Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Neofusicoccum parvum* Causing Stem and Branch Blight Disease of *Zanthoxylum bungeanum* in Sichuan, China

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The Chinese pepper *Zanthoxylum bungeanum* Maxim is a special economically important species and a traditional spice in China. It is widely used in medicine, food, timber, tourism, and soil and water conservation. In April 2019, a stem and branch blight disease of *Z. bungeanum* was discovered in Muli, Puge, and Yanyuan counties in Liangshan Prefecture (27°15'20" to 27°19'38" N, 101°44'58" to 102°04'10" E), causing approximately 15% yield loss in the three counties. Among all fields in Muli County, approximately 41.38%, 10.79%, and 2% of Chinese peppers exhibited mild, moderate, and severe branch blight, respectively. The symptoms started to occur from March to April. First, red-brown spots on the base of the stem, branches, or main trunks of young trees observed but were not obvious. In May, spots became gray-brown to dark brown ovals and gradually expanded into long strips. When the spots surrounded the branches, the branches above them withered and died, and the spots gradually expanded downward. Around June or July, scattered black dot-shaped fruiting bodies were observed on the lesion. The branches of infected trees were sampled systematically by cutting the branch at the junction of infected and healthy areas in 5 × 5 mm sections. Each sample was surface-sterilized with 3% NaClO and 75% alcohol for 60 s before rinsing three times with sterilized distilled water. Sterile filter paper was used to dry the tissue, and samples were cultured on potato dextrose agar (PDA) amended with streptomycin sulfate (50 µg/ml). Plates were incubated at 25°C in the dark. From the five isolates obtained, four exhibited the morphology described by Yu et al. (2015) for *Neofusicoccum parvum*. The colonies were white and fluffy at first and grew fast. After 5 days, the colony diameter reached 75.2 to 84.8 mm and produced yellow pigment. The mycelium in the middle of the colony began to turn gray and the entire colony turned dark gray 7 to 8 days post culturing as observed previously (Javier-Alva et al. 2009) and formed a black fruiting body at 20 days.

The width of the septate mycelium was 2.3 to 4.8 µm. The spores were round or fusiform, colorless, transparent, smooth, thin-walled, and 6.3 to 10.6 × 3.1 to 5.2 µm, similar to *N. parvum* (Yu et al. 2013). For molecular identification, DNA was extracted from four fungal isolates cultures using a plant genomic DNA extraction kit (Solarbio, Beijing). PCR was performed with the primers ITS1/ITS4 (White et al. 1990), EF446F/EF1035R (Inderbitzin et al. 2005), BTF/BTR2, and HspF3/HspR (Inderbitzin et al. 2010) for the ribosomal internal transcribed spacer region (ITS), elongation factor-1 alpha (EF1-alpha), beta-tubulin (TUB), and heat shock protein (HSP) genes, respectively. GenBank BLAST indicated that the ITS, TUB, HSP, and EF-1α sequences had 100, 99.01, 99.73, and 99.71% identity to *N. parvum*, respectively. Representative sequences were deposited in GenBank (ITS: MT355871; TUB: MT409397; EF-1α: MT409399; HSP: MT460413). A pathogenicity test was performed using *N. parvum* on ten 2-year-old potted *Z. bungeanum* plants at 22 to 28°C and 60% humidity indoors. The conidial suspension (1 × 10⁷ conidia/ml) collected from 25-day-old PDA cultures with 0.05% tween buffer was used for inoculation by brushing the wounded area of a branch scratched with sandpaper. Ten plants in pots were inoculated with sterile water as controls. Thirty days postinoculation, plants showed the same symptoms as the original diseased plants, and the controls remained asymptomatic. *N. parvum* was reisolated from the infected tissues and identified by morphological characteristics and DNA sequence analysis. The pathogenicity test was repeated three times with similar results, confirming Koch's postulates. This fungus is an important pathogen on a variety of woody hosts and represents a serious problem in vineyards worldwide (Mélanie et al. 2017). To our knowledge, this is the first report of *N. parvum* causing stem and branch blight of *Z. bungeanum* trees in China.

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