EPPO Datasheet: *Plenodomus tracheiphilus*

Last updated: 2024-03-11

IDENTITY

Preferred name: *Plenodomus tracheiphilus*
Authority: (Petri) Gruyter, Aveskamp & Verkley
Taxonomic position: Fungi: Ascomycota: Pezizomycotina: Dothideomycetes: Pleosporomycetidae: Pleosporales: Leptosphaeriaceae
Other scientific names: *Bakerophoma tracheiphila* (Petri) Ciferri, *Deuterophoma tracheiphila* Petri, *Phoma tracheiphila* (Petri) Kanchaveli & Gikashvili
Common names: dieback of citrus, mal secco of citrus, wilt of citrus
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EPPO Categorization: A2 list
view more categorizations online...
EU Categorization: RNQP ((EU) 2019/2072 Annex IV)
EPPO Code: DEUTTR



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Notes on taxonomy and nomenclature

Plenodomus tracheiphilus is a necrotrophic fungus causing a vascular wilt disease of citrus trees. Historically, the pathogen was described as *Deuterophoma tracheiphila* by Petri (Nigro *et al.*, 2011). Later, the fungus was transferred to the genus *Phoma* (section *Plenodomus*), whose members are characterized by their ability to produce ‘scleroplectenchyma‘ and often also pseudothecia (Boerema *et al.*, 1994). Recently, detailed molecular phylogenetic studies on the genus *Phoma* and related anamorph genera recognized that *Phoma* is a polyphyletic group (de Gruyter *et al.*, 2009). As a result, the section *Plenodomus* was redescribed and *Phoma tracheiphila* was designated as *Plenodomus tracheiphilus* (de Gruyter *et al.*, 2013).

