

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 7/21



Organization 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 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).
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- 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 pests¹
Protocoles de diagnostic pour les organismes réglementés

Ralstonia solanacearum

Specific scope

This standard describes a diagnostic protocol for *Ralstonia solanacearum*.

Introduction

Bacterial wilt caused by *Ralstonia solanacearum* was reported for the first time at the end of the 19th century on potato, tobacco, tomato and groundnut in Asia, southern USA and South America. The bacterium was described for the first time as *Bacillus solanacearum* by Smith (1896). In the years following, at least five pathogenic races and five biovars have been discriminated (Buddenhagen *et al.*, 1962). Race 1 occurs in tropical areas all over the world and attacks tobacco, many other solanaceous crops and many hosts in other plant families. It has a high temperature optimum (35 °C, as do race 2, 4 and 5). Race 2 occurs mainly in tropical areas of South America and attacks bananas and *Heliconia* (causing so-called Moko disease), but also in the Philippines (causing so-called bugtok disease on plantains). Race 3, occurring at higher altitudes in the tropics and in subtropical and temperate areas attacks potato, tomato, occasionally *Pelargonium zonale*, aubergine and capsicum, some solanaceous weeds like *Solanum nigrum* and *Solanum dulcamara*. A number of non-solanaceous weed hosts have also been found to harbour race 3 infections, often asymptotically (Pradhanang *et al.*, 2000; Strider *et al.*, 1981; Wenneker *et al.*, 1999; Janse *et al.*, 2004). This race has a lower temperature optimum (27 °C) and appears to be mostly biovar 2 A RFLP group 26 with a worldwide distribution (Cook & Sequeira, 1994), biovar 2 A RFLP group 27 (found in Chile and Colombia), or biovar 2T (sometimes also called 2 N, found in tropical areas in South America). Race 4 is particularly aggressive on ginger, race 5 (biovar 5) is specialized on *Morus*. Another recent classification of *R. solanacearum*, based on RFLP and other genetic fingerprinting studies (Hayward, 2000), is into Division

Specific approval and amendment

Approved in 2003-09.

I (biovars 3, 4 and 5 originating in Asia) and II (biovars 1, 2 A and 2T, originating in South America).

Within the EPPO region, the race which is now present and has potential for spread is race 3. This is the main race described in this protocol. Race 1 may be introduced with ornamental/herbal plants or plant parts of tropical origin and grown in glass-houses in temperate climates, like *Curcuma longa* (turmeric), *Anthurium* or *Epipremnum*. Worldwide, the most important hosts are: *Arachis hypogaea* (groundnut), *Heliconia* spp., *Lycopersicon esculentum* (tomato), *Musa paradisiaca* (banana and plantain), *Nicotiana tabacum* (tobacco), *Solanum melongena* (aubergine) and *Solanum tuberosum* (potato). For extensive host range, see Bradbury (1986). For host range of race 3, see Janse *et al.* (2004). For geographical distribution, see CABI (1999).

Identity

Name: *Ralstonia solanacearum* (Smith, 1896) Yabuuchi *et al.* (1995)

Synonyms: *Pseudomonas solanacearum* (Smith, 1896) Smith 1914; *Burkholderia solanacearum* (Smith, 1896) Yabuuchi *et al.* 1992; many other synonyms in Literature

Taxonomic position: Bacteria, Gracilicutes, *Proteobacteria* β subdivision. It belongs to rRNA homology group II (non-fluorescent) within the pseudomonads. *R. pickettii* (saprophyte or human facultative pathogen), *Pseudomonas syzygii* (causing Sumatra disease of cloves), and the so-called Blood Disease Bacterium (BDB, causing blood disease of banana in Indonesia) are closely related to *R. solanacearum* and may cross-react in serological and DNA-based detection methods. Subclassification of *R. solanacearum*, based on RFLP and other genetic fingerprinting studies (Hayward, 2000) is into Division I (biovars 3, 4 and 5 originating in Asia) and II (biovars 1, 2A and 2T, originating in S. America). Further taxonomic division mainly based on nucleic sequence analysis into phyllovars

