Diseases Caused by Fungi and Fungus-Like Organisms

First Report of Damping-Off Disease Caused by *Fusarium oxysporum* on *Pinus pinea* in Jordan

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Plant Dis. 105:4153, 2021; published online as <https://doi.org/10.1094/PDIS-10-20-2135-PDN>. Accepted for publication 19 June 2021.

Forests in Jordan cover 60% of the northern and 40% of the southern parts of the country. Natural forests constitute about 75% in the northern part and 25% in the southern part. There are many types of forest trees in Jordan, including pines (*Pinus* spp.). *Pinus pinea* is considered one of the most important components of Jordan's natural forests. Due to its adaptability, lack of environmental requirements, and ease of cultivation, its cultivation has been expanded in all parts of Jordan. *P. pinea* cultivation prevents soil erosion and combats desertification. *P. pinea* seeds are used in many popular foods. At the end of 2019, wilting and damping-off symptoms were noticed in 50% of *P. pinea* seedling nurseries. Six-month-old *P. pinea* seedlings with visible symptoms of damping-off were collected between May and July 2020 from a pine nursery in Amman Province, Jordan (32°0'40.4316" N, 135°52'20.3628" E). Thirty-two seedlings with different disease severity were selected for the isolation of root pathogens. Two root samples from each seedling were surface-sterilized using 1% sodium hypochlorite for 3 to 5 min and rinsed with sterile distilled water. Root samples were cut into small pieces (1 to 2 cm long) and placed on potato dextrose agar (PDA) supplemented with 5 mg/liter streptomycin sulfate. Petri dishes were incubated in a growth chamber at 25 ± 2°C for 7 days and subcultured by hyphal tipping. Cultural and conidial morphology of 7-day-old mycelia were observed with a microscope. For morphological identification of *Fusarium*, 200 measurements of microconidia, macroconidia, and chlamydospores were conducted. *Fusarium* isolates had purple-violet mycelium growth in PDA. Macroconidia had 3 to 5 septa with a foot-shaped basal cell. They were sickle-shaped, hyaline, and thin-walled, and 20 to 42 × 3.2 to 5.5 µm. Microconidia were short, elliptic to oval unicellular, with sharp unbranched monophyalides, and averaged 5.0 to 17.0 × 2.3 to 5.1 µm. Older mycelia developed many terminal chlamydospores (7.2 to 14.1 µm) that were intercalary and rough-walled. All the characteristics agreed with those recorded by Leslie and Summerell (2006) and Nelson et al. (1983) for *Fusarium oxysporum*. Representative isolate (FoxypineJO2020-01) was selected for molecular identification. The DNA was extracted, amplified using the translation elongation factor 1-α (EF1α) gene (EF1/EF2) (O'Donnell et al. 1998), and sequenced. Forward and reverse

sequences were received, assembled, and consensus sequence was produced using BioEdit (Hall 1999). The consensus sequence was searched with BLASTn on NCBI (<https://www.ncbi.nlm.nih.gov>) and was 100% similar with *F. oxysporum* accession KC622308.1. Phylogenetic analysis was conducted using MEGA 7.0.26 (Kumar et al. 2016) with 1,000 bootstrap values and correlated the representative isolate with accession KC622308.1. The isolated sequence was deposited in GenBank (MW057934). Koch's postulates were fulfilled using FoxypineJO2020-01 to confirm *F. oxysporum* as the causal agent of *P. pinea* damping-off. One-month-old seedlings of *P. pinea* were soaked in a spore suspension of 10⁶ spores/ml for 10 min. Seedlings were placed in 25 cm × 20 cm deep plastic pots filled with a sterile mixture of peat moss, perlite, and vermiculite (60:20:20). Thirty control seedlings were soaked in distilled water. Planted seedlings were incubated at 25 ± 2°C with a 12:12 h light/dark period. Seedlings of *P. pinea* inoculated with spores gradually showed symptoms similar to those of naturally infected plants. The inoculated pathogen was successfully reisolated from roots of the diseased seedlings. The uppermost leaves began to wilt, and the roots darkened 25 days after inoculation. By 40 days after inoculation, entire seedlings were discolored and dead. Furthermore, the roots became dark and peeled. These symptoms matched those described by Machón et al. (2009) and Luo and Yu (2020). Control seedlings remained asymptomatic. To our knowledge, this is the first report of *F. oxysporum* on *P. pinea* in Jordan. The pathogen can cause significant economic losses to *P. pinea* as well as to other *Pinus* spp. in nurseries and forests in Jordan. Therefore, for disease control in nurseries, it is important to determine the onset time, decrease the incidence (Gordon et al. 2015), and identify the infection source (Morales-Rodríguez et al. 2018). Future surveys need to be conducted on forest trees in selected forest and biosphere reserves that show tree decline to identify major forest fungal pathogens in Jordanian forests.

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The author(s) declare no conflict of interest.

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Keywords: *Pinus pinea*, *Fusarium oxysporum*, Jordan, damping off

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