

## PM 9/3 (3) *Ralstonia solanacearum*

**Specific scope:** This Standard describes a national regulatory control system for *Ralstonia solanacearum* that provides guidance on surveillance for the pest and its containment and eradication if found.

**Specific approval and amendment:** First approved in 2003–09. Revisions approved in 2011–09 and in 2023–09. Authors and contributors are given in the Acknowledgements section.

### 1 | INTRODUCTION

*Ralstonia solanacearum* has been described as distributing into four Phylotypes in a species complex (Prior & Fegan, 2005), and was reclassified by Safni et al. (2014) into three distinct species: *R. solanacearum* (Phylotype II), *Ralstonia pseudosolanacearum* (Phylotype I and III) and *Ralstonia syzygii* (Phylotype IV; EPPO, 2022a). Strains affecting potato are found in all phylotypes. This Standard applies to *R. solanacearum* (Phylotype II), which is the only species established in the EPPO Region (an EPPO A2 pest), where it causes brown rot of potato and bacterial wilt of tomato. However, the measures described in this Standard may also be applied to other species in the *Ralstonia solanacearum* species complex (RSSC), in particular *R. pseudosolanacearum*. Prevention of spread of the pest within the region is achieved mainly by international phytosanitary measures and the operation of certification schemes for seed potato. If a country can demonstrate that it is a pest-free area, or can establish pest-free areas within its territory, potatoes and host plants from these areas will not be subject to the restrictions which otherwise apply. Requirements for establishing pest-free areas are described in EPPO Standard PM 3/61 (EPPO, 2019). EPPO Standard PM 8/1 on commodity-specific phytosanitary measures specifies requirements for commodities of potatoes with respect to *R. solanacearum* (EPPO, 2017). The present Standard presents the basis of a national regulatory control system for the surveillance, containment and eradication of *R. solanacearum* with a focus on potato and tomato production systems.

### 2 | OUTLINE OF THE SYSTEM

A national regulatory control system is recommended to all EPPO countries for the surveillance,

containment and eradication of the pest if present, and it provides sufficient guarantees to allow export of potatoes within the region, in conformity with EPPO Standard PM 8/1. This system is described in the present Standard and takes into account EU Commission Implementing Regulation 2022/1193 on establishing measures to eradicate and prevent the spread of *Ralstonia solanacearum* (EU, 2022). It is also recommended that EPPO member countries at risk prepare a pest-specific contingency plan (based on EPPO Standard PM 9/10 *Generic elements for contingency plans*; EPPO, 2009) to ensure that the necessary management and operational arrangements are in place to deal with an outbreak.

Visual inspection of potato tubers alone is not adequate to prevent the spread of brown rot because the disease can often be latent. Brown rot control therefore depends primarily on the production and use of certified seed potatoes and on the testing of seed potato samples by internationally agreed methods for detection and identification of *R. solanacearum*. Whenever the disease is found, measures should be taken to contain and eliminate it from the production system, especially by delimitating a regulated area, by investigating possible sources of contamination and spread, and by restricting the cultivation of host plants of *R. solanacearum* for several years (including control of volunteer potatoes and relevant host weeds). Strict hygiene measures are also a key element in disease control.

*Ralstonia solanacearum* can also contaminate water courses, with bacterial levels building up and persisting in weeds such as *Solanum dulcamara*, an important secondary host, which may commonly be found growing in the margins of waterways. The concentration of the bacterium can increase in the water, particularly during summer, when water temperatures are higher, and potentially giving rise to infectious levels of *R. solanacearum*. Indeed, the use of contaminated water for irrigation has resulted in numerous outbreaks in both potatoes and tomatoes in the EPPO region. Depending on the local situation, eradication in surface water is difficult and often not possible. The irrigation of host plants with contaminated water from waterways should be prohibited.

The national regulatory control system is devised to ensure that countries, which demonstrate that they apply this control system, can export potatoes and other host plants

on the same basis as countries which have demonstrated that *R. solanacearum* does not occur in their territory.

### 3 | CONTROL SYSTEM

This control system for *R. solanacearum* has five objectives:

- to determine if the pest is present in the country and, if present, to locate it and determine its distribution.
- to prevent its spread.
- to prevent introduction in production systems, with a focus on potato and tomato.
- to eradicate incursions.
- to eradicate the pest from production systems in areas where it is present.