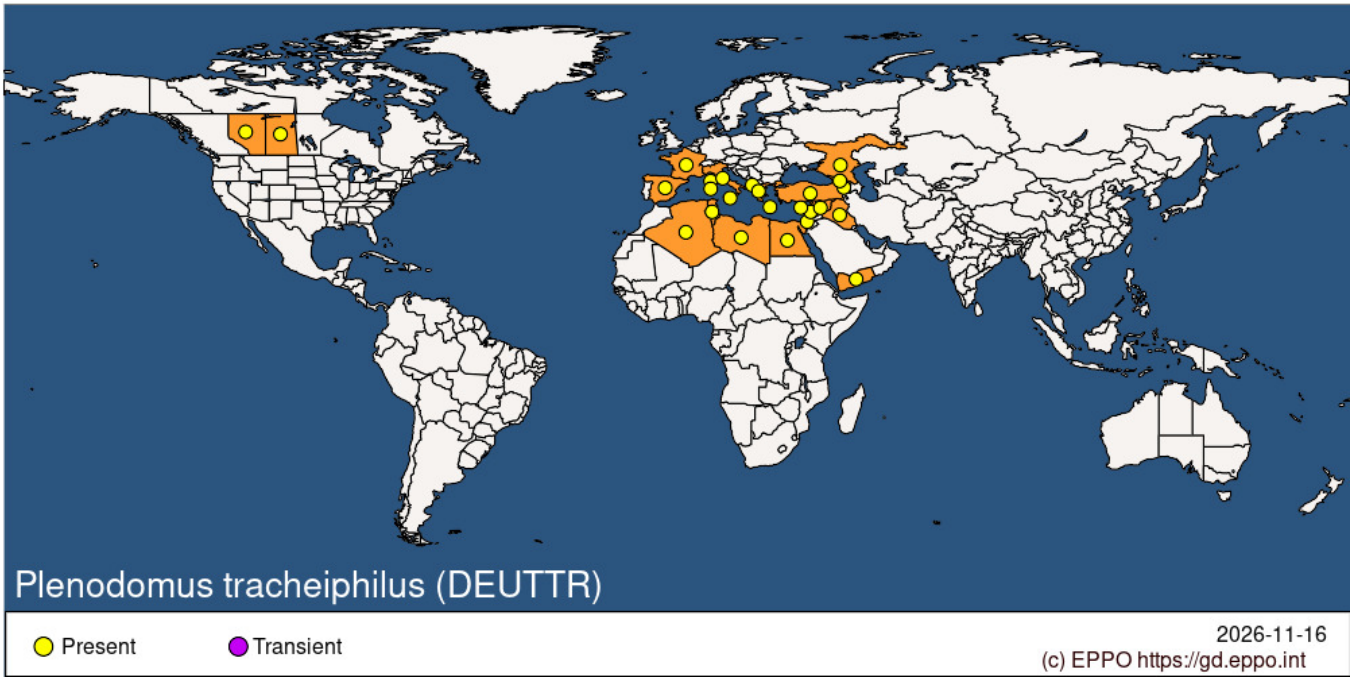
HOSTS

The main host of this pathogen is lemon (*Citrus* x *limon*). However, *P. tracheiphilus* has also been reported on citron (*Citrus medica*), bergamot (*C.* x *limon* var. *bergamia*), lime (*C.* x *latifolia*), sour orange (*C. x aurantium*), and rough lemon (*C.* x *limonia* var. *jambhiri*), other species and hybrids of *Citrus*, species of several other genera in the Rutaceae, such as *Eremocitrus, Fortunella, Poncirus, Atalantia*; and their interspecific and intergeneric hybrids can also serve as hosts with different degrees of resistance to the disease (EFSA, 2014; Sicilia *et al.*, 2022; Dimaria *et al.*, 2023). The information on relative susceptibility of citrus species to *P. tracheiphilus* is based on field observations of symptoms and it has not always been confirmed by pathogenicity tests (Nigro *et al.*, 2011; EFSA, 2014; Sicilia *et al.*, 2022). Most cultivars of oranges, mandarins (*Citrus deliciosa* and *C. reticulata*), clementines (*C. clementina*), and grapefruits (*Citrus* × *paradisi*) are considered to be only occasionally affected. A number of rootstocks such as sour orange (*C.* x *aurantium*), rough lemon (*C.* x *limonia* var. *jambhiri*), Volkamer lemon (*Citrus* x *limonia* var. *volkameriana*) and alemow (*Citrus* x *aurantiifolia* var. *macrophylla*) are very susceptible to the disease while other rootstocks, such as Cleopatra mandarin (*C. reshni*), trifoliate orange (*Poncirus trifoliata*), and, to a lesser extent, Troyer citrange (*C. sinensis* × *P. trifoliata*) have been reported to be resistant (Nigro *et al.*, 2011; de Gruyter *et al.*, 2013; Russo *et al.*, 2020; Sicilia *et al.*, 2022).

Host list: *Atalantia buxifolia, Citrus hybrids, Citrus karna, Citrus latipes, Citrus limonimedica, Citrus medica, Citrus myrtifolia, Citrus pennivesiculata, Citrus reshni, Citrus reticulata, Citrus taiwanica, Citrus trifoliata, Citrus webberi, Citrus x aurantiifolia var. macrophylla, Citrus x aurantiifolia, Citrus x aurantium var. clementina, Citrus x aurantium var. deliciosa, Citrus x aurantium var. paradisi, Citrus x aurantium var. sinensis, Citrus x aurantium, Citrus x junos, Citrus x limon var. bergamia, Citrus x limon var. limettioides, Citrus x limon var. meyerii, Citrus x limon, Citrus x limonia var. jambhiri, Citrus x limonia var. volkameriana, Citrus x limonia, Citrus x tangelo , Fortunella japonica, Fortunella sp., Ulmus sp.*

GEOGRAPHICAL DISTRIBUTION

'Mal secco', an Italian term meaning 'dry disease' is a severe vascular disease of citrus caused by *P. tracheiphilus* which appeared at the end of the XIX century (1894) in the Greek Aegean islands (Nigro *et al.*, 2011). In Italy, the disease was first reported in 1918 (Eastern Sicily), apparently following the introduction of infected plants from Greece and reached continental Italy, affecting the groves of all parts of Italy, and crossed again the sea to land in Sardinia (Nigro *et al.*, 2011; EFSA, 2014). In spite of the efforts to keep disease under control, it soon spread to most citrus-growing countries of the Mediterranean and Black Sea basins, but has not been reported from Portugal or Morocco (EPPO, 2015). It also spread to North Africa and West Asia (EFSA, 2014; CABI, 2022). Currently, the pest is present in most countries of the Mediterranean region and in some countries bordering the Black Sea.



