

EPPO Datasheet: '*Candidatus Phytoplasma ulmi*'

Last updated: 2022-07-05

IDENTITY

Preferred name: '*Candidatus Phytoplasma ulmi*'

Authority: Lee, Martini, Marcone & Zhu

Taxonomic position: Bacteria: Tenericutes: Mollicutes:
Acholeplasmatales: Acholeplasmataceae

Other scientific names: *Elm phloem necrosis phytoplasma*,
Elm yellows phytoplasma, *Phytoplasma ulmi* Lee, Martini, Marcone
& Zhu

Common names: elm yellows, phloem necrosis of elm, yellows of elm (US)

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EPPO Categorization: A1 list, A2 list

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EU Categorization: PZ Quarantine pest ((EU) 2019/2072 Annex III)

EPPO Code: PHYPUL



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Notes on taxonomy and nomenclature

'*Candidatus Phytoplasma ulmi*' is a member of the elm yellows phytoplasma group or 16SrV group, subgroup 16SrV-A (Lee *et al.*, 2004). Other members of this group are phytoplasmas causing mainly diseases of woody plants such as flavescence dorée of grapevine, alder yellows, Palatinate grapevine yellows, spartium witches'-broom, rubus stunt, eucalyptus little leaf, cherry lethal yellows, flowering cherry decline, peach yellows in India, jujube witches'-broom, Japanese raisin witches'-broom and sophora japonica witches'-broom (Jung *et al.*, 2003; Lee *et al.*, 2004; Arnaud *et al.*, 2007; Malembic-Maher *et al.*, 2011; Marcone, 2015, 2017; Marcone *et al.*, 2021). '*Candidatus Phytoplasma ulmi*' differs from other members of the elm yellows group including '*Candidatus Phytoplasma rubi*' (the rubus stunt agent) and '*Candidatus Phytoplasma ziziphi*' (the jujube witches'-broom agent) by less than 2.5% in 16S rDNA sequence similarity, the threshold for assigning species rank to phytoplasmas under the provisional status '*Candidatus*' (IRPCM, 2004). Recently, this threshold was lowered to 1.35% (Bertaccini *et al.*, 2022). However, supporting data for separating the above taxa at the putative species level were obtained by examining other molecular markers and considering biological properties, such as host range and insect vector specificity (Jung *et al.*, 2003; Lee *et al.*, 2004; Malembic-Maher *et al.*, 2011; Martini *et al.*, 2014; Bertaccini *et al.*, 2022).