#### 3.1 | Surveillance

The holding and handling of *R. solanacearum* should be prohibited, except under authorization (licence) from the NPPO; see EPPo Standard PM 3/64(1) *Intentional import of organisms that are plant pests or potential plant pests* (EPPo, 2006b). *R. solanacearum* should be considered as a notifiable pest. All persons suspecting or confirming the presence of the disease should notify this to the NPPO.

Surveillance for the presence of *R. solanacearum* in a country or area not known to have potato brown rot or bacterial wilt of tomato, is usually based on a systematic detection survey. The intensity of the surveillance should be based on risk assessment and should provide assurance for pest freedom covering the whole production system.

Surveys should be undertaken on potato, tomato, surface water and, possibly, other host plants. In addition, a targeted survey on liquid waste discharged from industrial processing or packaging premises may be appropriate but this should be based on a risk assessment of the possible reintroduction of the pest into production systems (see also [Appendix 1](#) for surveillance strategies in national surveillance programmes). Surveys should comprise the following elements:

##### 3.1.1 | Potato

In the case of seed potatoes, a survey should be undertaken on potato tubers, sampling from the growing crop or from tuber lots in storage. Surveys on farm saved seed potatoes should be based on the pest risk identified. When sampling of tubers is undertaken in the field (e.g. for early potatoes) it should be done as late as possible between desiccation of haulms and harvest. Samples should be taken at random to help ensure that they are

representative and that reliable results can be obtained (see ISPM No. 31 *Methodologies for sampling of consignments*; IPPC, 2016). An individual sample for analysis should be comprised of not more than 200 tubers. Additional tubers may be inspected by cutting them and examining the exposed cut surfaces for any sign of the disease in the vascular tissue. Growing potato crops may be inspected visually at appropriate times for typical signs and symptoms of the disease. ISPM No. 31 may be used as a basis for establishing sampling rates and for providing probability levels for detection at a given tuber sampling rate.

In the case of potatoes other than seed potatoes, the growing crop may be inspected visually at appropriate times for typical symptoms of the disease as described in PM 7/21. In the case of any suspicious symptoms, samples should be taken for laboratory testing. In addition, testing of harvested tubers should be carried out.

##### 3.1.2 | Tomato

Crops of tomato plants (grown outdoors or under protection) should be inspected visually during active growth. In the case of any suspicious symptoms of bacterial wilt of tomato, samples should be taken for testing.

##### 3.1.3 | Surface water

Samples from watercourses used for irrigation or pesticide spraying of host plants should be taken for testing, especially if these watercourses are close to production sites found to be infested with *R. solanacearum* or are close to the waste discharges from industrial potato processing, packaging premises, or domestic sewage outfalls. Surface water samples should be taken in the vicinity of *S. dulcamara* plants if present.

It should be considered that detection of *R. solanacearum* in surface water is most reliable during late spring, summer and early autumn when water temperatures exceed 15°C, which would encourage survival of *R. solanacearum* in the water if present. Repeated sampling at different times in late spring, summer and early autumn, at designated sampling points, will increase the reliability of detection by reducing the effects of climatic variation (e.g. after heavy rainfall, high flow rates can reduce detection).

##### 3.1.4 | Other host plants

Samples of other host plants (e.g. *Citrullus lanatus*—watermelon), including known important weed hosts (e.g. *S. dulcamara*, *S. nigrum* and *Urtica dioica*), should be taken in areas where the risk of spread of

*R. solanacearum* has been identified. In natural habitats, *R. solanacearum* can survive moderate winters in weed hosts or in the rhizosphere of non-host plants, which act as reservoirs of inoculum. *S. dulcamara* and *S. nigrum* are known to be major alternative hosts and can be very important in the epidemiology of the disease. *U. dioica* has also been found to be infected in some countries but the significance of this is not known.

### 3.1.5 | Solid and liquid waste

Samples from solid and liquid waste considered to pose a risk for potato and tomato production may be taken from processing and packing plants for testing.

### 3.1.6 | Detection and identification

Methods for processing, testing and identification of *R. solanacearum* from samples are described in EPPO Standard PM 7/21 (EPPO, 2022a).