EPPO Region: Albania, Algeria, Armenia, Cyprus, France (mainland, Corse), Georgia, Greece (mainland, Kriti),

Israel, Italy (mainland, Sardegna, Sicilia), Russian Federation (Southern Russia), Spain (mainland), Tunisia, Türkiye
Africa: Algeria, Egypt, Libya, Tunisia
Asia: Iraq, Israel, Lebanon, Syrian Arab Republic, Yemen
North America: Canada (Alberta, Saskatchewan)

BIOLOGY

P. tracheiphilus is a vascular pathogen of lemons and other species of the genera *Citrus, Fortunella, Poncirus, Eremocitrus, Atalantia,* and their hybrids. Frost, wind, hail, and cultivation practices that cause injuries to different organs of hosts favour infection. The fungus infects the plant via wounds, and then it enters the vessels and spreads inside the host, colonizing the neighbouring xylem tissues and moves upwards with the transpiration stream (Nigro *et al.*, 2011; Sicilia *et al.*, 2022). As a consequence of the xylem clogging, due to the presence of the fungal hyphae and the reaction of the host (gum production), the water and solute transport is compromised and water-stress symptoms appear (Perrotta and Graniti,1988; Nigro *et al.*, 2011). The rate and extent of xylem colonization is directly related to symptoms' severity and to the virulence of the different fungal strains (Nigro *et al.*, 2011). Infection spreads by conidia that are produced in pycnidia on withered twigs or by phialoconidia produced by phialides formed on free hyphae on exposed woody surfaces (including wood debris on soil), wounded plant tissues and within the xylem elements (Migheli *et al.*, 2009). Pycnidia are considered the primary mode of infection while phialoconidia are secondary ones and they are dispersed by the transpiration flow to distal parts of the plant where they cause additional damage (Ben-Hamo *et al.*, 2020). The pycnidia differentiate in autumn/winter on infected organs on the plant or on the ground and when the temperature averages approximately 10°C and citrus plants are dormant the pathogen is still active (Nigro *et al.*, 2011). Conidia are dispersed on infected plant tissue by wind and rain, and pathogen penetrates the xylem at a dormant stage of host and growth through the host branches into the main trunk, eventually reaching the roots (Ben-Hamo *et al.*, 2020). Conidia require 40 h of moisture at temperatures in the range of 14 to 28°C to germinate (EFSA, 2014). The range of temperature at which infection will occur is also considered to be between 14 and 28°C, the optimum temperature for growth of the pathogen and for symptom expression is 20–25°C, whereas the maximum temperature for mycelial growth is 28–30°C and during midsummer fungal growth can cease (Migheli *et al.*, 2009; Nigro *et al.*, 2011; Ben-Hamo *et al.*, 2020). During the period which is too cold for mycelial growth (<14°C), the pathogen can still produce pycnidia (at approximately 10°C) to infect plants (Ben-Hamo *et al.*, 2020). Infection periods depend on local climatic and seasonal conditions and infected trees can show first symptoms of the disease during the spring or early summer (Ben-Hamo *et al.*, 2020).The timing and length of these periods may vary according to the seasons, e.g. in Sicily (Italy) infections usually occur between September and April (Somma and Scarito, 1986) whereas in Israel the infection commences in early spring (late March) and continues until the beginning of June with breaks during midsummer (a hot period) and between early November and the end of December (a cold period), when the fungus does not develop (Migheli *et al.*, 2009; Ben-Hamo *et al.*, 2020).

For more information on the biology of the pathogen, see Migheli *et al.* (2009), Nigro *et al.* (2011), and Ben-Hamo *et al.* (2020).

DETECTION AND IDENTIFICATION

Symptoms

The symptomatology of the disease is characterized by the desiccation of twigs, branches, or the whole plants, as suggested by its internationally adopted name 'mal secco', meaning 'dry disease' in Italian (Sicilia *et al.*, 2022). The first symptoms appear mainly in spring as leaf vein chlorosis followed by leaf drop, and dieback of twigs and branches (Nigro *et al.*, 2011). The disease symptoms are induced by a phytotoxin malseccin (EFSA, 2014). A salmon pink or brown-reddish wood discoloration under the bark of withered twigs, infected branches, and the trunk is the most typical symptom of the disease, which is due to gum production within the xylem vessels (Magnano *et al.*, 1992; Sicilia *et al.*, 2022). Raised black points within lead-grey or ash-grey areas of withered twigs indicate the presence of pycnidia (Nigro *et al.*, 2011). The growth of sprouts from the base of the affected branches and suckers from the rootstock are a very common response of the host to the disease. Gradually the pathogen affects the entire tree, which eventually dies. In addition to the more common form of mal secco, two other forms of the disease can be distinguished. Root infections have been characterized by a chronic, slowly developing disease leading to a

browning of the heartwood called ‘mal nero’ (‘black disease’) and a sudden death syndrome called ‘mal fulminante’ which is a rapid form of the disease apparently due to root infection (Nigro *et al.*, 2011; Dimaria *et al.*, 2023).

