Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Boeremia exigua* var. *exigua* Causing Branch Blight on Walnut in China

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Walnut (*Juglans sinensis* L.) is an economically important tree. Its fruit is rich in omega-3 essential fatty acids, which are valuable nutritionally (Cheon et al. 2013). In March 2019, severe branch blight of walnuts (cv. Qingxiang) was observed in two fields in Ganquan Town, Gansu Province, China (N 33°56', E105°44'). The incidence was about 3% among 10,000 walnuts. Disease symptoms included fusiform or oval black lesions that gradually expanded on the branches, blight and dieback of branches, reddish brown dead branch bark with lots of small black spots (pycnidia), and defoliation. To isolate the pathogen, 30 diseased tissues collected from different trees were disinfected with 0.1% HgCl solution for 1 min, rinsed in sterilized water three times, and placed on potato dextrose agar (PDA) at 25°C in the dark. About 3 days later, four fungal isolates (3-3, 3-6, 3-9, and H3) with similar morphological characteristics were obtained. Their colonies, with regular margins on oatmeal agar, 6.1 to 6.8-cm diameter after 7 days, were loose, greenish olivaceous to olivaceous gray, velvety, and floccose to woolly. The conidia ($n = 60$) were 4.77 to 8.84 μm long (mean = 6.88 μm ; SD $\pm$ 0.91 μm) $\times$ 1.71 to 3.89 μm wide (mean = 2.81 μm ; SD $\pm$ 0.53 μm), cylindrical, ellipsoidal to oblong, hyaline, and aseptate. Pycnidia ($n = 25$) were 76.66 to 132.86 μm diameter (mean = 102.93 μm ; SD $\pm$ 12.15 μm), variable in shape and size, and mostly globose to subglobose. These characteristics were similar to *Boeremia exigua* var. *exigua* (Boerema et al. 2004). Pathogenicity tests of four isolates were performed three times on five healthy 2- to 3-year-old walnuts (cv. Qingxiang). Plants were wounded by insect needle (no. 6) and

inoculated with a 5-mm mycelium block grown on PDA medium or PDA medium as a control and kept moist in a climatic cabinet (>85% RH, 25°C). After 3 days, oval black lesions occurred on branches and gradually expanded, but the control was asymptomatic. Original isolates were reisolated from these diseased shoots. Genomic DNA of four isolates was extracted, and the internal transcribed spacer (ITS), β -tubulin (*tub2*) gene, and RNA polymerase II second largest subunit (*rpb2*) gene were amplified and sequenced using the primers ITS1/ITS4, Btub2Fd/Btub4Rd, and RPB2-5F2/fRPB2-7CR (Chen et al. 2015; White et al. 1990; Woudenberg et al. 2009), respectively. Sequences were deposited in GenBank (accession nos.: ITS, MT154621, MT154622, MT154623, MT154624; *tub2*, MT223481, MT223482, MT223483, MT223484; and *rpb2*, MW448152, MW448153, MW459982, MW459983) and compared with available sequences in NCBI. Results showed that ITS of four isolates had 100% sequence identity to *Boeremia* spp., *tub2* and *rpb2* had 100% sequence identity to *B. exigua* var. *exigua* (GenBank accession nos. MN983734 and MN983315) and *B. exigua* var. *linicola* (GenBank accession nos. MN983785 and MT920619). According to host specificity (Boerema and Verhoeven 1976), a 10^6 conidia/ml spore suspension of four isolates or sterile water was inoculated on the stem base of 2-month-old flax seedlings, which were placed in climatic cabinet (>85% RH, 25°C) for moisturizing and repeated three times. After 2 weeks, all inoculated plants still were asymptomatic, indicating that the four isolates were not *B. exigua* var. *linicola*. Therefore, they were identified as *B. exigua* var. *exigua* based on morphological characteristics, molecular analysis, and pathogenicity tests. To our knowledge, this is the first report of *B. exigua* var. *exigua* causing walnut branch blight worldwide, which will provide further guidance for prevention and control of walnut branch blight.

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