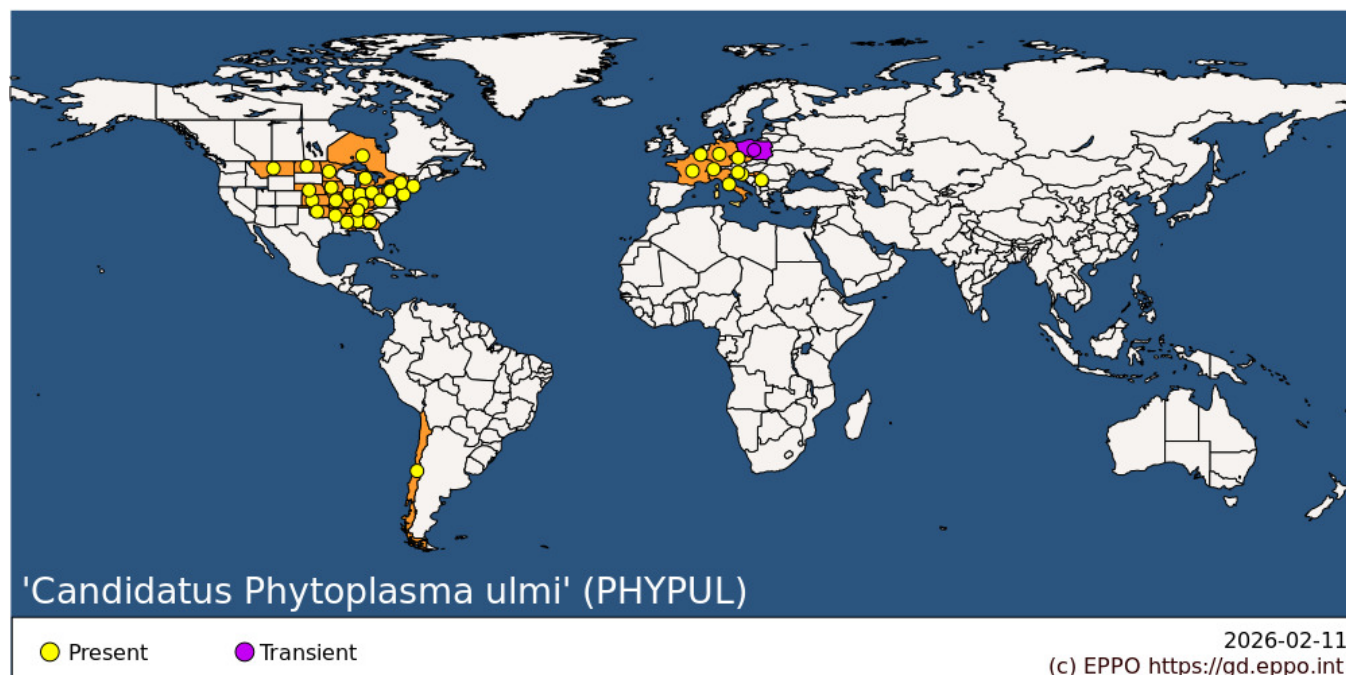
HOSTS

'*Candidatus Phytoplasma ulmi*' exhibits a high plant host specificity. In nature, this pathogen preferentially infects plants in the genus *Ulmus*. It was also identified in naturally infected plants of *Zelkova serrata* (Japanese zelkova) in Central Italy, which showed symptoms of yellowing, foliar reddening, witches'-brooms, reduced terminal growth and stunting (Romanazzi & Murolo, 2008; Murolo & Romanazzi, 2008). The pathogen has also been transmitted from diseased elm trees to the experimental phytoplasma host *Catharanthus roseus* (periwinkle) via dodder (*Cuscuta epithymum*, *C. ceanothi*) bridges (Braun & Sinclair, 1979; Mäurer *et al.*, 1993). In addition, a '*Candidatus Phytoplasma ulmi*'-related strain was identified in diseased plants of murta (*Ugni molinae*), a shrub of the Myrtaceae family, showing symptoms of witches'-brooms in Chile. The Chilean '*Candidatus Phytoplasma ulmi*'-related strain was experimentally transmitted from diseased murta plants to ryegrass (*Lolium multiflorum*) using the leafhopper *Amplipcephalus curtulus* (Arismendi *et al.*, 2011, 2014).

Host list: *Ugni molinae*, *Ulmus alata*, *Ulmus americana*, *Ulmus canescens*, *Ulmus chenmoui*, *Ulmus crassifolia*, *Ulmus davidiana* var. *japonica*, *Ulmus glabra*, *Ulmus laevis*, *Ulmus minor*, *Ulmus parvifolia*, *Ulmus pumila*, *Ulmus rubra*, *Ulmus serotina*, *Ulmus villosa*, *Ulmus wallichiana*, *Ulmus wilsoniana*, *Ulmus x hollandica*, *Zelkova serrata*

GEOGRAPHICAL DISTRIBUTION

Elm yellows is known to occur in North America and Europe. This disease was first described in Ohio in 1938 (Swingle, 1938). However, there is evidence that it was present in this US state as well as in Kentucky, Indiana and Illinois long before, perhaps as early as the late 1800s (Garman, 1893, 1899). Once known throughout the midwestern states, elm yellows spread into eastern states and southeastern Ontario (Matteoni & Sinclair, 1988; Sinclair, 2000). Until the 1980s, elm yellows was considered to be a North American disease. Conti *et al.* (1987) first reported the occurrence of this disease in Italy, although it had been observed in Italy since at least 1918 (for review see Marcone, 2017). Following this finding, phytoplasma diseases of elm have also been recorded in several other European countries (Marcone, 2015, 2017; De Jonghe *et al.*, 2019; Schneider *et al.*, 2020). Detection of elm yellows in Europe was first carried out on the basis of symptoms (Braun & Sinclair, 1979; Conti *et al.*, 1987). Later, molecular studies using mainly RFLP and sequence analyses of PCR-amplified rDNA showed that the phytoplasma diseases of elm in Europe and North America are caused by the same organism, the elm yellows agent '*Candidatus Phytoplasma ulmi*' (Lee *et al.*, 1993, 2004; Mäurer *et al.*, 1993; Marcone *et al.*, 1997).



