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# *Fomitiporia mediterranea* infecting citrus trees in Greece

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**Summary.** In recent years a serious disease of citrus (the orange cv. Washington navel, lemon and the common mandarin grafted on sour orange rootstocks) has been observed in southern Greek orchards. Affected trees decline, their leaves become yellow and fall early, and shoots and twigs die as the damage expands towards the trunk. Cross-sections of the trunks and large branches reveal a light-colored rot in the center, which is surrounded by brown hard necrotic wood. Symptoms start from pruned areas and spread to the rootstock wood, and then resemble esca of grapevine. From the white rotted areas, a fungus was isolated on PDA that formed cream-yellow to light-brown colonies with dense aerial mycelium. Fungal fruit-bodies formed abundantly on the trunks of diseased trees. The fungus was identified as *Fomitiporia mediterranea* by both traditional and molecular methods. Pathogenicity tests were performed by artificially inoculating orange, mandarin, lemon and sour orange trees with the fungus. Control holes were filled with two PDA plugs. Branches inoculated with the isolates from infected citrus showed wood discoloration that extended up to 20 cm above and 20 cm below the infection hole. The fungus was re-isolated from the discolored parts of the wood. Inoculations with isolates from grapevine and kiwi produced wood discoloration only 3–4 mm around the holes.

**Key words**: esca, *Fomitiporia punctata, Phellinus pseudopunctatus, Phellinus punctatus,* white rot.

## Introduction

Several citrus species are cultivated in southern Greece for consumption as fresh produce or for processing. The most common of these are *Citrus sinensis* (L.) Osbeck (orange), *C. limon* (L.) Burm. (lemon), *C. reticulata* Blanco (mandarin), and *C. reticulata*×*aurantium* (clementine). *C. aurantium* L. (sour orange) trees are usually used as rootstocks. The most severe fungal diseases of citrus in Greece are gummosis and brown rot caused by species of the genus *Phytophthora,* "mal secco" caused by *Phoma tracheiphila* (Petri) Kanchaveli & Gikashvili, and some post-harvest diseases (Annual Reports of the Benaki Phytopathological Institute (Anonymous, 1960–2004).

In recent years a serious disease of citrus (orange cv. Washington navel, lemon and common mandarin grafted on sour orange rootstocks) has been observed in orchards in Argolis and the island of Andros (southern Greece). Trees decline, leaves become yellow and fall early, and lastly shoots and twigs die as the damage expands to-

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wards the trunk. Seriously affected trees become unproductive and die. On the trunk or the branches rough cankers arise. Fungal fruit bodies form abundantly on the trunks and large branches of diseased trees, mostly on the cankers.

This study reports the identification of the pathogen associated with this new disease, the results of pathogenicity tests on citrus, and also the capacity of the *Fomitiporia* isolates from grapevine and kiwi to infect citrus species.

## Materials and methods

### Isolations from field samples

Fruit bodies and affected wood from diseased trees were collected from Argolis and Andros orchards in February, May, June, July, October and December 2002, 2003, and 2004. A total of 140 trees were analyzed in the whole. In the last year, 2004, 20 trees were also used for fungal isolation. The trunks and large branches of the trees were cut crosswise and lengthwise to observe the symptoms in the wood. Each sample was examined separately, taking small wood fragments aseptically from the diseased area of light-colored decay in the center and from the surrounding brown hard necrotic wood, and plating them on potato dextrose agar. Isolates were also obtained from the fruit bodies.

### Isolate identification and pathogenicity tests

Pure fungal cultures were identified by generating sequences of the ribosomal ITS1-5.8S-ITS2 region using primers ITS5, ITS1 and ITS4 following the methods of Fischer (2002).

Pathogenicity tests were performed in citrus orchards belonging to the Ministry of Agriculture in the Galata area (Peloponisos, southern Greece), using the method of Ippolito *et al*. (1998) with modifications. Tested trees were free of disease, with neither foliar symptoms nor wood deterioration. Three 15-year-old trees each of orange (cv. New hall, grafted on citrumelo), clementine (cv. Sra 63, grafted on sour orange), lemon (Lekka, local cultivar, grafted on *Poncirus trifoliata*) and sour orange were inoculated with two *Fomitiporia* sp. isolates, Fomit22 from orange, and Fomit23 from common mandarin. The pathogenicity of *Fomitiporia* isolates Fomit17 from grapevine and Fomit1 from kiwi on citrus was examined in the same way for comparison. The above isolates derived from the *Fomitiporia* collection of the Benaki Phytopathological Institute. Each isolate was inoculated at two points on one branch 6–8 cm thick, and another branch of the same tree was used as a control. Two holes 2–3 cm deep were drilled in each branch with a 10-mm-diameter auger. Inoculum consisted of two 7-mm-diameter mycelial plugs taken from the margin of a 23-day-old culture growing on PDA. Two plugs were inserted in each hole, while two PDA plugs were inserted in the control holes. Holes were filled with sterilized pieces of cheesecloth and were protected with Parafilm tape. Three trees for each of the four citrus species and five branches per tree (four inoculated, one control) were tested in the whole.

Samples from inoculated trees (one tree per citrus species with two replicates on each branch) were taken six, ten and 16 months after inoculation. Transverse and longitudinal sections were made and the extent of wood discoloration was recorded.

## Results

### Isolations from field samples

In all samples cross-sections revealed the light-colored white rot in the central area where the wood changed to a soft spongy mass (Fig. 1), which in some cases came close to the bark, causing cracks along the trunk. These rotted areas were surrounded by dark-brown areas of hard wood (Fig. 1). The necrosis generally started from large pruning wounds or from trees with frost damage and sometimes decreased in size with further growth of the trees. The decay extended upward and downward, in the latter case attacking the rootstock wood. In advancing edges of the decay only brown discoloration was observed. From both wood rot and fruitbodies a mycelium was isolated that formed cream-yellow to light-brown colonies, with a dense aerial mat on PDA.

