Diagnostic protocols for regulated pests[1]
Protocoles de diagnostic pour les organismes réglementés

Ralstonia solanacearum

Specific scope

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

Specific approval and amendment

Approved in 2003-09.

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 asymptomatically (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 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 glasshouses 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

[1]The 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; Wenneker *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; Wenneker *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	*Ralstonia solanacearum*	*Burkholderia cepacia*	*Burkholderia gladioli*	*Burkholderia caryophylli*	*Pseudomonas corrugata*	*Acidovorax avenae*
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).

Table 2 Tests for biovar determination in *Ralstonia solanacearum*

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

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 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

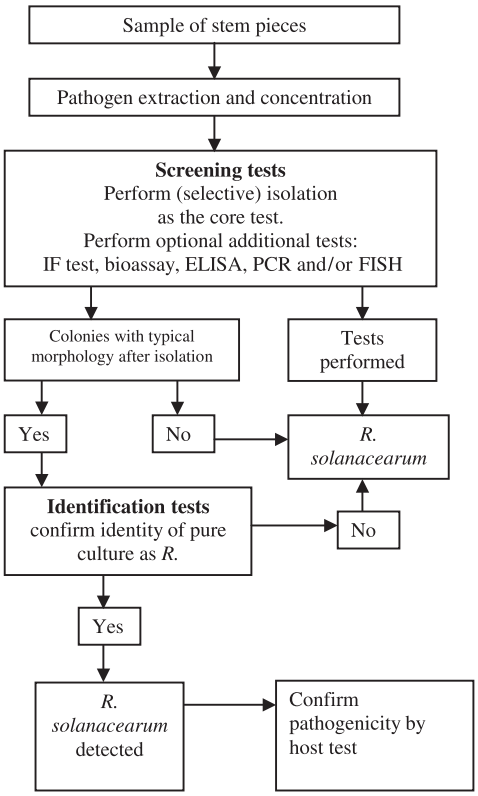

Fig. 1 Decision scheme for the detection and identification of *Ralstonia solanacearum* in samples of symptomatic potato, tomato or other host plants. Full details for methods and procedures, reagents and composition of media are necessary for successful application of the scheme and can be found in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

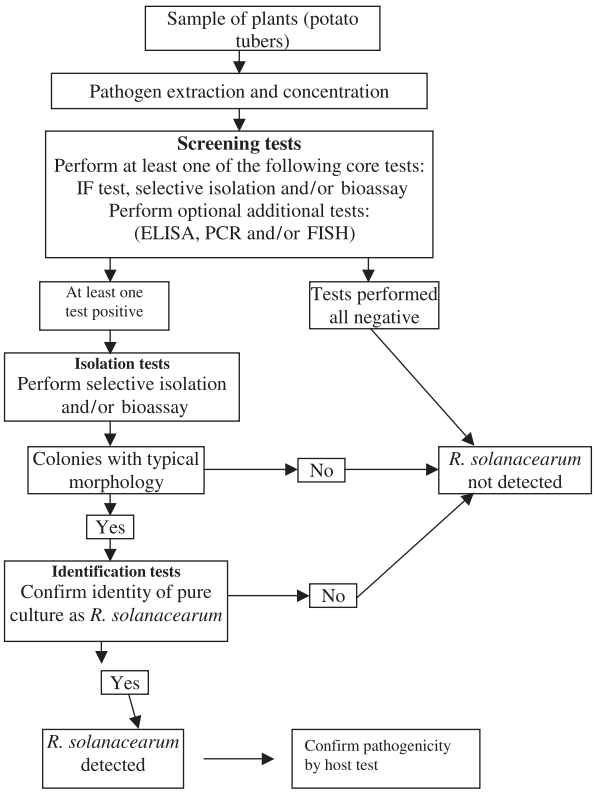

Fig. 2 Decision scheme for the detection and identification of *Ralstonia solanacearum* in samples of asymptomatic plants. Full details for methods and procedures, reagents and composition of media are necessary for successful application of the scheme and can be found in EU (1998). Further details and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

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 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.

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

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)
Musa acuminata	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

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

References

Boudazin G, Le Roux AC, Josi K, Labarre P & Jouan B (1999) Design of division-specific primers of *Ralstonia solanacearum* and application to the identification of European isolates. *European Journal of Plant Pathology* **105**, 373–380.

Bradbury JF (1986) *Guide to Plant Pathogenic Bacteria*. CAB International, Wallingford (GB).