## 3.2 | Determination of presence

If an outbreak is detected by routine testing, or if an outbreak is suspected, the NPPO should prohibit all movement of the material directly concerned, and of any suspect material, and may as appropriate take various additional safeguarding measures, such as prohibiting the use of surface water for irrigation of host plants and the movement of other potatoes or other host plants from the place of production<sup>1</sup> under investigation. Samples of suspect material should be submitted to the laboratory for confirmatory testing as soon as possible, following EPPO Standard PM 7/21 (EPPO, 2022a). The NPPO should ensure the preservation of appropriate specimens (e.g. the original sample, the original extract, IF-microscope slides or DNA prepared from extracts, relevant documentation pertaining to the sample or lot) until the completion of all tests. Cultures of the pest should be kept and stored appropriately for at least 1 month after finalization of the tests.

If the material presents a risk to another country, the NPPO of that country should be informed immediately of the suspect finding. Information for the potato, or other host plant lots exported should consist of at least:

- the variety name of the potato, or other host plant lot.
- for potatoes, the type (ware, seed, etc.) and where applicable the seed category.

<sup>1</sup>A place of production is defined as 'Any premises or collection of fields operated as a single production or farming unit. This may include production sites which are separately managed for phytosanitary purposes' (ISPM No. 5, 2010).

- the name and address of the consignor and the consignee.
- the date of delivery of the lot.
- the quantity delivered.

Additionally, the crop or lot identification number, the registration number of the grower or merchant, a copy of the phytosanitary certificate and a copy of the delivery note should be provided if available.

If the outbreak is associated with material from another country, evidence such as appropriate specimens or material and documentation should be kept for at least 1 year according to the requirements in ISPM No. 13 *Guidelines for the notification of non-compliance and emergency action* (IPPC, 2021), as appropriate.

The NPPO should investigate the extent and primary source of the outbreak. This investigation should include sampling and testing of at least all other lots of potatoes grown at the place of production and all clonally-related seed potato and ware potato stocks. In the case of an outbreak in tomato production, tomato plants from the same source should be investigated. Where relevant, the investigation should also include:

- places of production in contact with infested material through machinery, etc.
- places/sites of production using common sources of surface water for spraying or irrigation, or flooded, from a source suspected or confirmed to be contaminated.
- the surface water used for such irrigation (river, lake, reservoir, canal, etc.).

The NPPO should designate as 'infested', where appropriate:

- the lot from which the sample was taken
- the waste from an infested lot (e.g. soil, processing waste).
- vehicles, equipment and other articles (machinery, packing material, storage areas, etc.) which have been in contact with the lot.
- the place of production or production site where the lot was grown.

The extent of 'probable infestation'<sup>2</sup> should also be determined, considering:

- All potato stocks or other host plants clonally related to the infested lot. When one or more of these lots are found infested, all other lots in the clonal line should be declared probably infested.
- Knowledge on the location and timing of contact between the infested lot or possible inoculum, and surface water, due to flooding or (unintentional) use of

<sup>2</sup>No positive test result, but a strong presumption that infestation is possible.

water due to irrigation and spraying. Host plants, production sites, storage areas which may have been in contact with such water should be designated as probably infested. When there has been no risk of further spread to some other production sites, the extent of probable infestation should be restricted to the only those production sites at risk of coming into contact with infested surface water.

- Knowledge on the risk of contact-related spread. Host plants (e.g. seed and ware potato lots), vehicles, equipment and other articles (e.g. machinery, packing material, storage areas) that may have been in contact with infested lots and possible inoculum should be declared as probably infested.

In particular, in the case of latent infestations detected by laboratory testing and without visual symptoms in tubers (after cutting) of the infested lot, the risk of spread of bacteria from this lot to other lots through contact with residual infective material is low. In this case, at least the lot that was handled directly after the infested lot during planting, harvesting, sorting and other processing should be declared as probably infested as a precautionary measure.

Where there is no evidence of a clonally related infestation, and there are no other reasons to restrict the probable infestation, all other potatoes or host plants grown at the place of production concerned should be declared as probably infested.

