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THE OCCURRENCE OF *Stereum hirsutum* ASSOCIATED WITH WOOD ROTTING OF OLIVE TREES IN IRAN

Research note

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ABSTRACT

In summer 2021 (June–July), disease symptoms on trunks and shoots were observed in olive groves in the Tarom Sofla region, Qazvin province, Iran. The symptoms were light-pink discoloration and surface depression in the external layers of the wood. As the disease progressed, brown streaks of tissue appeared on the longitudinal sections of the wood. The identification of fungus was made based on PCR amplification of the rDNA-ITS region with the universal fungal primers ITS5 and ITS4. BLAST searches revealed 99.52% identity to *Stereum hirsutum*. Several species of basidiomycetes are known to live on wood as saprobionts or parasites. On olive trees, they cause white rot symptoms. Although they are not directly responsible for tree mortality; however, they can lead to structural deterioration of woody tissues. To the best of our knowledge, this is the first report of *S. hirsutum* associated with wood rotting of olive trees in the world.

Key words: olive, Stereum hirsutum, wood rot

Several species of basidiomycete are known to live on wood as saprobionts or parasites. Basidiomycetes existing on hardwood hosts, including olive species, cause white rot symptoms. White rot is often very prominent in older trees and can degrade all components of cell walls (Fischer & González García 2015). A considerable number of basidiomycetes causing white rot symptoms, including the genus *Stereum* inhabiting fallen or standing branches, trunks, and stumps, were collected from several tree species (Fischer 2006; Țura et al. 2008; Roccotelli et al. 2014). These fungi can cause decay and discoloration of wood tissues, in particular in the trunk, branches, and roots (Markakis et al. 2017). In a survey in the summer 2021 (June–July), disease symptoms were observed on trunks and shoots in olive groves in the Tarom, Sofla region, Qazvin province, Iran. The symptoms were lightpink discoloration and surface depression in the external layers of the wood (Fig. 1A). As the disease progressed, brown streaks of tissue appeared on the longitudinal sections of the wood (Fig. 1A). Infected olive trees 'Zard' were older than 20 years and grew under a regime of standard orchard hygiene and routine cultivation practices. Shoots with typical symptoms, mentioned above, were collected and transferred to the laboratory for fungal isolation. Affected shoots were surface disinfected with a 70% (vol/vol) ethanol solution.

Bark tissues (3–4 by 3 by 1–2 mm) were cut with a sterile scalpel, soaked in 0.5% sodium hypochlorite, rinsed with sterilized water three times, dried, and placed in Petri dishes containing potato dextrose agar (PDA). Petri dishes were incubated at 25 °C until fungal colonies were grown enough to be examined on the basis of morphological characteristics. Fungal colonies were orange-white with dense mycelium (Fig. 1B), and only one single type of colony was isolated from all samples. In order to confirm the identity of the fungus, total genome DNA was extracted from mycelia using the method described by Barnes et al. (2001). The rDNA-ITS region was amplified using PCR with the universal fungal primers ITS5 (forward) 5'-(GGAAGTAAAAGTCGTAACAAGG)-3' and ITS4 (reverse) 5'-(TCCTCCGCTTATTGATATGC)-3'. BLAST searches of the GenBank nucleotide database revealed 99.52% identity to Stereum hirsutum (Genebank Accession No KX578081.1) (Taudiere et al. 2017). The sequence of S. hirsutum isolate JPA was deposited in Genebank (MZ948811).

The infection test by S. hirsutum was conducted based on 10 replicates of detached branches (Moral et al. 2010), in the greenhouse with 50% humidity at 24 °C. Segments of branches 14-18-cm long and 10-15 mm in diameter were collected from healthy 'Zard' olive pots grown in the greenhouse of the Iranian Research Institute of Plant Protection. The bark surface of branches was disinfected with ethanol at the inoculation point situated in the middle of each branch. Holes 10 mm in diameter were made in the bark, just deep enough to remove the outer bark but leave the inner bark intact. A 10-mm-diameter mycelium plug of the fungus was placed into each hole, and the inoculated area was wrapped with parafilm. The control branches were placed with PDA plugs without the mycelia. After a month, the inoculated branches were carefully examined for lesions (Fig. 1C), and the symptoms were similar to those observed in the olive groves. Koch's postulates were satisfied after reisolating S. hirsutum from inoculated olive branches. No symptoms were observed in the control (Fig. 1C).



Figure 1. (A) The symptoms of the infection including a discoloration in the external layer of the wood and brown streaking tissue in the longitudinal section of the wood; (B) colony morphology of fungus *S. hirsutum* grown on PDA; and (C) the lesions in the inoculated branches with *S. hirsutum* at the greenhouse, and the control without symptoms

The fungus S. hirsutum is considered either a saprobe or more rarely a parasite, causing white rot in the living and dead woody tissues of its hosts. It may act as a weak facultative parasite affecting the external layers of the wood in the grapevine (Fischer & González García 2015), as well as a plant pathogen infecting peach trees (Cox 2004). It is suggested that under dry Mediterranean climate conditions, this fungus is highly adapted to live on old grapevines (Fischer & González García 2015). The olive orchard, from which we isolated the fungus S. hirsutum, was suffering from frost damage on the trunks and main branches, implying that abiotic stresses causing physical damage may promote the establishment of the fungus on the host plants. On a local scale, different factors including cultivar, abiotic stresses, plant age, and global climate change, because of their effects both on microbe ecology and epidemiology (Graniti et al. 2011), and host fitness, can affect the distribution and pathogenicity of some fungi.

Some basidiomycetes such as *Fomitiporia mediterranea* had been reported as the causal pathogen of olive (Fischer 2006; Paplomatas et al. 2006). Many species of wood-rotting basidiomycetes are not directly responsible for tree mortality; however, they can lead to structural deterioration of woody tissues and economic damage after several years. Identification of wood-decay fungi and their phytopathogenic role is necessary to provide successful disease diagnosis and control management. To the best of our knowledge, this is the first report of *S. hirsutum* associated with wood rotting of olive trees in the world.

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