Buddenhagen IW, Sequeira L & Kelman A (1962) Designation of races of *Pseudomonas solanacearum*. *Phytopathology* **52**, 726.

CABI/EPPO (1999) *Distribution Maps of Plant Diseases*. *Ralstonia solanacearum*. Map nos., 783–785. CAB International, Wallingford (GB).

Caruso P, Gorris MT, Cambra M, Palomo JL, Collar J & López MM (2002) Enrichment DASI-ELISA for sensitive detection of *Ralstonia solanacearum* in asymptomatic potato tubers using a specific monoclonal antibody. *Applied and Environmental Microbiology* **68**, 3634–3638.

Cook D & Sequeira L (1994) Strain differentiation of *Pseudomonas solanacearum* by molecular genetic methods. In: *Bacterial Wilt: the Disease*

and its Causative Agent, Pseudomonas solanacearum (Ed. Hayward AC & Hartman GL), pp. 77–93. CAB International, Wallingford (GB).

Danks C & Barker I (2000) On-site detection of plant pathogens using lateral flow devices. *Bulletin OEPP/EPPO Bulletin* **30**, 421–426.

Elphinstone JG, Hennessey JK, Wilson JK & Stead DE (1996) Sensitivity of different methods for the detection of *Pseudomonas solanacearum* in potato tuber extracts. *Bulletin OEPP/EPPO Bulletin* **26**, 663–678.

Elphinstone JG, Stanford HM & Stead DE (1998) Detection of *Ralstonia solanacearum* in potato tubers, *Solanum dulcamara*, and associated irrigation water. In: *Bacterial Wilt Disease: Molecular and Ecological Aspects* (Ed. Prior P, Allen C & Elphinstone J), pp. 133–139. Springer Verlag, Berlin (DE).

Elphinstone JG, Stead DE, Caffier C, Janse JD, López MM, Mazzucchi U, Müller P, Persson P, Rauscher E, Schiessendoppler E, Sousa Santos M, Stefani E, van Vaerenbergh J, *et al*. (2000) Standardization of methods for detection of *Ralstonia solanacearum*. *Bulletin OEPP/EPPO Bulletin* **30**, 391–395.

EPPO/CABI (1997) *Clavibacter michiganensis* subsp. *sepedonicus*. In: *Quarantine Pests for Europe*, 2nd edn, pp. 1071–1081. CAB International, Wallingford (GB).

EU (1998) Council Directive 98/57/EC of 20 July 1998 on the control of *Ralstonia solanacearum*. Annex II-test scheme for the diagnosis, detection and identification of *Ralstonia solanacearum*. *Official Journal of the European Communities,* no. L235, 8–39.

Hayward AC (2000) *Ralstonia solanacearum. Encyclopedia of Microbiology*, Vol. 4, 2nd edn, pp. 32–42. Academic Press, London (GB).

Janse JD (1991) Infra- and intraspecific classification of *Pseudomonas solanacearum* strains, using whole cell fatty acid analysis. *Systematic and Applied Microbiology* **14**, 335–345.

Janse JD, Arulappan FAX, Schans J, Wenneker M & Westerhuis W (1998) Experiences with bacterial brown rot *Ralstonia solanacearum* biovar 2, race 3 in The Netherlands. In: *Bacterial Wilt Disease: Molecular and Ecological Aspects* (Ed. Prior P, Allen C & Elphinstone J), pp. 146–152. Springer, Berlin (DE).

Janse JD, van den Beld HE, Ephinstone J, Simpkins S, Tjou-Tam Sin NNA & van Vaerenbergh J (2004) Introduction to Europe of *Ralstonia solanacearum* biovar 2, race 3 in *Pelargonium zonale* cuttings. *Journal of Plant Pathology* **87**, 147–155.

Lelliott RA & Stead DE (1987) *Methods for the Diagnosis of Bacterial Diseases of Plants*. Blackwell Scientific, Oxford (GB).

OEPP/EPPO (1990) EPPO Standards PM 3/26, *Pseudomonas solanacearum* inspection and test methods. *Bulletin OEPP/EPPO Bulletin* **20**, 255–262.

Pastrik KH, Elphinstone JG & Pukall R (2002) Sequence analysis and detection of *Ralstonia solanacearum* by multiplex PCR amplification of 16S–23S ribosomal intergenic spacer region with internal positive control. *European Journal of Plant Pathology* **108**, 831–842.

Pastrik KH & Maiss E (2000) Detection of *Ralstonia solanacearum* in potato tubers by polymerase chain reaction. *Journal of Phytopathology* **148**, 619–626.