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

and sequevars has been proposed (Poussier *et al.*, 2000; Taghavi *et al.*, 1996)

Bayer computer code: PSDMSO (PSDMS3 for race 3)

Phytosanitary categorization: EPPO A2 list no. 58, EU Annex designation I/A2

Detection

Disease symptoms

Potato

Foliage symptoms include rapid wilting of leaves and stems (Web Fig. 3), usually first visible at the warmest time of day. Eventually, plants fail to recover, become yellow and brown necrotic and die. As the disease develops, a streaky brown discoloration of the stem may be observed on stems above the soil line, and the leaves may have a bronze tint. Epinasty of the petioles may occur. A white, slimy mass of bacteria exudes from vascular bundles, when broken or cut. This slime oozes spontaneously from the cut surface of a potato stem in the form of threads, when suspended in water. Such threads are not formed by other bacterial pathogens of potato. This test is of presumptive diagnostic value in the field. Under cool growing conditions, wilting and other foliar symptoms may not occur.

On tubers, external symptoms may or may not be visible, depending on the state of development of the disease. Symptoms may be confused with those of ring rot due to *Clavibacter michiganensis* subsp. *sepedonicus* (EPPO/CABI, 1997). *R. solanacearum* can be distinguished by the bacterial ooze that often emerges from the eyes and stolon-end attachment of infected tubers. Soil may adhere to the tubers at the eyes (Web Fig. 4). Cutting the diseased tuber will reveal a browning and eventual necrosis of the vascular ring and immediately surrounding tissues. A creamy fluid exudate usually appears spontaneously on the vascular ring of the cut surface a few minutes after cutting (Web Fig. 5). In the case of ring rot, the tuber has to be squeezed in order to press out a mass of yellowish macerated vascular tissue and bacterial slime. Atypical symptoms have been described on potato (necrotic spots on the epidermis), possibly caused after lenticel infection. Plants with foliar symptoms caused by *R. solanacearum* may bear healthy and diseased tubers, while plants that show no signs of the disease may sometimes produce diseased tubers.

Tomato

The youngest leaves are the first to be affected and have a flaccid appearance, usually at the warmest time of day. Wilting of the whole plant may follow rapidly if environmental conditions are favourable for the pathogen. Under less favourable conditions, the disease develops less rapidly, stunting may occur and large numbers of adventitious roots are produced on the stem. The vascular tissues of the stem show a brown discoloration and, if the stem is cut crosswise, drops of white or yellowish bacterial ooze may be visible.

Pelargonium

First symptoms are wilting and subsequent chlorosis (often sectorial yellowing) of leaves. Stems may blacken and

eventually become necrotic. Internal vascular browning is often visible. In a later stage, leaves become brown necrotic and the whole plant desiccates and dies. In final stages, plants collapse totally.

Tobacco

One of the main symptoms is unilateral wilting and premature yellowing. Leaves on one side of the plant or even a half leaf may show wilting symptoms. In severe cases, leaves wilt without changing colour and stay attached to the stem. As in tomato, the vascular tissues show a brown discoloration when cut open. The primary and secondary roots may become brown to black.

Banana

Moko disease, caused by *R. solanacearum*, is easily confused with Panama disease caused by *Fusarium oxysporum* f.sp. *cubense*. A clear distinction is possible when fruits are affected: brown dry rot is seen only in the case of Moko disease. On young and fast-growing plants, the youngest leaves turn pale-green or yellow and collapse. Within a week, all leaves may collapse. Young suckers may be blackened, stunted or twisted. The pseudostems show brown vascular discoloration.

