



First report of *Schizophyllum commune* associated with apple wood rot in Tunisia

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During 2013-2014, an emerging disease was observed on apple (*Malus domestica*) trees leading to significant damage in Tunisia. This problem was particularly widespread in the region of Kasserine, the largest apple-producing region in Tunisia, where 80% of the orchards were affected. Diseased trees exhibited symptoms of severe dieback associated with wood rot. Masses of carpophores on twigs, scaffold branches and trunks, as well as bark darkening and necrotic leaves were observed (Fig. 1).

Samples of infected twigs and leaves were collected and brought in plastic bags to the laboratory. They were disinfected with 70% ethanol for one minute, rinsed in sterile distilled water and air-dried. Small pieces were removed from lesion margins and placed on malt extract agar (MEA) medium. Plates were incubated at 25°C under fluorescent light (12 h/day). Developing fungal colonies showed a white cottony mycelium and fan-shaped fruiting bodies after 65 days incubation (Fig. 2). Based on their macro- and micro-morphological traits, isolates were identified as *Schizophyllum commune* (Latham, 1970; Bergdahl & French, 1985; Takemoto *et al.*, 2010).

A pathogenicity test of one representative isolate was performed on detached apple stems (20 cm in length). Stems were surface sterilised for one minute with 70% alcohol and air-dried. They were wounded using a sterilised scalpel and inoculated with mycelial plugs (6 mm in diameter) which had been removed from the margin of a culture actively growing on MEA. Inoculated stem sections were sealed with paraffin film to prevent desiccation. Control stem sections were inoculated with MEA plugs only. Both inoculated and control stems were incubated in moist chambers maintained at 25°C and 95% relative humidity. After 30 days incubation, all inoculated stems developed symptoms similar to those observed under field conditions whereas control ones remained healthy. The pathogen was consistently re-isolated from symptomatic stems thereby fulfilling Koch's postulates.

For molecular identification, DNA from five isolates was extracted

according to the protocol of Mahuku (2004) and the ITS region was amplified using ITS1F and ITS4 primers. Amplicons of the expected size were sequenced (GenBank Accession Nos. KX555521-KX555525) and showed 99% identity with *S. commune* isolates KP326577 and KJ093499. *Schizophyllum commune* is a sap-rot basidiomycete and has also been reported as a plant pathogen of living trees including apple (Takemoto *et al.*, 2010), citrus (Russo *et al.*, 2012) and olive (Úrbez-Torres *et al.*, 2012). To our knowledge, this is the first report of *S. commune* causing wood rot on apple trees in Tunisia.

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Figure 1



Figure 2



Figure 3

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