Organisation Européenne et Méditerranéenne pour la Protection des Plantes
European and Mediterranean Plant Protection Organization

# Normes OEPP
# EPPO Standards

## Diagnostics
## Diagnostic

PM 7/43

Organisation Européenne et Méditerranéenne pour la Protection des Plantes
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## Approval

EPPO Standards are approved by EPPO Council. The date of approval appears in each individual standard. In the terms of Article II of the IPPC, EPPO Standards are Regional Standards for the members of EPPO.

## Review

EPPO Standards are subject to periodic review and amendment. The next review date for this EPPO Standard is decided by the EPPO Working Party on Phytosanitary Regulations.

## Amendment record

Amendments will be issued as necessary, numbered and dated. The dates of amendment appear in each individual standard (as appropriate).

## Distribution

EPPO Standards are distributed by the EPPO Secretariat to all EPPO member governments. Copies are available to any interested person under particular conditions upon request to the EPPO Secretariat.

## Scope

EPPO Standards on Diagnostics are intended to be used by NPPOs in their capacity as bodies responsible for the application of phytosanitary measures. Standards on diagnostic protocols are concerned with the diagnosis of individual pests and describe different methods which can be used to detect and identify pests of phytosanitary concern for the EPPO region. General Standards on diagnostics are in preparation on: (1) the purpose of diagnostic protocols (which may differ according to the circumstances of their use); and (2) reporting and documentation of diagnoses.

In 1998, EPPO started a new programme to prepare diagnostic protocols for the regulated pests of the EPPO region (including the EU). The work is conducted by the EPPO Panel on Diagnostics and other specialist Panels. The objective of the programme is to develop an internationally agreed diagnostic protocol for each regulated pest. The protocols are based on the many years of experience of EPPO experts. The first drafts are prepared by an assigned expert author(s). They are written according to a 'common format and content of a diagnostic protocol' agreed by the Panel on Diagnostics, modified as necessary to fit individual pests. As a general rule, the protocol recommends a particular means of detection or identification which is considered to have advantages (of reliability, ease of use etc.) over other methods. Other methods may also be mentioned, giving their advantages/disadvantages. If a method not mentioned in the protocol is used, it should be justified.

The following general provisions apply to all EPPO Standards on Diagnostics:

- laboratory tests may involve the use of chemicals or apparatus which present a certain hazard. In all cases, local safety procedures should be strictly followed
- use of names of chemicals or equipment in these EPPO Standards implies no approval of them to the exclusion of others that may also be suitable
- laboratory procedures presented in the protocols may be adjusted to the standards of individual laboratories, provided that they are adequately validated or that proper positive and negative controls are included.

## References

EPPO/CABI (1996) *Quarantine Pests for Europe*, 2nd edn. CAB International, Wallingford (GB).

EU (2000) Council Directive 2000/29/EC of 8 May 2000 on protective measures against the introduction into the Community of organisms harmful to plants or plant products and against their spread within the Community. *Official Journal of the European Communities* L169, 1–112.

FAO (1997) *International Plant Protection Convention* (new revised text). FAO, Rome (IT).

IPPC (1993) *Principles of plant quarantine as related to international trade*. ISPM no. 1. IPPC Secretariat, FAO, Rome (IT).

IPPC (2002) *Glossary of phytosanitary terms*. ISPM no. 5. IPPC Secretariat, FAO, Rome (IT).

OEPP/EPPO (2003) EPPO Standards PM 1/2(12): EPPO A1 and A2 lists of quarantine pests. *EPPO Standards PM1 General phytosanitary measures*, 5–17. OEPP/EPPO, Paris (FR).

## Definitions

*Regulated pest*: a quarantine pest or regulated non-quarantine pest.
*Quarantine pest*: a pest of potential economic importance to the area endangered thereby and not yet present there, or present but not widely distributed and being officially controlled.

