Diseases Caused by Fungi and Fungus-Like Organisms

Boeremia exigua Causes Leaf Spot of Walnut Trees (*Juglans regia*) in China

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Walnuts, an important perennial nut crop widely cultivated in China, are rich in protein, carbohydrates, renieratene, and other beneficial nutrients. China is the largest producer of walnuts in the world, with the largest planting area and output. At the end of April 2020, several unknown necrotic spots on the leaves of walnut trees were observed in a *Juglans regia* field in Sancha Town, Enshi, China (30.28°N, 109.64°E). Initially, lesions were black, small, and sunken and turned to yellowish-brown, irregular, and well surrounded by brown margins. In severe cases, leaf spots coalesced and resulted in withered and abscised leaves. To identify the pathogen, infected leaves were collected. Sections of leaves were aseptically excised from the margins of necrotic spots following surface sterilization and placed on potato dextrose agar (PDA) at 28°C. After 4 days, fungal isolates were obtained and purified by hyphal tip isolation. The isolates looked morphologically similar, producing colonies with dark gray hyphae, lobed margins, and white to light gray

aerial mycelium. After 15 days of incubation, subglobose, dark brown pycnidia (100 to 176 µm in width, 75 to 95 µm in length) were formed with an orifice in the center, producing conidia. Conidia (3.5 to 9.0 × 1.6 to 4.5 µm) were oval to round, and aseptate or occasionally 1-septate. These morphological characteristics led to the conclusion that the isolates may be identified as *Phoma* sp. (Boerema et al. 1976). A single isolate was randomly selected and designated for further verification. To confirm the identity, the internal transcribed spacer region (ITS), actin (ACT), and beta-tubulin genes were amplified and sequenced with primers ITS1/ITS4, ACT-512F/ACT-783R, and Bt2a/Bt2b, respectively (Groenewald et al. 2013; White et al. 1990). BLAST analysis of the ITS 505-bp sequence (GenBank accession no. MW282913), actin 269-bp sequence (GenBank accession no. MW201958), and beta-tubulin 347-bp sequence (GenBank accession no. MW273782) showed ≥99% homology with the sequences of *Boeremia exigua* available in GenBank (GenBank accession nos. AB454232, LT158234, and KR010463, respectively). Based on the above results, the strain HTY2 was identified as *B. exigua*. Pathogenicity was tested. Walnut plants were spray-inoculated with a spore suspension (5 × 10⁵ CFU/ml). Controls were inoculated as described above except with sterile distilled water in the dark at 25°C. After 7 days, lesions were evident at inoculation points and equivalent to those observed in the fields. Control leaves remained symptomless. The pathogenicity test was repeated three times, and the results were the same, fulfilling Koch's postulates. The pathogen has been reported on various plants around the world, causing a series of symptoms. Infected plants rarely died, but the presence of lesions decreased their fruit quality and yield. Previous identification of the disease is essential in formulating management strategies.

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