EPPO Region: Belgium, Croatia, Czechia, France (mainland), Germany, Italy (mainland), Poland, Serbia, Slovenia, Switzerland

North America: Canada (Ontario), United States of America (Alabama, Arkansas, Georgia, Illinois, Indiana, Iowa, Kansas, Kentucky, Massachusetts, Michigan, Minnesota, Mississippi, Missouri, Montana, Nebraska, New Jersey, New York, North Dakota, Ohio, Oklahoma, Pennsylvania, Tennessee, West Virginia)

South America: Chile

BIOLOGY

Elm yellows is a lethal or decline phytoplasma disease that affects several *Ulmus* (elm) species and hybrids. This disease was formerly called elm phloem necrosis to emphasize a prominent symptom, discoloration and death (= necrosis) of the current season's phloem in highly susceptible taxa such as *Ulmus americana* (American or white elm) (Swingle, 1938). Since elm yellows is lethal to North American *Ulmus* species, while some Eurasian genotypes are tolerant or resistant, it seems that the disease is of European origin (Sinclair, 2000). Tolerance or resistance is usually expected in regions where '*Candidatus Phytoplasma ulmi*' and its natural hosts co-evolved, while the lack of tolerance or resistance could indicate a recent introduction of the pathogen.

The white-banded elm leafhopper *Scaphoideus luteolus* is the only confirmed vector of '*Candidatus Phytoplasma ulmi*' in North America, although other vectors are likely to be involved in its natural spread. This likelihood is supported by the fact that numerous homopteran insects belonging to genera known to vector phytoplasmas have been found on elm and probably feed on it to some extent, and that *S. luteolus* is rare or absent in some areas where

severe elm yellows outbreaks occur (Sinclair, 1981, 2000). In New York State, of the various leafhoppers and other homopteran insects collected on sites of elm yellows occurrence and tested for ability to transmit the elm yellows agent to American elm seedlings, single transmissions were recorded for the leafhopper *Allygus atomarius* and the spittlebug *Philaenus spumarius* (Matteoni and Sinclair, 1988). ‘*Candidatus Phytoplasma ulmi*’ was detected by real-time PCR in several leafhoppers belonging to *Allygus*, *Colladonus*, *Empoasca*, *Erythroneura*, *Graphocephala*, *Homalodisca*, *Orientus*, *Scaphoideus*, and *Typhlocyba*, which were collected in the University Park Campus of Pennsylvania State University, USA (Herath *et al.*, 2010). However, it remains to be demonstrated if these leafhopper taxa can transmit the pathogen. Rosa *et al.* (2014) reported that 3 of 30 American elm seedlings exposed to individuals of the spittlebugs *Lepyronia quadrangularis* and *P. spumarius* and the leafhopper *Latalus* sp., collected from an elm yellows-infected red elm tree in the Pennsylvania State University campus, were infected by ‘*Candidatus Phytoplasma ulmi*’. Carraro *et al.* (2004) showed that *Macropsis mendax* is a natural vector of the elm yellows agent in Friuli Venezia Giulia (Northern Italy). This leafhopper is strictly monophagous, univoltine and overwinters as eggs on elm. It is unknown whether *M. mendax* is involved in the spread of the elm yellows agent in other parts of Europe and there is no information on its transmission efficiency. Additionally, ‘*Candidatus Phytoplasma ulmi*’ was detected by PCR in individuals of *Hyalesthes luteipes*, and *Iassus scutellaris*, *Allygidius furcatus* and *Cixius* sp., collected from elm yellows-affected elm trees in Serbia and France, respectively (Boudon-Padieu *et al.*, 2004; Jović *et al.*, 2010). However, no transmission experiments were carried out in either of these countries.

The elm yellows agent may also spread among closely spaced trees of the same species through natural root grafts. This mode of transmission has been considered an important cause of shade tree losses in urban epidemics in North America (Seliskar & Wilson, 1981; Sinclair, 1981). The pathogen has also been transmitted from diseased elm trees to the experimental phytoplasma host *Catharanthus roseus* (periwinkle) via dodder (*Cuscuta epithymum*, *C. ceanothi*) bridges and is efficiently transmitted among periwinkle plants by grafting (Braun & Sinclair, 1979; Mäurer *et al.*, 1993).