A regulated area should be demarcated, composed of places/sites of production designated as ‘infested’ and of places/sites of production designated as ‘probably infested’, including where relevant other places/sites of production in the vicinity of the designated contamination (e.g. irrigated with contaminated or probably contaminated surface water).

If surface water is found to be contaminated, it should be, demarcated and regulated. The extent of probable infestation of the surface water will depend on the results of water testing, taking into account the direction of flow of water, and where appropriate the presence of infected *S. dulcamara* and other weed hosts. The demarcation of the area may be adjusted following further investigations (example in [Appendix 1](#)).

In some cases, the regulated areas may extend into other countries, in which case the NPPOs of these countries should also establish an equivalent regulated area considering the geography, production links, clonal links and links through surface water systems (e.g. rivers, canals).

### 3.3 | Containment

As part of eradication measures (see [Section 3.4](#)), or in the case of an established population of the

pest when eradication is no longer considered feasible, the following measures for the containment of *R. solanacearum* should be applied:

- The planting of tubers or plants designated as ‘infested’ or ‘probably infested’ should be prohibited.
- All seed potatoes intended for marketing should meet the requirements laid down in EPPO Standard PM 4/28 *Certification scheme for seed potatoes* (EPPO, 1999, under revision); and additional tests for *R. solanacearum* should be carried out, either on each plant of the initial clonal selection or on representative samples of basic seed potatoes (or higher grades of propagation stock) prior to certification in the regulated area.
- If *R. solanacearum* is detected (and confirmed by testing) in the seed potato production system:
  - Other related or non-related seed potato clones should be tested systematically to investigate whether the infestation has spread clonally, via infested surface water or by contact.
  - If the source of infestation cannot be identified, sampling and testing for *R. solanacearum* in the seed potato production system should be intensified in accordance with sound scientific and statistical principles or at least each seed potato crop should be sampled and tested.
- Plants of tomato and other hosts intended for transplanting should be subject to regular visual inspections within the regulated area.
- The use of contaminated surface water for the cultivation of host plants (e.g. for irrigation or spraying of crops) should be prohibited.
- Cleaning and disinfection measures should be applied according to [Sections 3.4.1](#) and [3.4.3](#), in particular to all vehicles which have been used for transportation of infested and probably infested material in the containment area before they leave this area.
- If waste from industrial tomato or potato processing or packaging premises is found to be contaminated with *R. solanacearum*, the procedures detailed in EPPO Standard PM 3/66 *Guidelines for the management of plant health risks of biowaste of plant origin* should be followed to eliminate contamination and/or prevent spread (EPPO, 2022b).

### 3.4 | Eradication from production systems

To ensure that *R. solanacearum* is eradicated from production systems a programme of phytosanitary measures should be undertaken. Although practical measures in this section are described for potato and tomato, equivalent measures should be applied to other host crops.

### 3.4.1 | Measures for ‘infested’ and ‘probably infested’ material

Successful application of the disposal methods recommended below for all material designated as ‘infested’ or ‘probably infested’ will require careful implementation to ensure containment of the pest during treatment and its elimination prior to release of the treated material from containment. To ensure the effectiveness of the disposal procedures, regular monitoring and auditing should be carried out. In addition, if material is transported for processing a system of cleaning and disinfection (see below) should be in place, especially for vehicles used for transportation of this material.

#### 3.4.1.1 | *All tubers or plants of potato, or plants of tomato, designated as ‘infested’*

This material should be disposed of. Possibilities for disposal include:

- incineration.
- heat sterilization.
- industrial processing at a processing plant with appropriate waste facilities with a system of cleaning and disinfection of at least the departing vehicles, except when used for transportation of the same lot.
- controlled composting according to EPPO Standard PM 3/66 (EPPO, 2022b) at an officially approved composting site.
- heat treatment at an officially approved facility according to EPPO Standard PM 3/66.
- feeding to animals after steaming.
- disposal (e.g. deep burial) at an officially approved dedicated waste disposal site where there is no risk of the pest spreading (e.g. seepage to agricultural land or surface water).

Additionally, in cases where a crop is found to be infested during the growing season or has been planted prior to being designated as infested, destruction of the growing plants is recommended. For potato if progeny tubers have formed these should be harvested and disposed of appropriately. Alternatively, the planted potatoes could be harvested before emergence and disposed of according to 3.4.1.1.

