Xu; xubl@gsau.edu.cn