Work by Bertelli *et al.* (2002) has shown that ‘*Candidatus Phytoplasma ulmi*’ infections occurred in all the reproductive structures of elm yellows-affected elm trees such as flower buds, whole flowers, anthers, ovaries, unripe and ripe samaras, seeds and membranaceous wings of ripe samaras, as revealed by nested PCR tests. However, it is not known if infected seeds give rise to diseased seedlings.

‘*Candidatus Phytoplasma ulmi*’ apparently spreads in nature only from elm to elm, because plants of other genera growing near elm yellows-infected elm trees, which could serve as pathogen reservoirs, have so far not been found to harbor elm yellows phytoplasma infections.

DETECTION AND IDENTIFICATION

Symptoms

Symptoms of elm yellows vary among the elm (*Ulmus*) species. In those native to North America such as *U. americana* (American or white elm), *U. rubra* (red or slippery elm), *U. alata* (winged elm), *U. serotina* (September elm) and *U. crassifolia* (cedar elm) symptoms include leaf epinasty, leaf curl, chlorosis, premature casting of the leaves, a yellow to brown discoloration of the phloem in the roots and stem, and tree death, that usually occurs within 1 or 2 years from the appearance of foliar symptoms. Red elm, which usually dies in the second year of symptom expression, often shows witches’-brooms that occur over the entire crown and progressively increase in severity giving the tree a starved appearance. Discoloured phloem tissue of American, winged, September and cedar elms have a characteristic odour of wintergreen oil (methyl salicylate), whereas a pleasant scent like that of maple syrup is released by red elm. In *U. minor* (syn.: *U. carpinifolia*, European field elm), the most characteristic symptoms are pronounced witches’-brooms present at the tips of twigs and branches and at the root level. For this reason, the disease of European field elm is often called elm witches’-broom. Other symptoms include leaf epinasty, yellowing, stunting, small leaves, and premature leaf shedding. Brooming and stunting are also the typical symptoms of *U. glabra* (Scots elm) and *U. parvifolia* (Chinese elm) (Braun and Sinclair, 1979; Lee *et al.*, 1993; Murolo & Romanazzi, 2008). Symptoms of leaf yellowing or reddening, reduced terminal growth, witches’-broom formation, dieback and decline have also been observed in several other European and Asian elm species including *U. pumila* (Siberian elm), *U. chenmoui* (Chenmou elm), *U. villosa* (cherry bark elm), *U. laevis* (European

white elm), *U. wallichiana* (Himalayan elm), *U. wilsoniana* (Wilson elm), *U. japonica* (Japanese elm) and *U. x hollandica* (Dutch elm) (Conti *et al.*, 1987; Lee *et al.*, 1995; Mitterpergher, 2000; Jović *et al.*, 2008, 2011, Marcone, 2015). However, phloem discoloration is not known to occur in any of the European or Asian species. There are also reports on the presence of ‘*Candidatus Phytoplasma ulmi*’ in non-symptomatic trees belonging to some European and Asian elm genotypes (Lee *et al.*, 1995; Sinclair *et al.*, 2000; Sfalanga *et al.*, 2002; Katanić *et al.*, 2016; Schneider *et al.*, 2020).

Morphology

Elm yellows was initially thought to be caused by a virus (Swingle, 1938; Baker, 1948, 1949). In 1972, on the basis of transmission electron microscope (TEM) observations, Wilson *et al.* (1972) reported that the disease was associated with mycoplasma-like organisms, now named phytoplasmas, rather than with viruses. The phytoplasma bodies occurred only in phloem sieve tubes of diseased elm trees. They were primarily spherical or oval, but filamentous forms were also present. The phytoplasma bodies were bounded by a unit membrane and were found to possess, in their cytoplasm, dispersed strands resembling DNA and ribosome-like particles. Spherical or oval bodies ranged in diameter from 200 to 1000 nm. Filamentous forms were up to 2200 nm in length and showed a diameter, where constricted, of only 80 nm.