Isolation, including sampling and detection of latent infections

R. solanacearum may easily occur in a latent form and in temperate European countries infected but symptomless *Solanum dulcamara* growing along waterways with its roots in surface water is an important factor in epidemiology (Elphinstone *et al.*, 1998; Janse *et al.*, 1998; Weneker *et al.*, 1999). Several selective media for isolation from latently infected material or difficult substrates like soil, waste, or surface water have been described. SMSA medium as modified by Elphinstone *et al.* (1996) has been used successfully in Europe (e.g. Elphinstone *et al.*, 1998; Weneker *et al.*, 1999). Isolation from symptomatic material can easily be performed using YPGA non-selective medium or Kelman's tetrazolium medium. In some cases when secondary infections are present, isolation on selective media is necessary. A presumptive test in the field can be the water-streaming test as described under disease symptoms or a serological agglutination test using a field kit in the form of a lateral flow device (Danks & Barker, 2000). Many standard methods for detection (of latent infection), identification and preparation of media for *R. solanacearum*, used in official EU testing schemes, can be found in EU (1998), Lelliott & Stead (1987) and OEPP/EPPO (1990). Detection of latent infection is by performing an immuno-fluorescence test and/or selective plating on SMSA medium eventually combined with optional PCR assays, ELISA or fluorescent *in situ* hybridization tests which can be performed following enrichment procedures for added sensitivity. Details of these methods and some recently developed techniques can be found in Boudazin *et al.* (1999); Caruso *et al.* (2002); Pastrik & Maiss (2000); Pastrik *et al.* (2002); Seal *et al.* (1993); Weller *et al.* (2000); Wullings *et al.*, 1998). Standard samples should consist of 200 tubers per 25

Table 1 Differential biochemical characteristics for *Ralstonia solanacearum* and some related non-fluorescent plant pathogenic bacteria, belonging to *Acidovorax*, *Burkholderia* and *Pseudomonas*

Test	<i>Ralstonia solanacearum</i>	<i>Burkholderia cepacia</i>	<i>Burkholderia gladioli</i>	<i>Burkholderia caryophylli</i>	<i>Pseudomonas corrugata</i>	<i>Acidovorax avenae</i>
Diffusible pigment	+	+	+	+	-	-
Oxidase	-	+	V	+	+	+
Arginine dihydrolase	-	-	-	+	-	-
Nitrate reduction	-	-	-	+	+	+
Growth at 41 °C	-	V	V	+	-	+
Oxidation of:						
Galactose	+ / V	+	+	-		
Glycerol	+ W	-	-	+ W		
Mannose	+ / V	-	+	-		
Utilization of:						
Cellobiose	V	+	+	V	-	
Trehalose	V	V	+	+		
D-Arabinose	-	+	+	+	-	-
D-Tartrate	- / V	- / W	+	-	V	
Mannitol	V	+	+	+		+
Sorbitol	V	+	+	+		+
L-Rhamnose	-	-	-	-	-	
Levulinate	V	+	- / W	-		
Sucrose	+	+	+	+		-
Glucose	+	+	+	+	+	+
Benzoate	V	- / V	+	-		
n-Propanol	+	+	-	+ W	-	-
β-Alanine	V	-	-	-	-	+
Betaine	-	+	+	+		
L-Arginine	-	+	+	+		
L-Lysine	-	+	+	V		
Heptanoate	-	+	+	-		
D-Fucose	-	+	+	+		
D-Raffinose	-	V	-	+		

+ = positive reaction; - = negative reaction; V = variable; W = weak.

tons. Many test methods have been standardized and ring-tested in EU Member States in an EU project (SMT CT97-2197) in which 20 laboratories co-operated. Figures 1 and 2 provide flow schemes for detection (of latent infection also) and identification. Correct application of these methods may require training from one of the laboratories involved in the above-mentioned project (EU, 1998; Elphinstone *et al.*, 2000).

