



First report of *Diplodia sapinea* on *Cedrus libani* in Turkey

F. Oskay¹, A. Lehtijärvi², H.T. Dogmuş-Lehtijärvi³ and S. Woodward⁴

¹ Çankırı Karatekin University, Faculty of Forestry, 18200, Çankırı, Turkey;; ² Bursa Technical University, Faculty of Forestry, 16310 Yıldırım-Bursa, Turkey;; ³ Süleyman Demirel University, Faculty of Forestry, TR 32260 Çünür, Isparta, Turkey;; ⁴ University of Aberdeen, School of Biological Sciences, Cruickshank Building, Aberdeen AB24 3UU, Scotland, UK

*E-mail: fundaoskay@karatekin.edu.tr

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Cedar of Lebanon (*Cedrus libani*) is an ecologically and economically important conifer with a natural distribution in southern Turkey. Currently, no serious diseases affect *C. libani* in its native range, although it is susceptible to pathogens such as *Heterobasidion annosum* and *Diplodia pinea* in inoculation experiments (Lehtijärvi *et al.*, 2011). Recent reports from the Balkans and Northern Europe, however, suggest that *C. libani* is susceptible to foliage and shoot diseases such as *Diplodia pinea*, *Dothistroma septosporum* and *Sirococcus tsugae* (Mullet *et al.*, 2016; Zlatkovic *et al.*, 2016).

During disease surveys in July 2017 in the Atatürk Arboretum, İstanbul (41°10'30.53"N, 28°59'5.22"E), dieback was observed on branches of a young *C. libani* (Fig. 1). Abundant black pycnidia were observed on dead needles under a binocular microscope (Fig. 2). Examination under a compound microscope revealed the presence of non- or one-septate, light to dark brown, oblong-ellipsoid conidiospores, characteristic of the genus *Diplodia* (Figs. 3-4). Isolations were made by plating spore masses onto 2% malt extract agar in 90 mm diameter Petri dishes under aseptic conditions and incubating at room temperature for ten days in the dark. Blackish-dark grey colonies with grey aerial mycelia were subcultured on 2% malt extract agar covered with cellophane membrane and incubated as described above. DNA was extracted from mycelia using an E.Z.N.A. fungal DNA extraction kit (Omega Bio-tek, USA) and amplified by PCR using species-specific primers for both *D. pinea* and *D. scrobiculata* (Smith & Stanosz, 2006). The *D. pinea* primers amplified a product of 700 bp. The ITS region was also amplified and sequenced (White *et al.*, 1990), and the sequence of a representative isolate was deposited in GenBank (Accession No. MH368280). Comparison of the ITS sequences obtained from *C. libani* showed 100% nucleotide identity with those of reference strains of *D. sapinea* (e.g. NR_152452.1).

Inoculations were made on two-year-old *C. libani* plants to determine the pathogenicity of the fungus, with five replicate plants each for stem and shoot inoculations. A circular 4-mm wound was made on stems approximately 10 cm above the root collar, by removing the bark tissues with a surface-sterilised cork borer. For shoot inoculations, a small wound of c. 3 mm was made on the shoots using a scalpel to remove the bark, at c. 5 cm from the tip of the terminal shoot. Agar plugs (4 and 2 mm diameter for stem and shoots, respectively) taken from the margins of an actively growing *D. sapinea* colony on potato dextrose agar (PDA), were placed

mycelium-side onto the wounds. All treatments were wrapped in plastic paraffin film. Controls (three replicates for both stem and shoot inoculations) were wounded, as described above, and treated with sterile PDA plugs. All plants were incubated at 24°C for eight weeks in a climate chamber. Following incubation, dieback symptoms were observed on all inoculated plants; foliage and stems were dead above the inoculation point (Fig. 5). Lesion extension towards the root collar was 24.2 ± 2.0 mm for stem and 26.0 ± 1.16 mm for the shoot inoculations. The pathogen was successfully re-isolated from symptomatic stem and shoot tissues, thus fulfilling Koch's postulates.

To our knowledge, this is the first report of *Diplodia sapinea* causing dieback on *C. libani* in Turkey.

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Figure 1



Figure 2

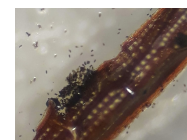


Figure 3

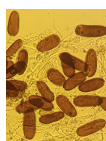


Figure 4

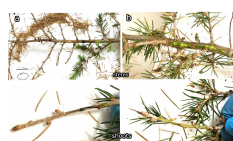


Figure 5

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