Morphology

Pycnidial conidiomata (60–165 x 45–140 µm diameter) bear a neck, are sparsely produced, pale yellow, surrounded by aerial mycelia, thin-walled and open irregularly at maturity. Conidiogenous cells monophialidic, integrated, hyaline cylindrical or flask-shaped, determinate with well-defined collarettes. Conidia variable in shape and size (0.5–1.5 × 2–4 ?m), hyaline, cylindrical, ellipsoidal, reniform, clavate, straight or curved, aseptate, occasionally 1-septate, usually with 2 or more guttules. Sometimes conidia are extruded through ostioles in whitish cirri. Larger conidia 2.5–11.5 × 1–3 µm (mean [± S.D.] = 6.8 [± 2.13] × 2.3 [± 0.44] µm) (Zhao *et al.*, 2021) are usually named phialoconidia and produced by phialides (12–30 × 3–6 µm) and borne on free hyphae growing on exposed wood surfaces, wounded plant tissues, and within the xylem elements of the infected host; they are hyaline, unicellular, uninucleate and sometimes binucleate or trinucleate, straight or curved, with rounded apices (3–8 x 1.5–3.0 µm) (de Gruyter *et al.*, 2009). No teleomorph is known (de Gruyter *et al.*, 2009).

Culture characteristics. On oatmeal agar media the colonies reach 25–35 mm diameter after 7 days, they are flat with small aerial mycelia; pigmentation of mycelial mat and medium variable, depending on strain and ranging from pale pink or bright orange to dark olive brown. On application of NaOH the reddish pigments turn blue (the presence of helminthosporin and cynodontin have been demonstrated). Colonies on potato dextrose agar are 35–36 mm in diameter after 7 days, and the growth rate is 3.8–6.0 mm per day at 23 ± 2°C (EPPO, 2015). The mycelium is initially hyaline and after a few days becomes brown or pinkish red, sometimes orange to olivaceous or olivaceous grey, reverse tan or from pale to dark red wine colour. Colonies on malt extract agar (2%) are 28–30 mm in diameter after 7 days, greyish blue or pale olivaceous grey, reverse from orange to dark brown, margin yellow (Zhao *et al.*, 2021). After 10–12 days, phialoconidia are produced and should be mounted in distilled water and observed under the microscope (EPPO, 2015).

For more information on morphology, see EPPO (2015) and Zhao *et al.* (2021).

Detection and inspection methods

P. tracheiphilus can be detected following the EPPO diagnostic protocol PM 7/048 (3): *Plenodomus tracheiphilus* (formerly *Phoma tracheiphila*) (EPPO, 2015).

The pest can be identified based on the typical wood discoloration (characteristic salmon-pink or orange-reddish discoloration of the wood) and species’ morphological structures (immersed, flask-shaped or globose pycnidia within lead-grey or ash-grey area on infected twigs) (EPPO, 2015). Detecting the disease based only on the symptoms is unreliable due to symptom variations, similar symptoms to other citrus pathogens and latent infections caused by rapid pathogen movement before symptoms appear (Migheli *et al.*, 2009; EFSA, 2014). Therefore, for reliable detection and identification of the pathogen, laboratory testing of the affected plant tissues should be performed. The fungus can be easily isolated from infected xylem material on agar media and samples (twigs and leaves) can be taken at any time of the year in the field (EPPO, 2015). When sporulation occurs, identification is possible based on symptoms together with cultural and morphological characters whereas in the absence of sporulation, identification should be based on cultural characters and a molecular method.

A conventional PCR test and a real-time PCR test were developed for detection in planta directly from symptomatic twigs, leaves and fruits following the EPPO diagnostic protocol (EPPO, 2015). The conventional PCR is also used for differentiation of the pest from other citrus pathogens; it utilizes a pair of *P. tracheiphilus*-specific primers (Ezra *et al.*, 2007). The real-time PCR test was developed for the quantification of the pathogen in the host as well as for its detection in latently infected (asymptomatic) plant tissues and for direct detection in soil (Demontis *et al.*, 2008; EPPO, 2015).

