Diseases Caused by Fungi and Fungus-Like Organisms

First Report of Twig Blight Caused by *Fusarium proliferatum* on 'Yanfu 3' Apple

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Apple (*Malus pumila* Mill.) originates from Asia and is cultivated worldwide in temperate and subtropical environments. In September 2018, twig blight was observed on 'Yanfu 3' apple cultivar, a descendent of 'Fuji' via 'Changfu2', in an orchard located at Weihai, Shandong Province, China (37°5' N; 122°1' E). The incidence of symptomatic plants was 5 to 15% of 6,000 trees in four individual orchards. Lesions that caused the twig blight initially appeared on bud bases from the previous year and developed sporadic white spots 3 to 5 mm in diameter, which expanded rapidly to cover the entire necrotic tissue with light pink mold. Finally, the branch beyond the necrotic parts shriveled and died. For isolations, stem sections (5 × 3 mm) were surface sterilized with 75% ethanol for 30 s and 1% sodium hypochlorite (NaOCl) for 1 min. The sterilized tissues were rinsed three times with sterilized water, placed on potato dextrose agar amended with 0.02% streptomycin sulfate, and incubated in the dark for 7 days at 24°C. Isolated fungal colonies were subcultured by hyphal tip transferring technique. Four isolates were obtained from five symptomatic plants. The fungal colonies initially had white velvety aerial mycelia that then turned pale yellow. The color of the colonies on the back of the plates was pale yellow, becoming dark brown with age. Two types of single-cell conidia were observed. Microconidia, abundant in aerial mycelia, were oval, elliptical, or oblong, without septa, 5.5 to 12.1 × 1.9 to 3.6 µm ($n = 80$);

macroconidia were sickle-shaped, straight, slightly pointed at the top, mostly two to three septate, with size varying from 17.3 to 25.2 × 2.3 to 4.4 µm ($n = 20$). Morphology of colonies and conidia was identical with *Fusarium proliferatum* (Nayyar et al. 2018). Three sequences were used for molecular identification: internal transcribed spacer (ITS), beta-tubulin gene (TUB2), and 18S rRNA. They were deposited in GenBank with accession numbers MN594807, MN603101, and MN638941, respectively. In BLAST searches, the obtained sequences revealed 100, 99.67, and 97.17% homology with the corresponding region for ITS (strain MD-L-19, MG372010), TUB2 (strain ITEM2400, LT841257.1), and 18S rRNA (strain ITEM2400, LT841264) of *F. proliferatum* from published research (Brankovics et al. 2017; Evidente et al. 2008), respectively. To conduct pathogenicity tests, four actively growing bud bases per plant from five healthy 5-year-old apple plants were superficially inoculated by spraying with spore suspension (1.0×10^6 conidia/ml) of the isolates according to the method previously described (Ma et al. 2019). Ten bud bases sprayed with sterile distilled water served as controls. After 7 to 10 days, in a 12-h light/dark photoperiod at $25 \pm 2^\circ\text{C}$ and 95% relative humidity, all of the inoculated sites showed symptoms similar to ones in the apple orchards. Controls remained asymptomatic. The experiment was repeated twice. The pathogen was reisolated from infected bud bases and identified as the original one, thereby fulfilling Koch's postulates. *F. proliferatum* has also been reported on garlic, onion (Salvalaggio et al. 2013), rice (Lei et al. 2019), soybean (Chang et al. 2015), and wheat (Meca et al. 2010). To our knowledge, this is the first report of *F. proliferatum* causing bud-base rot and twig blight on apple in China or worldwide.

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