## Outline of requirements

EPPO Standards on Diagnostics provide all the information necessary for a named pest to be detected and positively identified by an expert (i.e. a specialist in entomologist, mycology, virology, bacteriology, etc.). Each protocol begins with some short general information on the pest (its appearance, relationship with other organisms, host range, effects on host, geographical distribution and its identity) and then gives details on the detection, identification, comparison with similar species, requirements for a positive diagnosis, list of institutes or individuals where further information on that organism can be obtained, references (on the diagnosis, detection/extraction method, test methods).

## Existing EPPO Standards in this series

Forty-one EPPO standards on diagnostic protocols have already been approved and published. Each standard is

numbered in the style PM 7/4 (1), meaning an EPPO Standard on Phytosanitary Measures (PM), in series no. 7 (Diagnostic Protocols), in this case standard no. 4, first version. The existing standards are:

PM 7/1 (1) *Ceratocystis fagacearum*. *Bulletin OEPP/EPPO Bulletin* **31**, 41–44

PM 7/2 (1) *Tobacco ringspot nepovirus*. *Bulletin OEPP/EPPO Bulletin* **31**, 45–51

PM 7/3 (1) *Thrips palmi*. *Bulletin OEPP/EPPO Bulletin* **31**, 53–60

PM 7/4 (1) *Bursaphelenchus xylophilus*. *Bulletin OEPP/EPPO Bulletin* **31**, 61–69

PM 7/5 (1) *Nacobbus aberrans*. *Bulletin OEPP/EPPO Bulletin* **31**, 71–77

PM 7/6 (1) *Chrysanthemum stunt pospiviroid*. *Bulletin OEPP/EPPO Bulletin* **32**, 245–253

PM 7/7 (1) *Aleurocanthus spiniferus*. *Bulletin OEPP/EPPO Bulletin* **32**, 255–259

PM 7/8 (1) *Aleurocanthus woglumi*. *Bulletin OEPP/EPPO Bulletin* **32**, 261–265

PM 7/9 (1) *Cacoecimorpha pronubana*. *Bulletin OEPP/EPPO Bulletin* **32**, 267–275

PM 7/10 (1) *Cacyreus marshalli*. *Bulletin OEPP/EPPO Bulletin* **32**, 277–279

PM 7/11 (1) *Frankliniella occidentalis*. *Bulletin OEPP/EPPO Bulletin* **32**, 281–292

PM 7/12 (1) *Parasaissetia nigra*. *Bulletin OEPP/EPPO Bulletin* **32**, 293–298

PM 7/13 (1) *Trogoderma granarium*. *Bulletin OEPP/EPPO Bulletin* **32**, 299–310

PM 7/14 (1) *Ceratocystis fimbriata* f. sp. *platani*. *Bulletin OEPP/EPPO Bulletin* **33**, 249–256

PM 7/15 (1) *Ciborinia camelliae*. *Bulletin OEPP/EPPO Bulletin* **33**, 257–264

PM 7/16 (1) *Fusarium oxysporum* f. sp. *albedinis*. *Bulletin OEPP/EPPO Bulletin* **33**, 265–270

PM 7/17 (1) *Guignardia citricarpa*. *Bulletin OEPP/EPPO Bulletin* **33**, 271–280

PM 7/18 (1) *Monilinia fructicola*. *Bulletin OEPP/EPPO Bulletin* **33**, 281–288

PM 7/19 (1) *Helicoverpa armigera*. *Bulletin OEPP/EPPO Bulletin* **33**, 289–296

PM 7/20 (1) *Erwinia amylovora*. *Bulletin OEPP/EPPO Bulletin* **34**, 159–172

PM 7/21 (1) *Ralstonia solanacearum*. *Bulletin OEPP/EPPO Bulletin* **34**, 173–178

PM 7/22 (1) *Xanthomonas arboricola* pv. *corylina*. *Bulletin OEPP/EPPO Bulletin* **34**, 179–182

PM 7/23 (1) *Xanthomonas axonopodis* pv. *dieffenbachiae*. *Bulletin OEPP/EPPO Bulletin* **34**, 183–186