Plant leaves should be inspected in the field in spring for the presence of vein chlorosis, which is an early symptom of the disease and anytime for desiccation of twigs, branches, or the whole plant and orange to pink wood discoloration of the xylem (Migheli *et al.*, 2009). On fruits, browning of vascular bundles can be observed in the area of insertion of the peduncle (EPPO, 2020). General background information on inspection of consignments is given in the EPPO Standard PM 3/90 (1) (EPPO, 2020).

PATHWAYS FOR MOVEMENT

Plants for planting are likely to be the main pathway for the introduction and spread of this pest. Therefore, the pathogen can spread over long distances via the movement of infected host plants for planting (rootstocks, grafted plants, scions, budwood, etc.), fruit peduncles and leaves, particularly latently infected (asymptomatic) material. Conidia of *P. tracheiphilus* generated in pycnidia on diseased plant parts such as twigs, branches, peduncles, leaves, etc. are typically spread over relatively short distances through mechanisms including rain-splash, overhead irrigation, water surface flow or wind-driven rain (Migheli *et al.*, 2009; Nigro *et al.*, 2011); birds and insects have been suspected to be vectors (Perrotta and Graniti, 1988). Pruned material or soil containing infected plant debris (particularly twigs or branches) can be a source of inoculum and a potential pathway for the introduction of the disease into new areas, as the pathogen can survive on those plant parts for up to 4 months (De Cicco *et al.*, 1987).

PEST SIGNIFICANCE

Economic impact

P. tracheiphilus can cause major yield losses and can lead to death of twigs, thus seriously reducing the volume of the citrus tree canopy. The disease can also lead to the death of the whole tree (Migheli *et al.*, 2009; Nigro *et al.*, 2011). In the Mediterranean region, *P. tracheiphilus* is the most destructive fungal disease of lemons with a highly significant impact on the citrus industry in areas where the host plants are widely grown and the pathogen is present (Migheli *et al.*, 2009; Nigro *et al.*, 2011). In Tunisia, up to 100% of trees of a susceptible lemon cultivar were affected in some orchards (Ziadi *et al.*, 2012). Also, a lasting adverse impact of *P. tracheiphilus* was documented in Türkiye in 1956 (Mersin District), leading to the demise of roughly 20 000 lemon trees over a span of 15 years (EFSA, 2014). In general, injury to the tree through severe, cold weather may predispose it to fungal attack and destructive outbreaks of *P. tracheiphilus* may occur after frost spells and hailstorms in spring (Perrotta and Graniti, 1988). The disease reduces the quantity and quality of lemon production in the areas where the pathogen is present and limits the use of susceptible species and cultivars. The economic impact from *P. tracheiphilus* encompasses both immediate and secondary detrimental outcomes. These include expenses related to pruning branches and twigs, removing dead trees, lower fruit quality due to the utilization of resistant cultivars such as cv. Monachello, and the added cost of applying extra fungicide sprays (Migheli *et al.*, 2009; Nigro *et al.*, 2011).

Control

After entering the host, the pathogen penetrates the plant's vessels, making it unfeasible to eradicate using cultural methods or chemicals. Cultural techniques and chemical approaches implemented in EU Member States where the pest is present primarily focus on diminishing sources of infection and safeguarding vulnerable above-ground plant components from harm (EPPO, 2004; Migheli *et al.*, 2009; Nigro *et al.*, 2011; Caserta *et al.*, 2019).

P. tracheiphilus can be kept under control by pruning of diseased twigs/branches as soon as the first symptoms appear, timely removal of suckers and/or dead citrus trees (EPPO, 2004, 2020; Migheli *et al.*, 2009). Common practices aimed either at saving the infected trees or at reducing the inoculum include cutting whole branches and grafting the pollarded tree with resistant cultivars or species. Burning of the pruned branches and twigs is recommended in order to eliminate possible sources of inoculum. Injury during cultural practices should be avoided. In some citrus-growing EU countries (e.g., Spain, France, and Italy), citrus plants are produced under certification programmes (EFSA, 2014) preventing the introduction and further spread of *P. tracheiphilus* in new areas through the use of citrus planting material produced in certified nurseries.

Chemical control is not widely used except in nurseries. The use of fungicide sprays in citrus-growing regions within

the EU is considered to be ineffective in managing the disease within already infested areas for both 'mal nero' and 'mal fulminante' forms. Copper fungicides and mancozeb were the most common products used but both fungicides have been associated with environmental concerns (EFSA, 2014) and mancozeb is not currently (as per March 2024) approved for use in EU (https://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/start/screen/active-substances/details/277). Systemic plant protection products are effective only as a preventive measure (EPPO, 2004; Migheli *et al.*, 2009).