Poussier S & Luisetti J (2000) Specific detection of biovars of *Ralstonia solanacearum* in plant tissues by Nested-PCR-RFLP. *European Journal of Plant Pathology* **106**, 255–265.

Poussier S, Trigalet-Demery D, Vanderwalle P, Goffinet B, Luisetti J & Trigalet A (2000) Genetic diversity of *Ralstonia solanacearum* as assessed by PCR-RFLP of the hrp gene region, AFLP and 16S rRNA sequence analysis, and identification of an African subdivision. *Microbiology* **146**, 1679–1692.

Poussier S, Vandewalle P & Luisetti J (1999) Genetic diversity of African and worldwide strains of *Ralstonia solanacearum* as determined by PCR-Restriction Fragment Length Polymorphism analysis of the *hrp* gene region. *Applied and Environmental Microbiology* **65**, 2184–2194.

Pradhanang PM, Elphinstone JG & Fox RTV (2000) Identification of crop and weed hosts of *Ralstonia solanacearum* biovar 2 in the hills of Nepal. *Plant Pathology* **49**, 403–413.

Saddler GS (1994) IMI Descriptions of Fungi and Bacteria, 1220. *Burkholderia solanacearum*. *Mycopathologia* **128**, 61–63.

Seal SE, Jackson LA, Young JPW & Daniels MJ (1993) Detection of *Pseudomonas solanacearum*, *Pseudomonas syzygii*, *Pseudomonas pickettii* and Blood Disease Bacterium by partial 16S rRNA sequencing: construction of oligonucleotide primers for sensitive detection by polymerase chain reaction. *Journal of General Microbiology* **139**, 1587–1594.

Smith JJ, Kibata GN, Murimi ZK, Lum KY, Fernandez-Northcote E, Offord LC & Saddler GS (1998) Biogeographic studies on *Ralstonia solanacearum* race 1 and 3 by genomic fingerprinting. In: *Bacterial Wilt Disease, Molecular and Ecological Aspects* (Ed. Prior P, Allen J & Elphinstone J), pp. 50–55. Springer, Berlin (DE).

Strider DL, Jones RK & Haygood RA (1981) Southern bacterial wilt of geranium caused by *Pseudomonas solanacearum*. *Plant Disease* **65**, 52–53.

Taghavi M, Hayward C, Sly LI & Fegan M (1996) Analysis of the phylogenetic relationships of strains of *Burkholderia solanacearum*, *Pseudomonas syzygii*, and the blood disease bacterium of banana based on 16S rRNA gene sequences. *International Journal of Systematic Bacteriology* **46**, 10–15.

van der Wolf JM, Bonants PJM, Smith JJ, Hagenaar M, Nijhuis E, van Beckhoven JRCM, Saddler GS, Trigalet A & Feuillade R (1998) Genetic diversity of *Ralstonia solanacearum* race 3 in Western Europe determined by AFLP, RC-PFGE and Rep-PCR. In: *Bacterial Wilt Disease, Molecular and Ecological Aspects* (Ed. Prior P, Allen J & Elphinstone J), pp. 44–49. Springer, Berlin (DE).

Weller SA, Elphinstone JG, Smith N & Stead DE (2000) Detection of *Ralstonia solanacearum* from potato tissue by post enriched TaqMan PCR. *Bulletin OEPP / EPPO Bulletin* **30**, 381–384.

Wenneker M, Verdel MSW, Van Beuningen AR, Derks JHJ & Janse JD (1999) *Ralstonia (Pseudomonas) solanacearum* race 3 (biovar 2) in surface water and natural weed hosts: first report on stinging nettle (*Urtica dioica*). *European Journal of Plant Pathology* **105**, 307–315.

Wullings BA, van Beuningen AR, Janse JD & Akkermans ADL (1998) Detection of *Ralstonia solanacearum*, which causes brown rot of potato, by fluorescent *in situ* hybridization with 23S rRNA-targeted probes. *Applied and Environmental Microbiology* **64**, 4546–4554.

Yabuuchi E, Kosako Y, Yano I, Hotta H & Nishiuchi Y (1995) Transfer of two *Burkholderia* and an *Alcaligenes* species to *Ralstonia* gen. nov.: proposal of *Ralstonia pickettii* (Ralston, Palleroni and Douderoff 1973) comb.nov., *Ralstonia solanacearum* (Smith 1896) comb. nov. & *Ralstonia eutropha* (Davis 1969) comb. nov. *Microbiology and Immunology* **39**, 897–904.