Identification

Description and biochemical characteristics

Gram-negative rods with a polar tuft of flagella, non-fluorescent but diffusible brown pigment often produced. Polyhydroxybutyrate (PHB) is accumulated as cellular reserve and can be detected by Sudan Black staining on nutrient-rich media or the Nile Blue test, also in smears from infected tissues (EU, 1998); levan not formed from sucrose; gelatin hydrolysis negative or weak; starch and esculin not hydrolysed, nitrate reduced by nearly all strains, many produce gas (denitrification), oxidative metabolism of glucose only, no growth at 4° or 40 °C; growth

Table 2 Tests for biovar determination in *Ralstonia solanacearum*

Utilization of:	Biovar				
	1	2	3	4	5
Maltose	-	+	+	-	+
Lactose	-	+	+	-	+
Cellobiose	-	+	+	-	+
Mannitol	-	-	+	+	+
Sorbitol	-	-	+	+	-
Dulcitol	-	-	+	+	-

is weak at pH 8 with no growth at pH 4 or 9; oxidase and catalase positive; arginine dihydrolase, lecithinase (egg yolk) and lipase (Tween 80) negative. Most strains produce tyrosinase, the main exceptions being those isolated from the family *Musaceae*. Growth in 1% NaCl broth but little or no growth in 2% NaCl. This species belongs to rRNA group II. It is readily distinguished from other members of the group by failure to grow at 40 °C (Table 1). For carbon compounds utilized, see Bradbury (1986), Saddler (1994). Table 1 provides discriminating characters for *R. solanacearum* and some related plant pathogens, Tables 2

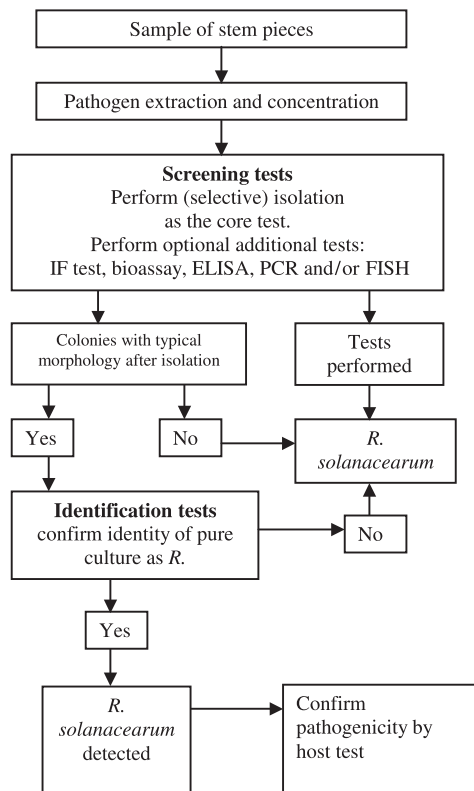


Fig. 1 Decision scheme for the detection and identification of *Ralstonia solanacearum* in samples of symptomatic potato, tomato or other host plants. Full details for methods and procedures, reagents and composition of media are necessary for successful application of the scheme and can be found in EU (1998). Further details and training may be necessary, as mentioned under 'Isolation, including sampling and detection of latent infections'.

and 3 for biovars, and Table 4 for races. Media used are specified in EU (1998). Methods for identification using IF, ELISA, PCR, fatty-acid analysis, FISH, RFLP, REP-PCR and AFLP fingerprinting have been described in detail or sources are indicated in EU (1998) and are described in Janse (1991); Smith *et al.* (1998); Poussier *et al.* (1999); Poussier & Luisetti (2000); Poussier *et al.* (2000); van der Wolf *et al.* (1998).

