Diseases Caused by Fungi and Fungus-Like Organisms

The First Report of *Juglans regia* Leaf Spot Caused by *Fusarium proliferatum* in China

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Juglans regia is planted widely in China for its significant economic value as a woody oil species. In August 2019, leaf spots were discovered in a walnut orchard in Tang County, Baoding City, Hebei Province. In the early stages of the disease, irregular brown spots appeared on the edges of leaves, causing them to shrivel; in a later stage, the disease spread inward along the edges to the whole leaves, which then withered. Diseased leaves were collected from the orchard. Tissues (3 to 4 mm²) were selected at the diseased and healthy junction for each diseased leaf, and their surfaces were sterilized for 30 s with 0.1% HgCl₂. The leaves were incubated on potato dextrose agar (PDA) at 28°C in darkness; each diseased leaf was repeated 10 times and purified after 6 days. The diameters of the suspected *Fusarium* isolates reached 8.3 cm, and the aerial mycelia were white to cream and wool-like, turning violet and dark purple with age. On PDA, the macroconidia exhibited three to five septa and were straight or slightly curved, with a size of 21.6 to 47.4 × 2.5 to 4.6 μm (average: 31.9 × 3.5 μm, *n* = 50). The morphological features of these fungi were noted to be consistent with those of *Fusarium proliferatum* (Leslie and Summerell 2006; Zhao et al. 2019). For molecular analysis, DNA was extracted from the mycelium and specific primers were used to further amplify the translation elongation factor (*EF-1α*) and RNA polymerase II largest-subunit (*RPB2*). The amplicons were sequenced and deposited in GenBank under accession numbers MZ891582 and MZ891583.

BLAST analysis revealed that these sequences showed more than 99% sequence identification with *F. proliferatum*. We used *Fusarium* multilocus sequence typing (MLST) for the identification of this strain, and the results showed that *EF-1α* and *RPB2* genes have more than 99% similarity with the database *F. proliferatum* data, with 100% sequence duplication. Sequences were deposited in GenBank under accession numbers MH582346 and MH582347 (*EF-1α*) and MH582193, MH582151, MH582196, and MH582172 (*RPB2*). We created a multigenic tree using *Fusarium* MLST. Based on the results of the morphology tests and the molecular sequences, the isolates from the diseased leaves were identified as *F. proliferatum*. To confirm the pathogenicity, healthy leaves of walnut trees planted in pots were inoculated using 50 μl of conidia suspension (1.0×10^7 CFU) from a pure clone, or inoculated using only distilled water as a control. All treatments were repeated three times and placed under 28°C and 100% relative humidity for 5 days. The inoculated plants showed the same symptoms as those observed in the original diseased plants, while the control plants were asymptomatic. The same fungus was reisolated from the inoculated leaves. The reisolated pathogen from diseased plants was identical to the purified strain used for inoculation, confirming Koch's postulates. According to the above results, *F. proliferatum* was identified as the pathogen behind the *Juglans regia* leaf spots, and it is clearly different from other pathogens, such as *Colletotrichum gloeosporioides*. It has been reported that *F. proliferatum* can lead to necrotic leaf lesions and the bulb rot of stored onions (Beck et al. 2021), and light brown discoloration and dryness on palm leaves and branches (Alanbeh et al. 2021). *F. proliferatum* has also been found to have the highest colonization ability on jujube leaves and is becoming a serious pathogen on jujube (Saleh et al. 2016). To our knowledge, this is the first report of walnut leaf spots caused by *F. proliferatum* in China.

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