

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

# **Normes OEPP EPPO Standards**

Diagnostic protocols for regulated pests  
Protocoles de diagnostic pour les  
organismes réglementés

PM 7/22



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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 Diagnostic Protocols for Regulated Pests are intended to be used by National Plant Protection Organizations, in their capacity as bodies responsible for the application of phytosanitary measures to detect and identify the regulated pests of the EPPO and/or European Union lists.

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 diagnostic protocols:

- 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.

## 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 Diagnostic Protocols for Regulated Pests 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

Nineteen 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

Several 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 ‘intercomparison’ 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 PM7. They will in future be subject to review by EPPO procedures, on the same terms as other members of the series.

**Diagnostic protocols for regulated pests<sup>1</sup>**  
**Protocoles de diagnostic pour les organismes réglementés**

## ***Xanthomonas arboricola* pv. *corylina***

### **Specific scope**

This standard describes a diagnostic protocol for *Xanthomonas arboricola* pv. *corylina*

### **Specific approval and amendment**

Approved in 2003-09.

### **Introduction**

Bacterial blight of hazelnut was described for the first time in the USA (Oregon) in 1913 on *Corylus maxima*. Subsequently, the same disease has been reported also on *C. avellana*, the most important hazelnut-producing species, in the following European countries: Italy, France, Netherlands, Russia (southern), Serbia and Montenegro, Switzerland, Turkey and United Kingdom (EPPO/CABI, 1997). Outside Europe, it has been recorded on *C. avellana* in Algeria, USA (Oregon, Washington), Canada (British Columbia), Chile, Australia (Victoria, Western Australia). It can also cause damage to *C. pontica* and *C. colurna*. The bacterium has a narrow host range, infecting only *Corylus* spp. Greatest losses on *C. avellana* cultivars are seen in 1–4 year-old orchards, where up to 10% of mortality has been recorded.

### **Identity**

**Name:** *Xanthomonas arboricola* pv. *corylina* (Miller *et al.*) Vauterin *et al.*

**Synonym:** *Xanthomonas campestris* pv. *corylina* (Miller *et al.*) Dye

**Taxonomic position:** Bacteria, Gracilicutes, Proteobacteria

**Bayer computer code:** XANTCY

**Phytosanitary categorization:** EPPO A2 list no. 134

### **Detection**

There are no diagnostic techniques (i.e. ELISA, IFAS, PCR) specifically developed for routine detection of *X. a. corylina*. No standardized antisera have been produced, nor have any selective or semiselective bacterial culture media been developed that might help the isolation procedure. Consequently,

rapid detection of the pathogen is not possible and diagnostic procedures have still to rely on the observation of disease symptoms, microscopical examination of the symptomatic tissues, isolation from the plant using common media for xanthomonads, pathogenicity and confirmation tests.

### **Disease symptoms**

Symptoms can be observed both in the nursery and in the field (Web Fig. 1). In the nursery, bud dieback and necrosis of the shoot tips can be noticed in spring on twigs over one-year old. Later, the shoots may wither completely. If the pathogen does not girdle the twig, it can cause cankers 10–25 cm long. The leaves show oily polygonal lesions which may subsequently coalesce. In the field, dieback of buds and new lateral shoots, and cankers along the twigs, are frequently observed in spring and summer. The fruits show typical ‘black heel’ symptom and browning. The involucre of the shell frequently shows oily lesions. Hazelnut organs that show symptoms of bacterial blight are: leaf (tiny angular necrotic lesions), shell (round or elongated black necrotic lesions), involucre of the shell (oily or necrotic round spots 2–4 mm in diameter), lateral twigs (partial or total dieback), twig (partial or total dieback, longitudinal canker mainly developing from a bud), branch (longitudinal canker), sucker (longitudinal canker).

### **Microscopic examination**

Small pieces of tissues (1–2 mm) showing symptoms of bacterial blight (i.e. oily or necrotic spots on leaves or on the involucre of the shell) are cut and put in a drop of sterile physiological saline (SPS; 0.85% NaCl in distilled water) on a microscope slide, covered with a cover-slip and examined with a phase-contrast microscope. Observation of abundant bacterial cells diffusing from the plant tissue indicates presumptive bacterial blight infection.

<sup>1</sup>The Figures in this Standard marked ‘Web Fig.’ are published on the EPPO website [www.eppo.org](http://www.eppo.org).

## Isolation

Isolation of the pathogen from symptomatic leaves is often difficult. Pieces of tissue (1–2 mm × 2–4 mm) taken from the margin of the lesion are crushed in a sterile mortar containing 3 mL of SPS. After 15 min, an aliquot of 100 µL is spread onto GYCA medium (glucose 10.0 g; yeast extract 5.0 g; calcium carbonate 30.0 g; agar 20.0 g; distilled water to 1.0 L) or onto YPGA medium (yeast extract 5.0 g; bacto peptone 5.0 g; glucose 10.0 g; agar 20.0 g, distilled water to 1.0 L; pH 6.5–7.0), to be preferred to YDC for the primary isolation. The plates are incubated at 25–27 °C for 3–4 days. Mucoid, yellow-pigmented colonies 2–3 mm in diameter with round margin are selected for the pathogenicity and confirmation tests.