#### 3.4.1.2 | *All tubers or plants of potato, or plants of tomato, designated as ‘probably infested’*

This material should also be disposed of, by one of the means noted in 3.4.1.1.

Alternatively:

- Tubers may be used as ware potatoes under the control of the NPPO for direct consumption, provided that they are packed for direct delivery and use without repacking. Packing should be done on a site with appropriate waste disposal facilities.

- On-farm composting at the place of production is also suitable provided it is conducted under official supervision to ensure that the entire quantity of material is adequately composted by exposure to a temperature of at least 55°C during an uninterrupted period of at least 2 weeks, and returned to the site of production.
- Tubers may also be returned to the production site of origin during winter and left on the surface to be killed by exposure to frost. This should be under official control, and there should be no significant risk of any movement of the material away from the production site.
- Anaerobic digestion for production of bio-gas at an officially approved site may also be a suitable method for disposal. However, the process needs to be validated before it is used as a phytosanitary control treatment. A suggested treatment is to subject the entire volume of material in a thermophilic digester, to a minimum temperature of 55°C, maintained over a period of 24h without interruption with a hydraulic dwell time in the reactor of at least 20 days.

All equipment and other objects classed as ‘infested’ or ‘probably infested’ should be thoroughly cleaned and disinfected (see below) before further use (unless authorized otherwise by the NPPO), or destroyed.

### 3.4.2 | Solid and liquid waste

Any remaining waste associated with and arising from the ‘infested’ material should be disposed of under conditions which ensure that no further risk for spreading the bacteria remains:

- Solid waste should be either
  - Disposed of at an officially approved dedicated waste disposal site, or
  - incinerated, or treated by another measure (authorized by the NPPO) ensuring that further spread of the bacterium is excluded.
- Prior to disposal, liquid waste containing suspended solids should be subjected to filtration or settlement processes to remove such solids, which should be disposed of according to the requirement for solid waste. Liquid waste should be heated to a minimum of 60°C throughout the entire volume consistently, for at least 30min prior to disposal, or treated by another measure (authorized by the NPPO) ensuring that further spread of the bacterium is excluded. Other time–temperature schedules may be approved based on scientific evidence.

Solid and liquid waste, e.g. wash water, arising from sources other than ‘infested’ material and considered to pose a risk for the potato production may be sampled

from the processing and packing facilities and sent to the laboratory for testing.

### 3.4.3 | Measures applied in the regulated area

#### 3.4.3.1 | *Measures applied at infested places of production*

All machinery, vehicles and storage facilities which have or might have been in contact with ‘infested’ or ‘probably infested’ potatoes and other hosts, or production sites, should be immediately and thoroughly cleaned and disinfected according to EPPO Standard PM 10/1 *Disinfection procedure in potato production* before being used or moved (EPPO, 2006a). In addition, any machinery, vehicles and storage facilities involved in potato production shall be cleaned and disinfected in the year of the infection and after the first subsequent growing year.

Volunteer potato plants, and other natural hosts of *R. solanacearum*, should be eliminated from all sites of the place of production, according to EPPO Standard PM 3/89 *Control of volunteer potato plants* (EPPO, 2020).

#### 3.4.3.1.1 | *Measures for infested production sites.*

- No potatoes or other host plants of the pest, or crops on which there is an identified risk of the pest spreading, should be grown for at least 4 years and until no volunteer potato plants are found for two consecutive years.
- When potatoes are grown for the first time after the infestation was found, only ware potatoes should be produced (with a laboratory test carried out on the harvested tubers).
- When tomato or other host plants are grown for the first time, the plants should be tested for *R. solanacearum* at the appropriate growth period.
- When the second crop of potatoes are grown after an appropriate rotation cycle, either seed potatoes or ware potatoes may be produced and a survey should be conducted (in the case of seed potatoes, there should be at least a 2-year period before potatoes are grown).

Alternatively:

- Infested production sites may be left in bare fallow, used for cereals or under permanent pasture with frequent close cutting or intensive grazing, or as grass for seed production, for 3 years, followed by planting in the succeeding 2 years with non-host plants of the pest for which there is no identified risk of the pest surviving or spreading.
- When potatoes are grown for the first time, either seed or ware potatoes may be produced, provided no volunteer potato plants were found for two consecutive

years, with a laboratory test carried out on the harvested tubers.