PM 7/24 (1) *Xylella fastidiosa*. *Bulletin OEPP/EPPO Bulletin* **34**, 187–192

PM 7/25 (1) *Glomerella acutata*. *Bulletin OEPP/EPPO Bulletin* **34**, 193–200

PM 7/26 (1) *Phytophthora cinnamomi*. *Bulletin OEPP/EPPO Bulletin* **34**, 201–208

PM 7/27 (1) *Puccinia horiana*. *Bulletin OEPP/EPPO Bulletin* **34**, 209–212

PM 7/28 (1) *Synchytrium endobioticum*. *Bulletin OEPP/EPPO Bulletin* **34**, 213–218

PM 7/29 (1) *Tilletia indica*. *Bulletin OEPP/EPPO Bulletin* **34**, 219–228

PM 7/30 (1) *Beet necrotic yellow vein benyvirus*. *Bulletin OEPP/EPPO Bulletin* **34**, 229–238

PM 7/31 (1) *Citrus tristeza closterovirus*. *Bulletin OEPP/EPPO Bulletin* **34**, 239–246

PM 7/32 (1) *Plum pox potyvirus*. *Bulletin OEPP/EPPO Bulletin* **34**, 247–256

PM 7/33 (1) *Potato spindle tuber pospiviroid*. *Bulletin OEPP/EPPO Bulletin* **34**, 257–270

PM 7/34 (1) *Tomato spotted wilt tospovirus*. *Bulletin OEPP/EPPO Bulletin* **34**, 271–280

PM 7/35 (1) *Bemisia tabaci*. *Bulletin OEPP/EPPO Bulletin* **34**, 281–288

PM 7/36 (1) *Diabrotica virgifera*. *Bulletin OEPP/EPPO Bulletin* **34**, 289–294

PM 7/37 (1) *Thaumetopoea pityocampa*. *Bulletin OEPP/EPPO Bulletin* **34**, 295–298

PM 7/38 (1) *Unaspis citri*. *Bulletin OEPP/EPPO Bulletin* **34**, 299–302

PM 7/39 (1) *Aphelenchoides besseyi*. *Bulletin OEPP/EPPO Bulletin* **34**, 303–308

PM 7/40 (1) *Globodera rostochiensis* and *Globodera pallida*. *Bulletin OEPP/EPPO Bulletin* **34**, 309–314

PM 7/41 (1) *Meloidogyne chitwoodi* and *Meloidogyne fallax*. *Bulletin OEPP/EPPO Bulletin* **34**, 315–320

Some of the Standards of the present set result from a different drafting and consultation procedure. They are the output of the DIAGPRO Project of the Commission of the European Union (no. SMT 4-CT98-2252). This project involved four 'contractor' diagnostic laboratories (in England, Netherlands, Scotland, Spain) and 50 'inter-comparison' laboratories in many European countries (within and outside the European Union), which were involved in ring-testing the draft protocols. The DIAGPRO project was set up in full knowledge of the parallel activity of the EPPO Working Party on Phytosanitary Regulations in drafting diagnostic protocols, and covered regulated pests which were for that reason not included in the EPPO programme. The DIAGPRO protocols have been approved by the Council of EPPO as EPPO Standards in series PM 7. They will in future be subject to review by EPPO procedures, on the same terms as other members of the series.

**Diagnostics**[1]
**Diagnostic**

# *Pseudomonas syringae* pv. *persicae*

## Specific scope

This standard describes a diagnostic protocol for *Pseudomonas syringae* pv. *persicae*.

## Specific approval and amendment

Approved in 2004-09.

## Introduction

Bacterial dieback of peach caused by *Pseudomonas syringae* pv. *persicae* (EPPO/CABI, 1997; OEPP/EPPO, 1992) was described for the first time in 1967 on nectarine and peach in France and almost simultaneously on nectarine, peach and Japanese plum (*Prunus salicina*) in New Zealand (Young, 1988). The same pathogen was also isolated once in the UK in 1966, from *Prunus cerasifera*. The disease occurs mainly on the above-ground parts of trees and affects shoots, branches, leaves and fruits. Not all affected stone fruit species display the recognized symptoms.

