New Disease Reports

First report of *Phoma tracheiphila* causing severe mal secco disease on a mandarin hybrid (cv. Ortanique) grafted onto Citrumelo rootstock in western Greece

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The Greek citrus industry covers an area of almost 59,000 ha located mainly in the central and southern parts of Greece. One of the major phytosanitary problems for the industry is a disease of Citrus spp. and hybrids, caused by the fungal pathogen Phoma tracheiphila, which mainly affects lemon trees (Citrus limon) causing significant yield reductions of up to 60%. It has been estimated that 60-100% of the trees can be affected within 20 years after planting (Thanassoulopoulos & Manos, 1992). P. tracheiphila is considered to be widespread in Greece (first observed in 1894) but it is listed as a II/A2 quarantine organism by the European Commission as it is absent from citrus plantations in some EU Member States (e.g. Spain and Portugal) (EPPO, 2014). Three disease syndromes are associated with the fungus depending upon the site of infection. These are 'mal secco', the most common form of the disease, arising from infection in the canopy (wilt and dieback); 'mal nero' infection at the base of the trunk (brown discoloration of the heartwood), and 'mal fulminante', infection in the roots (sudden wilt of branches or the whole tree). All three syndromes result in desiccation of twigs, branches, or the whole plant (Nigro *et al.*, 2011).

In addition to lemon (and other citrus species/hybrids), mandarin (C. reticulata) and some of its hybrids can also become affected by the pathogen (Nigro et al., 2011). Among rootstocks, sour orange (C. aurantium), the most widespread lemon rootstock in Greece, is considered to be very susceptible to infection (Solel & Oren, 1975). By comparison, Citrumelo, a citrus hybrid (C. paradisi x Poncirus trifoliata), is reportedly less susceptible to P. tracheiphila (Nigro et al., 2011), and it is recommended as a citrus rootstock due to its resistance to Citrus tristeza virus (Moreno et al., 2008). During surveys in citrus orchards in Katohi, western Greece in 2014, decline of a mandarin hybrid (C. reticulata x sinensis, cv. Ortanique) grafted onto Citrumelo rootstocks was observed, with approximately 90% of the trees being affected in an area of 20 ha. Symptoms first appeared in the spring in the apical parts of branches and sprouts including epinasty and shedding of leaves, followed by wilt and dieback of twigs and branches (Fig. 1A). Often individual branches were affected and symptoms appeared on one side of the plant (Fig. 1B). In some cases infection progressed rapidly affecting the entire tree. Infected shoots, twigs, secondary branches and trunk showed the characteristic salmon-pink or orange-reddish discoloration of the wood (Fig. 2).

Consistent orange-pink pigmented fungal colonies isolated from infected xylem on potato dextrose agar (PDA) were identified as putative P.



tracheiphila based on the formation of mature black pycnidia. DNA was extracted from infected twigs, bark, wood, and from PDA cultures, following PCR using the primer pair PtFOR2/Pt-REV2 developed by Balmas *et al.* (2005); a 380 bp fragment was generated in each case. The identified sequence (KP025953) was 100% identical to *P. tracheiphila* isolates Pt-42 (AY531666), and IPt-5 (DQ993285) among others. This is the first report of *P. tracheiphila* occurring on cv. Ortanique grafted onto Citrumelo rootstocks. Cultural and phytosanitary controls on this highly susceptible combination are likely to require intensification to help prevent the spread of the pathogen.

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Figure 2

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Figure 1