Diseases Caused by Fungi and Fungus-Like Organisms

First Report of Dieback on Fig (*Ficus carica*) Caused by *Lasiodiplodia theobromae* in North Al-Batinah Governorate of Oman

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Fig (*Ficus carica* L.) is widely grown in Oman mainly for its nutritional value and health benefits. In May 2020, a survey was conducted for fig trees that were showing symptoms of decline in three farms where more than 1,500 fig trees were grown. A disease incidence of 3 to 4% of fig trees showing symptoms of dieback of the twigs, death of entire branches, and discoloration of vascular tissues was observed in Sohar, North Al-Batinah governorate, Oman. Disease severity was 25 to 40% in most infected trees. The death of severely affected trees was observed in about 3% of orchards. Symptomatic samples were collected and isolation of the causal agent was performed. Infected tissues were cut into small pieces and surface-sterilized with 1% NaOCl for 3 min. The tissues were rinsed with sterile distilled water three to four times, blotted dry on sterile filter paper, and placed on potato dextrose agar (PDA) medium under aseptic conditions. After 2 to 3 days of incubation of plates at 27°C, a pure culture of the fungus was obtained by the hyphal tip isolation technique. Two identical isolates were grown in the PDA medium. The fungal colony was dark gray to black on PDA and the fungus produced dense aerial mycelium and numerous dark colored pycnidia after 25 days at an ambient temperature of 23 to 25°C and natural photoperiod. Pycnidia were ruptured and 20 emerged conidia were measured. The mature conidia were 1-septate, brown, ellipsoid, and with a thick cell wall and longitudinal striation. The average length of 20 conidia was 22.5 to 25 (24.9) µm, and the width was 12.5 to 15 (15) µm. To identify the fungus, DNA was extracted from the mycelium using the phenol-chloroform-isoamyl alcohol method, following Al-Mahmooli et al. (2015). The internal transcribed spacer (ITS) region of

rDNA and translation elongation factor 1- α (*TEF*) gene were amplified using ITS-4 and ITS-5 primers for ITS (White et al. 1990) and EF1-983F and EF1-2218R and EF1-728F and EF1-986R primers for *TEF* (Alves et al. 2008), and the amplified products were sequenced. BLASTn analysis of the ITS sequence of the fungal isolate revealed 100% identity with reference sequences of *Lasiodiplodia theobromae* (Pat.) Griffon & Maubl (MT075438.1). The sequence of the *TEF* gene was 99% identical to the sequences of *L. theobromae* (XM_035519539.1 and MN461169.1). The nucleotide sequences were submitted to GenBank (accession nos. MW590660 for ITS and MZ159970, MZ159971, and OM654917 for *TEF*). For phylogenetic analysis, we constructed a combined ITS-*TEF* dataset, following Alves et al. (2008). Using maximum likelihood phylogenetic analysis of the dataset, our specimen fell in a clade formed by the members of *L. theobromae*. Pathogenicity of the fungus on three replicates (three fig seedlings) was established by artificial inoculation of 2- to 3-month-old potted fig seedlings with the fungus by making pin prick injuries on the stem at 5 cm above the soil level with a sterile inoculation needle, placing mycelial discs containing spores and pycnidia (15 × 15 mm) from 20-day-old colonies grown on PDA on the injured surface, and wrapping with Parafilm, which was removed 48 h after establishing infection. The three inoculated plants showed withering and drying of leaves 14 days after inoculation (DAI) beginning near the inoculation site and spreading to the younger leaves. Eventually, seedlings were showing dieback, and death of entire seedlings occurred at 25 to 28 DAI. The mock-inoculated plants remained symptomless. The fungus reisolated from the diseased plants was confirmed by sequencing of ITS regions. The prevalence of dieback could be a potential threat to fig cultivation in Oman. To our knowledge, this is the first report of dieback disease caused by *L. theobromae* on fig in Oman.

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