Diagnostics[1]
Diagnostic

Candidatus *Phytoplasma pyri*

Specific scope

This standard describes a diagnostic protocol for Candidatus *Phytoplasma pyri* (Pear decline phytoplasma).

Specific approval and amendment

Approved in 2005-09.

Introduction

Candidatus *Phytoplasma pyri* is widespread throughout Europe. The severity of the disease depends on a number of factors including variety, rootstock and the age of the trees. The disease is spread by the pear psyllid *Cacopsylla pyricola* (Davies *et al*., 1992). It is likely that the species *C. pyri* and *C. pyrisuga* can also act as vectors.

Identity

Name: Candidatus *Phytoplasma pyri*.
Synonym: Pear decline phytoplasma.
Taxonomic position: *Bacteria, Firmicutes, Mollicutes, Acholeplasmatales, Acholeplasmataceae. P. pyri* is in the 16SrX phytoplasma group. It was proposed as a '*Candidatus*' by Seemüller & Schneider (2004).
EPPO code: PHYPPY.
Phytosanitary categorization: EPPO A2 list: no. 95, EU Annex designation: I/A2.

Detection

Disease symptoms

The most easily recognized symptoms occur in late summer with the development of premature autumn leaf colour on affected trees. Most cultivars develop a premature red colour (Web Figs 1 and 2), but some may develop a premature yellow colour. There may be some leaf cupping or curling and there is usually premature leaf drop. The following spring, affected trees suffer from weak growth and sparse pale foliage. The severity of the spring symptoms can vary from absence to death. There may be a line of necrotic tissue in the bark at the graft union between scion and rootstock.

The premature autumn leaf colour symptoms associated with pear decline may also have several other causes. Water logging, root damage, ring barking caused by feeding animals, some bacterial cankers, rootstock and variety incompatibility can all give rise to symptoms resembling those caused by phytoplasma infection.

P. pyri is found in mature sieve tubes in the phloem of affected trees but can only be detected in late summer, autumn and early winter (Schaper & Seemüller, 1982). It is not usually present in spring but may be detected in the roots of affected trees at all times of the year if the trees are grafted onto *Pyrus* rootstocks or are growing on their own roots. If the trees are grafted on the more widespread quince rootstocks, detection in the roots is unreliable. The pathogen may also be unevenly distributed through the tree, requiring several different parts of the tree to be examined. It is advisable to examine the bark from 2 to 3-old wood from three different parts of the tree together with one trunk sample (EPPO, 1996).

Identification

DAPI staining

Thin sections of young tissues (petioles of young leaves, or phloem tissues of shoots, branches and roots) are stained with 1 µg/mL DAPI solution (4′6 diamidino-2-phenylindole). Sections are observed under a fluorescence microscope. A bluish fluorescence (at 460 nm) in the sieve tubes indicates the presence of phytoplasmas (Seemüller, 1976). This method, previously the only one available, requires good experience of observing

[1]The figures in this Standard marked 'Web Fig.' are published on the EPPO website www.eppo.org.

slides and is not always sufficiently sensitive. The advantages of this method include rapidity and low cost, but it is not specific.

PCR assay

Molecular techniques which are both sensitive and specific are now available. DNA is extracted using the method of Ahrens & Seemüller (1992). Diagnosis can be performed all year round using different parts of the plant. Best results are obtained if DNA is extracted from leaf midribs or petioles collected from late spring to end of summer (June – end of September). If tests have to be done in winter, roots can be used to extract DNA. At the end of the growing period, *P. pyri* moves from the apical part of the plant to roots where it overwinters.

Different types of universal primers are able to amplify phytoplasmal DNA extracted from phloem. The most frequently used are the ones described by Lorenz *et al*. (1995) and Lee *et al*. (1998). Both are able to amplify a product by PCR from all phytoplasmas including *P. pyri*. The amplification product should then be digested by restriction enzymes *Alu*I and *Rsa*I to ensure that the phytoplasma belongs to group AP, as described by Seemüller *et al*. (1998); or to group X, according to the Lee classification of Lee *et al*. (1998).

Specific primers are also available and the obtained amplification product can be cleaved by restriction enzymes *Ssp*I and *Sfe*I for a suitable differentiation of AP and PD strains (Lorenz *et al*., 1995). More details on molecular tests are given in EPPO Standard PM 7/62(1).

The presence of symptoms does not give sufficient evidence for identification. Laboratory testing by PCR is essential. This may be followed by RFLP or PCR using a different set of primers if confirmation is required. A test on an indicator plant (EPPO Standard PM 4/27(1)) may be performed if necessary (e.g. in case of dispute).

Reporting and documentation

Guidelines on reporting and documentation are given in EPPO Standard PM7/– (in preparation).

Further information

Further information on this organism can be obtained from:

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This protocol was originally drafted for EPPO by D.L. Davies, Horticulture Research International, East Malling (UK).

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