The most effective approach for managing *P. tracheiphilus* is to utilize citrus cultivars or clones which are resistant to the pathogen or grafted onto resistant rootstocks, but unfortunately this is not easily achieved. In certain regions of Italy and Greece, the resistant or tolerant lemon cultivars have replaced the susceptible cultivar Femminello but they are of inferior quality. These resistant cultivars have shown reduced yield, fruit quality, or overall agronomic performance compared to the susceptible ones. Initially, *in vitro* selection was considered a promising method for developing lemon clones with disease tolerance, however, to date, no commercially available lemon cultivar produced using this breeding technique exists, and there is limited information on their yield, fruit quality, agronomic traits and adaptation to different environmental conditions (Russo *et al.*, 2020).

Another technique is the use of acibenzolar-S-methyl which is known to activate plant defence mechanisms against fungi. This has been preliminarily tested on citrus against *P. tracheiphilus* showing a strong reduction in 'mal secco' symptoms (Leonardi *et al.*, 2023). However, this active substance is no longer authorized in the EU (https://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/start/screen/active-substances/details/1053) (as per July 2024).

Phytosanitary risk

P. tracheiphilus is included in the EPPO A2 List of pests recommended for regulation. The pathogen is also considered to be of a quarantine concern by several regional plant protection agencies worldwide, including the Asia and Pacific Plant Protection Commission (APPPC), and the Comité Regional de Sanidad Vegetal para el Cono Sur (COSAVE) (Migheli *et al.*, 2009; Nigro *et al.*, 2011). *P. tracheiphilus* has been classified as a regulated non-quarantine pathogen in European Union (EFSA, 2014).The fungus, however, does not currently occur in some citrus-growing countries of the EPPO region (e.g., Portugal and Morocco). This is possibly a result of the severe restrictions on movement of citrus propagating. There are no obvious climatic or cultural factors limiting potential establishment of *P. tracheiphilus* in uninfected areas.

PHYTOSANITARY MEASURES

When *P. tracheiphilus* is not present in a country and regulated as a quarantine pest, appropriate measures may consist of production of host plant in pest-free areas or within a certification scheme (see below) requiring them to be derived in direct line from material which has been growing permanently in an insect-proof glasshouse or in an isolated cage on which no symptoms of *P. tracheiphilus* have been observed (as it was suggested in the past by the EU Council Directive 2000/29/EC; EU, 2000).

When *P. tracheiphilus* is already present in a country and treated as a regulated non-quarantine pest (RNQP), then the following phytosanitary measure can be recommended: (a) the hosts should be produced in areas known to be free from *P. tracheiphilus*; or (b) the hosts have been grown in a site of production that was found free from *P. tracheiphilus* over the last complete growing season, by at least two visual inspection at appropriate times, during that growing season, and any symptomatic plants in the immediate vicinity have been rogued out and destroyed immediately; or (c) no more than 2 % of hosts in the lot showing symptoms during at least two visual inspections at appropriate times to detect the pest during the last growing season, and those symptomatic hosts and any other symptomatic hosts in the immediate vicinity have been rogued out and destroyed immediately (Picard, 2018; RNQP, 2018; EU, 2019).

A system for the production of vegetatively propagated plants for planting was also recommended (EPPO, 1995). For the production of certified pathogen-tested trees and rootstocks of *Citrus*, *Eremocitrus*, *Poncirus*, *Fortunella*, *Atalantia* and their hybrids, the successive steps should be taken as described in the Certification scheme for pathogen-tested citrus trees and rootstocks (EPPO, 1995). Host plants should be visually inspected every year for possible mutations or back mutations and in countries where *P. tracheiphilus* is present, propagation stock should be kept on fields on which none of plants infected by *P. tracheiphilus* have been grown for at least 5 years (EPPO,

1995). Mother plants of pathogen-tested trees and rootstocks should be covered with a net (white plastic net normally used to protect trees from wind and hail damage) to avoid contamination by *P. tracheiphilus* (EPPO, 1995). Moreover, the EPPO Standard on good plant protection practice for *Citrus* should be recommended for controlling *P. tracheiphilus* (careful pruning and burning of withered plant material, soil cultivation in late autumn or winter, protection of orchards from wind, spraying systemic fungicides is recommended after hail or frost damage as either soil drenches or foliage treatments) (EPPO, 2004).

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