Detection and inspection methods

Elm yellows-affected trees of elm species native to North America can be identified on the basis of visual assessment of characteristic symptoms such as epinasty, foliar yellowing, yellow discoloration and necrosis of root and stem phloem, odour of oil of wintergreen, defoliation and death, whereas the most characteristic symptoms observed in affected trees of most European and Asian elm species are witches’-brooms. The odour of oil of wintergreen, which can be detected by sniffing at the cambial surface of a freshly collected inner bark sample or at the mouth of a small container in which the sample has been enclosed for a few minutes (= wintergreen test), is diagnostic for elm yellows in American, winged, September and cedar elm trees. However, this odour is absent in infected red elm trees. It is not known whether the odour of maple syrup released by elm yellows-diseased red elms also occurs in red elms affected by other pathogens. Masking of specific symptoms by unrelated pathogens or adverse environmental factors may complicate diagnosis of diseased trees. For instance, a North American elm that survives winter after showing elm yellows-induced foliar yellowing typically produces short, thin twigs with small leaves in the following year and dies within the growing season. In spring, these symptoms can be confused with those of Dutch elm disease. In large American elm trees, water shortage can induce early cessation of cambial growth, partial dehydration of inner bark, and foliar symptoms that mimic those of elm yellows (Sinclair, 2000). Therefore, field observations have to be confirmed by other means including microscopic examination and the application of molecular methods.

Phytoplasma infections have been detected microscopically in the phloem sieve tubes of elm yellows-affected elm trees using TEM and DAPI fluorescence methods (for references see Marcone, 2017). However, these methods are limited when the phytoplasma population is very low and unevenly distributed among the plant host organs, as it is often true for North American species, such as *U. americana* and *U. rubra*. In contrast, European and Asian species including *U. minor*, *U. laevis*, *U. parvifolia* and *U. pumila* are relatively high-titre hosts. Moreover, microscopic methods are not appropriate in epidemiological studies to identify plant reservoirs or insect vectors for a given phytoplasma because they do not attain pathogen identification. No immunological detection methods are available for ‘*Candidatus Phytoplasma ulmi*’.

Currently, PCR technology is most widely used for phytoplasma detection. Universal phytoplasma primers as well as group- and pathogen-specific primers have been developed, targeting ribosomal or non-ribosomal DNA sequences. Primers amplifying rDNA sequences are the most extensively used. Sensitivity of detection can be increased by the use of nested PCR which is one of the best means for detecting extremely low-titre phytoplasma infections. Information on primer sequences and primer combinations for detection of ‘*Candidatus Phytoplasma ulmi*’ can be found elsewhere (Lee *et al.*, 1993, 1995, 2004; Marcone *et al.*, 1997; Arnaud *et al.*, 2007; Bertaccini *et al.*, 2019; Martini *et al.*, 2019). Due to the close relationship of ‘*Candidatus Phytoplasma ulmi*’ with other elm yellows group phytoplasmas, specific detection of the elm yellows agent by PCR tests is difficult. However, ‘*Candidatus Phytoplasma ulmi*’ can clearly be distinguished from the other elm yellows group phytoplasmas by RFLP analysis of PCR-amplified 16S rDNA sequences employing *RsaI*, *HpaII* and *BfaI* restriction endonucleases (Lee *et al.*, 2004; Martini *et al.*, 2014). Real-time PCR (qPCR) Taq-Man tests using nonribosomal primers and primers directed to 16S-23S spacer region sequences, respectively, have been developed for specific detection of ‘*Candidatus*

Phytoplasma ulmi' (Herath *et al.*, 2010; Schneider *et al.*, 2020).

PATHWAYS FOR MOVEMENT

'*Candidatus* Phytoplasma ulmi' is spread locally by insect vectors and through natural root grafts among closely spaced elm trees of the same species. The use of infected plant material is responsible for long-distance movement of the pathogen. As is the case for other phytoplasmas, '*Candidatus* Phytoplasma ulmi' is not sap-transmissible and abiotic factors are not involved in natural spread of the pathogen. Although '*Candidatus* Phytoplasma ulmi' DNA has been detected in seeds from elm yellows-infected elm trees, there is no evidence that this phytoplasma is a seed-borne pathogen.