Pathogenicity test

Pathogenicity can be determined by inoculating a suspension (10^6 cell mL^{-1}) of a 48-h nutrient agar/YPGA culture into 5–10 susceptible tomato (e.g. cv Moneymaker) or aubergine (e.g. cv Black Beauty) plants at, preferably, the third true leaf stage or slightly older. Incubation should be for up to two weeks at 25–28 °C under high relative humidity conditions. Symptoms are wilting and/or epinasty, chlorosis, sometimes only stunting. The bacterium should be reisolated from plants by taking a stem or petiole section above the inoculation point and placing it in a small volume of sterile distilled water or 50 mM phosphate buffer, plating on YPGA and/or SMSA medium, and observing for typical colonies (EU, 1998). Colonies can be used for

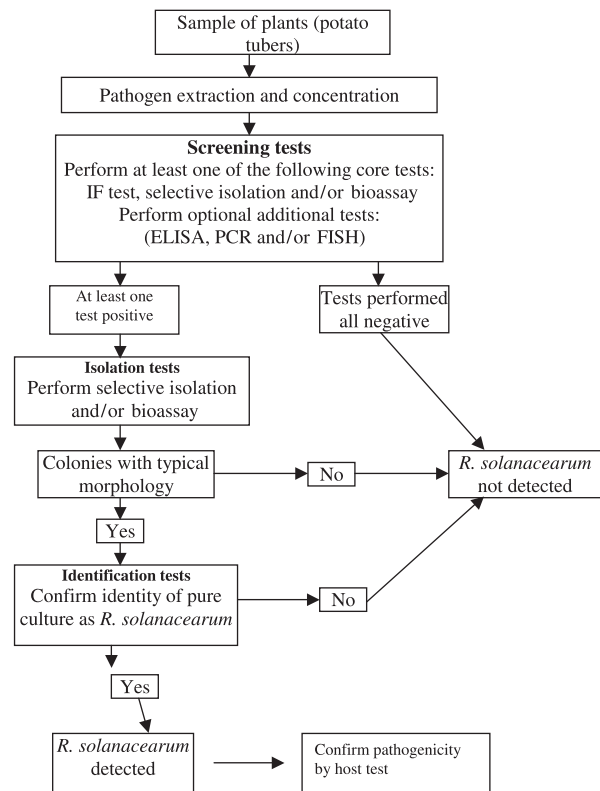


Fig. 2 Decision scheme for the detection and identification of *Ralstonia solanacearum* in samples of asymptomatic plants. Full details for methods and procedures, reagents and composition of media are necessary for successful application of the scheme and can be found in EU (1998). Further details and training may be necessary, as mentioned under 'Isolation, including sampling and detection of latent infections'.

Table 3 Tests for differentiation of subphenotypes of biovar 2 of *Ralstonia solanacearum*

Test	Biovar 2A RFLP group 26	Biovar 2A RFLP group 27	Biovar 2T or 2N
Utilization of trehalose	–	+	+
Utilization of inositol	+	–	+
Utilization of D-ribose	–	–	+
Pectolytic activity	Low	Low	High

confirmation by IF, PCR or FISH. For race determination, also tobacco (e.g. cv. White Burley) and *Musa acuminata* can be inoculated and incubated as described above. Symptom development in *M. acuminata* may take longer than two weeks.

Reference material

Reference strains at the National Collection of Plant Pathogenic Bacteria, CSL, York (GB) are: UK NCPPB 325 (Type strain, biovar 1); NCPPB 4156 (biovar 2), NCPPB 3996 (biovar 3), NCPPB 4029 (biovar 4), NCPPB 4011 (biovar 5).

Table 4 Race determination in *Ralstonia solanacearum** (Janse, 1991)

Race	1	2	3
Reaction in:			
Tomato/aubergine	Wilting	No reaction	Wilting
Tobacco cv. White Burley plants (stem inoculation)	Wilting	No reaction	No reaction
Tobacco cv. White Burley leaves (hypersensitivity test)	Necrosis (48 h) and wilting (7–8 days)	HR (12–24 h)	Chlorosis (2–8 days)
<i>Musa acuminata</i>	No reaction	Wilting	No reaction

*Race 4, pathogenic to ginger and a few other hosts and race 5, pathogenic to mulberry only, not included.