## Identity

**Name:** *Pseudomonas syringae* pv. *persicae* (Prunier, Luisetti & Gardan, 1970; Young, Dye & Wilkie, 1978)
**Synonyms:** *Pseudomonas mors-prunorum* subsp. *persicae* (Prunier *et al*., 1970)
**Taxonomic position:** *Proteobacteria*, Gamma subdivision, order *Pseudomonadales*, family *Pseudomonadaceae*, genus *Pseudomonas* (Garrity, 2005)
**EPPO computer code:** PSDMPE
**Phytosanitary categorization:** EPPO A2 list no. 145, EU Annex designation II/A2

## Detection

### Disease symptoms

In nectarine and peach, symptoms include shoot dieback, limb and root injury, tree death, leaf spots and fruit lesions. On Japanese plum symptoms are mainly confined to dieback, occasional limb death, and leaf spots (Young, 1995). Dieback of terminal shoots can occur already in autumn and in spring following the development of girdling lesions from nodal infections. Small elliptical lesions may develop at internodes. The rootstock can also be infected showing symptoms similar to those on woody shoots. Leaf infection results in small, angular, water-soaked spots, the tissue of which becomes brown. The necrotic tissue subsequently falls out, causing a 'shot hole' effect. On fruits, small, round, dark, oily spots occur. These can be spread within the fruit tissue, causing sunken, deforming lesions that ooze gum.

Some symptoms of bacterial dieback due to *P. s. persicae* can be confused with those of bacterial canker of stone fruits (*Pseudomonas syringae* pv. *syringae*, *Pseudomonas syringae* pv. *mors-prunorum*) and symptoms of leucostoma canker (*Leucostoma* spp.) or frost injury. Distinctive characteristics of dieback are discoloration of wood in branches above the necrosis and the absence of an obvious boundary between the morbid and healthy bark in the lower parts of the tree. Bacterial dieback can be disseminate with infected plants for planting or contaminated pruning tools.

### Isolation

Bacteria can be isolated directly from diseased tissue by cutting out the tissue from the border between apparently healthy tissue and the necrotic area. Prior to removing, the tissue should be disinfected with ethanol. Small pieces of such tissue are crushed with small amount of sterile water. Small pieces of tissue can also be shaken for a few minutes in a tube of phosphate buffer or sterile water.

After a few minutes, the suspension is streaked onto King's B medium containing: 15.0 g Difco bacto agar, 20.0 g Difco proteose peptone no. 3; 1.5 g $K_2HPO_4$; 1.5 g $MgSO_4$, 10 mL glycerol; 1000 mL $H_2O$, pH 7.2. The colony morphology of

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

 

bacteria is observed after 3–4 days of incubation at 24°C. *P. s. persicae* colonies are irregular, small (2–3 mm in diameter), grey, flat, translucent. Comparison with the reference strain on the same medium is recommended.

### Description of the pathogen

Gram-negative short rod, motile by 3–6 polar flagella, obligatorily aerobic with an optimum growth temperature of about 24°C.

## Identification

### Biochemical tests

*P. s. persicae* belongs, like *P. s. syringae* and *P. s. mors-prunorum*, to LOPAT Group Ia of the determinative scheme of Lelliott *et al*. (1966). It can be distinguished from the other two pathovars attacking stone fruits on the basis of REP profiles. It grows significantly more slowly on King's B medium than the other two pathovars. Some strains from *P. cerasifera* rootstocks in the UK have genetic fingerprints (REP-PCR) that are identical to those of *P. s. persicae*. Host tests on peach have not been performed and the significance of this finding is not yet known. Detailed differences between the three pathovars of *P. syringae* occurring on stone fruits are shown in Table 1.