- When tomatoes or other hosts are grown for the first time, the plants should be tested for *R. solanacearum* at the appropriate growth period.

#### 3.4.3.1.2 | *Measures for other production sites.* For potato production

If official investigations (Section 3.2) have shown that the source of infestation in the place of production was not by contact with other tuber lots, but clonally or by irrigation with infested water courses, the following conditions should apply to the first potato crop cultivated after the reported infestation:

- Certified seed potatoes should be planted on production sites where no potatoes or other cultivated solanaceous host plants have been grown for at least 2 years.
- The tubers produced on these sites should be tested before marketing.

In other cases, the following conditions should apply:

- For three consecutive years, laboratory tests should be performed on tubers harvested from these production sites.
- In the first year, either no host plants of *R. solanacearum* should be planted or only ware potatoes produced from certified seed potatoes.
- In the second and third years, either seed or ware production from certified seed potatoes is permitted.

Alternatively, in the second and third year, potatoes grown under official control and tested for *R. solanacearum* may be planted instead of certified seed potatoes. In the third year, also potatoes grown under official control from certified seed potatoes may be planted instead of certified seed potatoes.

For tomato production

- In the first year, plants may be planted for fruit production, only.
- In the second and third years tomato crops can be grown for either the production of plants for planting or for fruit.

In each year an official inspection of the growing crop at appropriate times should be conducted.

3.4.3.1.3 | *Measures for protected crop production where changing of the growing medium is possible.* Host plants should not be planted until the production site (e.g. unit in the facility) has been subjected, under official control, to measures to eliminate *R. solanacearum*, including the removal of all host-plant material, change of the growing medium, controls on irrigation and

spraying programmes, cleaning and where appropriate disinfection of the unit and all equipment.

3.4.3.2 | *Further measures applied in the whole regulated area (i.e. within both 'infested' and 'probably infested' places of production)*

3.4.3.2.1 | *In the year of the infection.* Machinery and storage facilities used in production systems of host plants should be cleaned and disinfected (but within infested places of production stricter measures are required, see 3.4.3.1).

3.4.3.2.2 | *For 3 years.*

- Potato and tomato production, handling and storage should be kept under official supervision.
- Harvested seed potato and ware potato stocks should be kept separate or cleaning and disinfection should be carried out between the handling of seed potato and ware potato stocks.
- Only certified seed potatoes, or potatoes grown under official control, should be planted (but with additional restrictions within infested places of production, see above).
- Only tomato seed or tomato plants grown under official control from seeds, should be planted.
- Harvested seed potato crops on probably infested places of production should be tested for *R. solanacearum*.
- An official survey should be conducted annually (see Section 3.1: Surveillance).
- All seed-potato stocks within the regulated area should, if appropriate, be replaced over a suitable period of time.

### 3.4.4 | Measures for the regulated surface water

- Regulated surface water should not be used for irrigation or spraying host crops, unless appropriately treated.
- Where a complete water course has been regulated, and it is likely to remain contaminated (e.g. due to the presence of infested *S. dulcamara*) no further testing is necessary. In all other situations, samples of surface water should be taken and tested within and around the regulated areas at appropriate times each year according to EPPO Standard PM 7/21(2) (EPPO, 2022a).
- Where appropriate, all potato and tomato crops should be inspected and in the area around the water course, the seed potatoes tested.
- Samples of *S. dulcamara*, *S. nigrum* or *U. dioica* may also be tested.
- According to the results of these inspections and tests, the regulated area around the water course may be adjusted.
- In cases where host crops may have been in contact with contaminated surface water, these crops should be designated as 'probably infested'

- Conditions for the partial or complete 'descheduling' of regulated areas, in the case of negative results, are under consideration.

