Diseases Caused by Fungi and Fungus-Like Organisms

First Report of Wilt of European Hornbeam (*Carpinus betulus*) Caused by *Fusarium oxysporum* in China

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European hornbeam (*Carpinus betulus* L.) has been used as an important ornamental species for urban landscaping since the Italian Renaissance (Rocchi et al. 2010). In May 2019, 15% of 3,000 *C. betulus* trees with wilted leaves and root rot were observed in a field (about 26 ha) in Pizhou, Jiangsu Province, China. Internal discoloration of the stem began with brown to black discoloration of the vascular system and gradually spread to inward areas. Roots and stems from symptomatic plants were washed free of soil, surface sterilized with 0.8% NaOCl, rinsed three times in sterile H₂O, and blotted dry with a paper towel. Small segments (0.5 cm long) were cut from the discolored vascular tissues and then put on potato dextrose agar (PDA) at 25°C in darkness. After 4 days, fungal colonies were observed on the PDA. Pure cultures were obtained by monospore isolation, and nine morphologically similar fungal isolates (EJ-1 to EJ-9) were obtained. All purified cultures were incubated on PDA at 25°C in darkness as the initial isolation. Colonies of the nine isolates on PDA displayed entire margins and showed abundant pink aerial mycelia initially and turned to light violet with age. Microconidia were elliptical or oval in shape, zero septate, and (5.2 to) 8.7 (to 12.5) × (3.5 to) 3.6 (to 5.5) µm. Macroconidia were falciform, zero to four septate, straight to slightly curved with a notched foot cell, and (17.1 to) 20.5 (to 28.4) × (3.8 to) 4.1 (to 4.6) µm. These morphological characteristics resemble *Fusarium oxysporum* (Leslie and Summerell 2006). Genomic DNA of each isolate was extracted from mycelia using a CTAB method (Möller et al. 1992). The *RPB2*, *TEF1*, and *cmdA* genes were amplified and sequenced with the primers 5f2/7c (Liu et al. 1999), EF-1Ha/EF-2Tb (Carbone and Kohn 1999),

and Cal228F/CAL2Rd (Groenewald et al. 2013), respectively. The sequences were deposited in GenBank. A maximum likelihood phylogenetic analysis based on *RPB2*, *TEF1*, and *cmdA* sequences using MEGA7 revealed that the isolates were placed in the *F. oxysporum* species complex with 98% bootstrap support. Based on the morphological and molecular characters, all nine isolates were identified as *F. oxysporum*. A pathogenicity experiment was conducted using thirty 2-year-old *C. betulus* seedlings potted in sterile peat: 27 for inoculation (three replicate plants per isolate) and three for a negative control. The treated plants were planted in the peat mixed with 50 ml of a conidial suspension of each isolate, respectively. The negative control was inoculated with sterilized water. Conidia were harvested from colonized plates of PDA using sterilized water and adjusted to a concentration of 1 × 10⁷ conidia/ml. All 30 seedlings were incubated in a greenhouse at 25°C with a relative humidity of 80% and a 12-h photoperiod. The inoculated seedlings displayed wilt symptoms within 30 to 40 days, and they eventually died within 75 to 85 days after inoculation. Control plants remained symptomless. *F. oxysporum* was successfully reisolated from the vascular tissues of symptomatic plants, and sequences of *RPB2*, *TEF1*, and *cmdA* of reisolates matched those of the original isolates. No pathogen was isolated from the tissues of control plants. The experiment was repeated twice with similar results, fulfilling Koch's postulates. *F. oxysporum* is an important soilborne pathogen and can cause disease in many economically important plants, such as yellowwood (Graney et al. 2017), hickory (Zhang et al. 2015), and larch (Hassan et al. 2019). To our knowledge, this is the first report of wilt on *C. betulus* caused by *F. oxysporum* in China.

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