For propagative material not showing symptoms, no methods have been standardized. The buds that harbour the pathogen during winter as well as during the growing season (Gardan & Devaux, 1987) are presumably the best candidate organs for checking the presence of the bacterium. The same isolation procedures and the bacterial culture media can be used as above, with 1–3 buds per mortar.

## Identification

Table 1 shows the biochemical characteristics of *X. a. corylina* which are helpful in the identification of isolates. For growth on SQ medium (Lee *et al.*, 1992), isolates are streaked onto SQ medium (succinic acid disodium salt 10.0 g; quinic acid 5.0 g; K<sub>2</sub>HPO<sub>4</sub> 1.5 g; (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> 1.0 g; agar 15.0 g; distilled water to 1 L; pH 7.2–7.5). After autoclaving, 7.5 mL of autoclaved 20% MgSO<sub>4</sub>·7H<sub>2</sub>O solution is added. The plates are incubated for 4–

**Table 1** Biochemical characteristics of *Xanthomonas arboricola* pv. *corylina*

Characteristic	Reaction	Characteristic	Reaction
Utilization of:		Presence of oxidase	–
– L-arabinose	+	Esculin hydrolysis	+
– D-arabinose	+	Starch hydrolysis	+
– glucose	+	Growth at 35 °C	+
– galactose	+	Tobacco hypersensitivity	+(after 48 h)
– mannose	+	Growth on SQ medium	+
– sucrose	+		
– maltose	+		
– trehalose	+		
– cellobiose	+		
– glycerol	+		
– L-xylose	–		
– D-xylose	–		
– rhamnose	–		
– lactose	–		
– raffinose	–		
– adonitol	–		
– mannitol	–		
– inuline	–		
– sorbitol	–		
– dulcitol	–		
– erythritol	–		

6 days at 28 °C and the diffusion of a deep green colour around the bacterial streak is considered as a positive reaction.

## Pathogenicity test

Inoculation of buds, from October to June, is the most suitable method for confirming the pathogenicity of isolates suspected to be *X. a. corylina* (Gardan & Devaux, 1987). Bacteria grown for 48 h on GYCA medium are suspended in SPS to an optical density corresponding to 1 × 10<sup>8</sup> cfu mL<sup>-1</sup>. The buds are pricked with a sterile needle, and 10 gL of the bacterial suspension is placed on the wound. Symptom development can vary according to the month of inoculation. However, appearance of a necrotic lesion should be expected from 14 days to one month after inoculation. A positive control (i.e. pathogenic strain) should be included in the test. Inoculation through wounds along the twig is less successful.

## Reference material

The *X. a. corylina* type-strain NCPPB 935, isolated in Oregon (US) from *C. maxima*, proved weakly pathogenic to *C. avellana* and deviated phenotypically and genotypically from other *X. a. corylina* strains obtained from *C. avellana* (Scortichini *et al.*, 2002). For comparison purposes, it is recommended to use NCPPB 2896, isolated from *C. avellana* and showing the typical characteristics of the pathovar.

## Possible confusion with similar species

*X. a. corylina* is genetically similar to but pathogenically distinct from the other *X. arboricola* pathovars: *celebensis*, *fragariae*, *juglandis*, *poinsetticola* type *C*, *populi* and *pruni*. Comparison with such pathovars for detection purposes are merely indicative.

## Requirements for a positive diagnosis

The procedures for detection and identification described in this protocol should have been followed. The presence of *X. a. corylina* is suspected when the colony morphology on the bacterial culture media and the confirmatory tests are those typical of the pathovar. Final confirmation requires a pathogenicity test on *C. avellana* cultivars by artificial inoculation.

## Report on the diagnosis

A report on the execution of the protocol should include:

- results obtained by the recommended procedures
- information and documentation on the origin of the infected material
- a description of the disease symptoms, if any are evident on the sample
- a table with the tests performed and the results obtained in comparison with those of the reference strain
- comments as appropriate on the certainty or uncertainty of the identification.

## Further information

Further information on this organism can be obtained from: M. Scortichini, Istituto Sperimentale per la Frutticoltura, Via di Fioranello, 52.1-00040 Ciampino Aeroporto (Roma), Italy. Tel. +39 0679348147; Fax +39 0679340158; E-mail: mscortichini@hotmail.com

## Acknowledgements

This protocol was originally drafted by: M. Scortichini, Istituto Sperimentale per la Frutticoltura, Ciampino Aeroporto (Roma) (IT).

## References

- EPPO/CABI (1997) *Quarantine Pests for Europe*, 2nd edn. CAB International, Wallingford (GB).
- Gardan L & Devaux M (1987) La bactériose du noisetier (*Xanthomonas campestris* pv. *corylina*): biologie de la bactérie. *Bulletin OEPP/EPPO Bulletin* **17**, 241–250.
- Lee YA, Hildebrand DC & Schroth MN (1992) Use of quinate metabolism as a phenotypic property to identify members of *Xanthomonas campestris* DNA homology group 6. *Phytopathology* **82**, 971–973.
- Scortichini M, Rossi MP & Marchesi U (2002) Genetic, phenotypic and pathogenic diversity of *Xanthomonas arboricola* pv. *corylina* strains, question the representative nature of the type strain. *Plant Pathology* **51**, 374–381.

**Fig. 1.** Typical spots induced by *Xanthomonas arboricola* pv. *corylina* on the husk of hazelnuts.