Possible confusion with similar species

For discrimination of *R. solanacearum* from some related plant pathogenic species, see Table 1. Symptoms of bacterial wilt/brown rot caused by *R. solanacearum* may be confused with other wilting symptoms caused by, e.g. *Fusarium* spp., *Verticillium* spp., *Erwinia chrysanthemi* and *Clavibacter michiganensis* subsp. *sepedonicus* (see under Symptoms). The water-streaming test, described under symptoms, and the observation of spontaneous slime drop formation after cutting tubers/rhizomes are often of good value for a preliminary diagnosis. Serological field kits, which incorporate highly specific monoclonal antibodies, are commercially available (e.g. from CSL) for rapid on-site diagnosis. Definitive diagnosis should always be obtained through laboratory tests on a pure culture.

Requirements for a positive diagnosis

The procedures for detection and identification described in this protocol, and the decision scheme in Fig. 1 or 2, should have been followed. When the organism is diagnosed for the first time, or in critical (import/export) cases, the following are required:

- disease symptoms, morphological and biochemical characteristics of the pathogen and its pathogenic properties should be in accordance with the descriptions in the protocol
- for isolation of the bacterium and descriptions of morphological, biochemical and pathogenic characteristics, the procedures and requirements of the protocol should be followed
- in the case of latent infections, after an initial screening test, the pathogen should be isolated through selective plating before or after inoculation of tissue extract into tomato or aubergine plants and correctly identified, including a pathogenicity test with the pure culture on tomato or aubergine. The biovar or genotype should be determined
- a negative control should always be included and a positive control where possible. Plants inoculated with the positive control strain should be kept apart from other test plants.

Report on the diagnosis

A report on the execution of the protocol should include:

- information and documentation on the origin of the infected material

- a description of the disease symptoms (if applicable, preferably with photographs)
- a description of the morphological, biochemical and pathogenic characteristics of the bacterium
- an indication of the magnitude of the infection
- details of the diagnostic methods employed
- comments as appropriate on the certainty or uncertainty of the identification.

The original sample (with labels, if applicable), sample extract, IF-slide and pure culture should be kept under proper conditions (for at least two months) with correct registration and should be available in case of doubt.

Further information

Further information on this organism can be obtained from: Department Bacteriology, Plant Protection Service, PO Box 9102, 6700 HC, Wageningen, the Netherlands, Fax 0031-317 421701, E-mail: j.d.janse@minlnv.nl; Plant and Environmental Bacteriology Team, Plant Health Group, Central Science Laboratory, Sand Hutton, York YO41 1LZ, UK

Acknowledgement

This protocol was originally drafted by: J.D. Janse, Plant Protection Service, Wageningen (NL).

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Fig. 1. Foliar symptoms caused by *Ralstonia solanacearum*. Courtesy Dr Janse, PD Wageningen (NL).



Fig. 2. Bacterial ooze from cut stem suspended in water (left) and potato eye (right). Courtesy Dr Janse, PD Wageningen (NL).

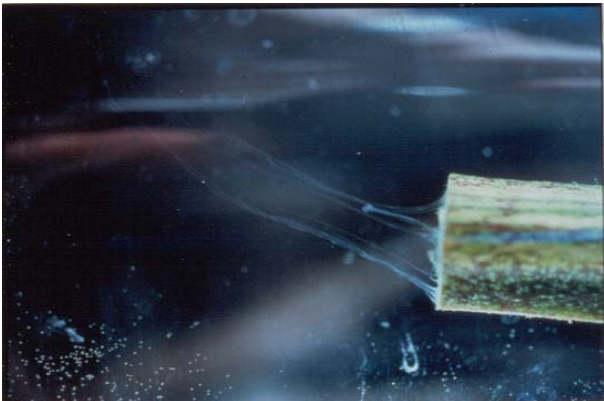


Fig. 3. Symptoms of *R. solanacearum* on cut tubers. Courtesy Dr Janse, PD Wageningen (NL).