The characteristics of the fruit bodies (Fig. 2) were as follows: woody perennial, initially resupinate, later becoming cushion-shaped or even ungulate on vertical surfaces, 11–18 cm in diameter; pore surface initially ash-gray, later gray-brown, ochre brown, and the older parts black-brown and cracked; pores rounded, 5–7 per mm, with entire dissepiments; tubes concolorous, up

Fig. 1. Cross-section of orange-tree trunk infected with *Fomitiporia mediterranea*. Light-colored decay in the center, surrounded by brown hard necrosis.

Fig. 2. Fruit body of *Fomitiporia mediterranea* on the canker of an orange-tree trunk.

to 5 mm long, stratified in successively formed layers; context thin, golden brown to brown; hyphal system dimitic, skeletal hyphae thick-walled and dark-brown in KOH, generative hyphae hyaline and thin-walled, septa clampless; hymenial setae in examined specimens present but rather rare, short, acute, ventricose, often with swollen base, thick-walled, 17–25×8–9 $\mu$m; cystidioid elements also present, dark brown, thin-walled, ventricose with long rostrum; basidia 4-spored, broadly clavate, without basal clamp; basidiospores subglobose to broadly ovoid, 6.5–7.5×5.5–7 $\mu$m, hyaline, smooth, strongly dextrinoid in Melzer's reagent.

**Isolate identification and pathogenicity tests**

After amplification of the ribosomal ITS1-5.8S-ITS2 region, sequences were generated for selected isolates and were found to be consistent with those of the recently described species *Fomitiporia mediterranea* M. Fischer (Fischer, 2002).

There were significant differences in virulence among fungal isolates from the different hosts, as judged by the length of wood discoloration. The branches of orange, lemon, mandarin and sour orange inoculated with the fungal isolates from mandarin and orange showed a brown wood discoloration that extended up to 20 cm above and below the infection hole. These pathogens were also re-isolated at a distance of 20 cm from the inoculation site. On these same hosts, isolates from grapevine and kiwi produced a brown wood discoloration only 3–4 mm around the inoculation sites. The controls did not show any discoloration. The pathogen was re-isolated from the inoculated trees but not from the controls.

## Discussion

The white rot decay symptoms on the trunks and large branches of orange, common mandarin and lemon were similar to those associated with esca of grapevine and represent a significant problem causing severe losses on the citrus species. When these symptoms were noticed by growers in the past, they used to remove the diseased plant-parts from the trees. This did not prevent the disease from continuing to the remaining parts of the trees, killing numerous trees every year. In the Argolis area citrus fields are

often adjacent to olive orchards and vineyards, or they have been established on discontinued vineyards. According to growers the damage is greater in orchards exposed to frost. Frost events cause injuries to the branches that are points of fungal entry, and so is one of the main factors allowing infections to arise.

The decay described in the present paper has many aspects in common with the one associated with esca of grapevine (Mugnai *et al.,* 1999; Chiarappa, 2000; Elena *et al*., 2003). Recently, wood discoloration and rot symptoms similar to those of esca of grapevine have been observed also in kiwi and olive in Greece (Di Marco *et al.,* 2000; Elena and Paplomatas, 2002; Paplomatas *et al*., 2002). Shoots and twigs died as the damage spread towards the trunk. This infection has been observed in all major kiwi-growing areas and in olive orchards of the Peloponisos. Cross-sections of infected trunks and shoots revealed a light-colored decay in the center, which was surrounded by brown hard necrotic wood.

*Fomitiporia mediterranea* is the main pathogen causing white rot in esca-infected diseased grapevine in Europe. This taxon was also isolated from the two types of wood decay occurring in kiwi and olive in Greece. *F. mediterranea* was distinguished from the morphologically very similar *Fomitiporia punctata* (Fries) Murrill on the basis of molecular and sexual data (Fischer, 1996, 2000, 2002; Wagner and Fischer, 2001).

Besides *F. mediterranea*, which is most often isolated from white-rotted vine wood, *Phaeomoniella chlamydospora* (W. Gams, Crous, M.J. Wingf. & L. Mugnai) Crous & W. Gams also occurs in darkened woody tissue of grapevine (Mugnai *et al.,* 1999; Elena and Paplomatas, 2002; Paplomatas *et al*., 2002; Elena *et al*., 2003). The etiology of esca itself remained undefined for a long time and even today is still partly unclear (Graniti *et al.,* 2000).

The results of the pathogenicity tests and some similarities in the symptoms and pathogens isolated from the hosts mentioned above quite clear. In all cases the decay of the wood starts from pruning wounds and the wood rot is of the same type. Nevertheless in grapevine the brown hard necrosis surrounding the decay is associated also to species different from *F. mediterranea*.

A wood rot of citrus, causing identical symptoms and severe losses, was first reported in Italy on clementine 'Fedele' trees (also, but in less severe form, on clementine 'De Mulles', 'Moureal' and 'Comune') grafted on sour orange rootstock (Ippolito *et al*., 1998). This rot extended to the trunk of the trees but stopped at the rootstock wood of sour orange. The fungus isolated from the rotted wood was originally identified as *Phellinus punctatus* (Ippolito *et al*., 1998), but in the light of new data it is most likely to have been *F. mediterranea* (Fisher, 2002). Pathogenicity tests with this fungus on lemon, clementine, sweet and sour orange branches reproduced symptoms of wood discoloration and wood rot in all plants except sour orange.

To our knowledge this is the first report of *F. mediterranea* causing wood rot of citrus in Greece.

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