**Table 1** Biochemical characters of *Pseudomonas syringae* pv. *persicae* in comparison with pathovars *syringae* and *mors-prunorum*

| Test[1] | pv. *syringae* | pv. *mors-prunorum* | pv. *persicae* |
|---|---|---|---|
| Fluorescence on King's B medium | + | + or – | – |
| Fluorescence on CSGM[2] | + | + | + |
| Levan production | + | + | + |
| Gelatine hydrolysis | + | + | – |
| Aesculin hydrolysis | + | | – |
| Acid production from: | | | |
| Inositol | + | + | – |
| Sorbitol | + | + | + |
| Erytritol | + or – | + or – | – |
| Utilisation of: | | | |
| DL lactate | + or – | – | – |
| D(–) tartrate | + or – | – | – |
| L(+) tartrate | – | + | – |

[1]Fluorescence – appearance of green or blue pigment which diffuses into medium visible under UV-light; levan production – occurrence of mucoid colonies on sucrose-rich medium; gelatin hydrolysis – liquefaction of solid medium; aesculin hydrolysis – dark brown discoloration of the medium; remaining tests – yellow discoloration of medium. For preparation of media and performance of tests (see Lelliott & Stead , 1987; Fahy & Persley 1983; Schaad, 1988).

[2]Casamino-sucrose-gelatin medium (Lelliott & Stead, 1987).

### Pathogenicity tests

*Preparation of inoculum*

For preparation of inoculum, 24–48 h-old cultures of tested isolate grown on King's B medium are used. Bacteria are rinsed from the medium surface with sterile water. Cell number in the bacterial suspension can be determined turbidimetrically or by the plate-count method (serial dilution in water and plating on King's B medium). The suspension should be adjusted to about $10^7$ c.f.u. $mL^{-1}$.

*Hypersensitivity test on tobacco*

The bacterial suspension is injected into the intercellular spaces (between veins) of 2 tobacco leaves (cv. 'White Burley' or 'Hicks') with a hypodermic needle. Fully developed leaves can be more easily injected than younger ones. After injection, the intercellular spaces become water-soaked for a short time but within about 1 h the leaf regains its original state. If the suspension contains *P. s. persicae* the injected tissue becomes necrotic in 24 h or less. The reference strain should be used as a control.

*Pathogenicity on shoots*

The pathogenicity of the bacterial isolate can be determined by inoculation of young dormant (one-year-old) shoots of young trees of susceptible cultivars of peach, nectarine or plum, growing under standard conditions in a non-heated glasshouse (during the period from mid September to the end of January). Shoots can be inoculated by introduction of the drop of bacterial suspension (about $10^7$ c.f.u. $mL^{-1}$) onto a wound made to the xylem by a single transverse incision with a scalpel) or on a fresh leaf scar. The inoculated wound or leaf scar should be wrapped with plastic tape for 5 days. Necrosis should be observed and measured in the following spring, by comparison with the controls (treatment of wound or leaf scar with sterile water, or with the reference strain) should be made in the following spring. For each isolate, at least 5 inoculations should be done.

## Reference strain

Type strain LMG 5184 (CFBP 1573; = ICMP 5846; = NCPPB 2761).

## Reporting and documentation

Guidance on reporting and documentation is given in EPPO Standard PM7/– (in preparation).

## Further information

Further information on this organism can be obtained from:

Dr J.M. Young, New Zealand, Landcare Research, Private Bag 92170, Auckland (New Zealand); E-mail: youngj@landcare.cri.nz.

## Acknowledgements

This protocol was originally drafted by Dr P. Sobiczewski, Research Institute of Pomology and Floriculture, Skierniewice (PL) and Dr L. Gardan, INRA, Beaucouzé (FR).

## References

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Garrity GM (ed.) (2005) *Bergey's Manual of Systematic Bacteriology*, 2nd edn. Springer, Berlin (DE).

Lelliott RA, Billing E & Hayward AC (1966) A determinative scheme for the fluorescent plant pathogenic pseudomonads. *Journal of Applied Bacteriology* **29**, 470–489.

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