PEST SIGNIFICANCE

Economic impact

Elm yellows is common in eastern North American states where several severe epidemics spread at rates of 5 to 8 km per year in some areas and destroyed a large number of native elm trees (Sinclair, 2000). In some states, elm yellows epidemics occurred together with the Dutch elm disease and exacerbated the latter by providing additional breeding material for elm bark beetles, the insects responsible for the spread of the Dutch elm disease (Lanier *et al.*, 1988; Sinclair, 2000). Amongst diseases of elm, elm yellows is second only to Dutch elm disease in importance and has disrupted several elm conservation and improvement programmes, based on the development of Dutch elm disease-resistant cultivars. Elm yellows continues to pose a threat to susceptible elm populations, including those that have survived Dutch elm disease. In Italy, significant elm yellows epidemics have been observed in some experimental fields established during the 1980s in Northern and Central Italy to test the adaptability of a number of elm species and various hybrid clones to local environmental conditions (Mittempergher, 2000). In one of the experimental fields, 30% of trees were infected five years after the first evidence of the disease, reaching nearly 80% within fourteen years. Elm yellows epidemics observed in Southern and Northern Italy on European field elm and Siberian elm showed a disease incidence greater than 80% (for reviews see Marcone, 2017; Marcone *et al.*, 2021).

Control

Attempts to control elm yellows disease by the application of tetracycline treatments have only been made in the USA. Work by Sinclair's group (Sinclair, 2000) showed that remission of foliar symptoms and resumption of normal growth occurred in potted elm yellows-affected Chinese elms treated with periodic soil drenches of oxytetracycline, but symptoms reappeared after the treatments stopped. Injections of oxytetracycline solutions into American elm saplings shortly before or at the time of graft-inoculation with the elm yellows agent, prevented development of symptoms in most of the treated plants, whereas injections one year after inoculation caused at best only temporary remission of symptoms. Injections of the same antibiotic into naturally infected large American elms, before or after onset of foliar symptoms, seldom resulted in temporary remission and did not prevent the disease (Sinclair, 2000). In large American elm trees, root necrosis is often so extensive when foliar symptoms appear that injections are not effective. At present, the application of tetracycline antibiotics may be appropriate for the treatment of particularly valuable trees, but it is not allowed in Europe.

As is the case for other phytoplasma diseases, the most promising approach to control elm yellows would be through the use of resistant plants. However, selection and breeding of elm trees for resistance to both elm yellows and Dutch elm diseases, which are also fully satisfactory from the silvicultural point of view, is an expensive, long-term project (Griffiths, 2013). Tolerant taxa may be suitable for planting in areas where elm yellows occurs, because natural reservoirs of elm yellows phytoplasma inoculum are already present, whereas Dutch elm disease-resistant cultivars that exhibit severe elm yellows symptoms may be recommended for planting in areas where the disease has not been found (Sinclair, 2000). Several elm genotypes which are resistant to the Dutch elm disease have been examined for elm yellows resistance or tolerance by graft-inoculation experiments. The inoculated trees of these genotypes greatly differed in their response to '*Candidatus* Phytoplasma ulmi' (Sinclair *et al.*, 2000). Diseased trees of 'Frontier', 'Pathfinder' and 'Patriot' showed foliar yellowing and reddening, witches'-brooms, reduced terminal growth and stunting. Since phloem necrosis and death were not observed, these elms may be rated as tolerant. Only 2 out of 20

inoculated ‘Prospector’ trees became infected, one of which died, whereas none of the inoculated ‘Homestead’ trees was infected. The latter trees showed localized phloem necrosis as a defence reaction that prevented spread of the pathogen, suggesting thus resistance of ‘Homestead’ (Sinclair *et al.*, 2000).

The use of certified pathogen-free plants is recommended for establishing new plantations. Other control measures including removal of diseased trees, effective control of the insect vector, and/or pruning of natural root grafts among closely spaced trees, may reduce disease incidence in urban and landscape areas (Marcone, 2017). However, these methods are impractical and difficult in forest ecosystems. Provision of good growing conditions, especially an adequate water supply, may improve the performance of declining and witches’-broom-affected elm trees (Mittempergher, 2000).

Phytosanitary risk

Because elm plants are produced by vegetative propagation, spread of the elm yellows phytoplasma in latently infected, symptomless planting material is a major risk. Therefore, nursery stocks must be tested regularly with highly sensitive and specific PCR tests to ensure that they are free from infections. The propagation material in the nursery must also be protected from natural infection by vector control.

PHYTOSANITARY MEASURES

The incidence of elm yellows disease can be reduced if proper attention is given to the mentioned control measures. Additional guidance can be found in the EPPO Standard on commodity-specific phytosanitary measures for *Ulmus* (EPPO, 2020).

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