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This Standard was originally drafted by Mr M.H.C.G. Steeghs (Plant protection service, NL) in 2001 taking into consideration the EU Control Directive 98/57/EC. The first revision was prepared in 2010 by Ms P. Müller (JKI, DE) to give alternative methods for disposal of infested material. In 2022, a new revision was prepared by an Expert Working Group (EWG) composed of Ms K. Fraser (SASA, GB), Ms F. Migliorini (SFR-ER, IT), Ms L. Stevens (DEFRA, GB) and Mr. M. van Sabben (NVWA, NL), and comments received from Ms. D. Kaemmerer (LfL Bayern, DE). The EWG considered all the changes recently made to the EU Control Directive. The Standard was reviewed by the Panel on Phytosanitary Measures for Potato.

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*Ralstonia solanacearum* and *Ralstonia syzygii* and reclassify current *R. syzygii* strains as *Ralstonia syzygii* subsp. *Syzygii* subsp. Nov., *R. solanacearum* Phylotype IV strains as *Ralstonia syzygii* subsp. *Indonesiensis* subsp. Nov., banana blood disease bacterium strains as *Ralstonia syzygii* subsp. *Celebesensis* subsp. Nov. and *R. solanacearum* Phylotype I and III strains as *Ralstonia pseudosolanacearum* sp. Nov. *International Journal of Systematic and Evolutionary Microbiology* 64, 3087–3103.

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## APPENDIX 1 - SURVEILLANCE STRATEGY FOR *RALSTONIA SOLANACEARUM* IN NATIONAL PROGRAMMES

This Appendix provides elements to consider for the surveillance in surface watercourses and in potato tubers, and for investigations following a suspicion or an outbreak. Examples of sampling rates applied in EPPO countries are also provided.

### 1. Surveillance strategy for surface watercourses

The surveillance strategy in surface watercourses is very dependent on the country situation. The following elements are considered relevant when establishing such a surveillance:

- geography (speed of rivers, connections between rivers, etc.),
- natural versus stabilized riverbanks,
- presence of the main host weeds (e.g. *S. dulcamara*, *S. nigrum*),
- water temperature,
- effluents from processing plants, sewage works, etc.
- solanaceous crop production,
- weather conditions (heavy rainfall in the days prior to sampling may act as a dilution factor).

Results from surveillance in surface watercourses are more erratic, and absence should be declared based on a pluriannual results. In the presence of the main host weeds (e.g. *S. dulcamara*, *S. nigrum*), it may be decided

**TABLE A1** Examples of strategies developed for the surveillance of surface water courses in the EPPO region.

<p>In the <b>Netherlands (NL)</b>, since the late 1990s, a general surveillance with systematic sampling of the surface water has been conducted, taking 1 sample per 10×10 km. After any finding, a demarcation survey is performed with samples taken in the surrounding area. This surveillance resulted in a number of areas where the surface water was designated as 'infested'. Once areas with 'infested' surface water were established, the surveillance grid was adapted:</p> <ul style="list-style-type: none"> <li>- within areas with 'infested' surface water, samples were only taken near the outer borders.</li> <li>- Outside areas with 'infested' surface water, the surveillance grid was maintained, with additional samples taken along the borders of the infested areas.</li> </ul> <p>Over the years, a number of areas with 'infested' surface water slowly increased in size because of new findings near the borders of the infested area. Stakeholders questioned the system because the status of the area with 'infested' surface water was never revised. It was then decided to survey each infested area for 3 consecutive years by sampling (only) inside the areas. Based on the results obtained, the total area considered as 'infested' could be decreased by more than 20%.</p> <p>To avoid constantly adjusting the limits of areas with 'infested' surface water, it has been decided that extending the limits or creating a new area is only performed when a sampling point is found to be infested during two consecutive sampling sessions. Decreasing the size of an area with 'infested' surface water is only possible when during three consecutive years a sampling point has been found free from <i>R. solanacearum</i>. When decreasing the size of an area, results of other relevant sampling points are taken into consideration. Since 2005 – by law – the use of surface water from these areas is prohibited for the production of potatoes, tomatoes, and other host plants. In addition, in the Netherlands, there is a general ban for the use of surface water to produce seed potatoes. Together, this significantly reduced the number of potato lots found to be infested.</p>
<p>In <b>Scotland (GB)</b>, surveillance is based on risk analysis. All new irrigation sources identified are targeted and tested the consecutive year. Every source that has been used for irrigation in the past is tested over a 3-year cycle. The rivers deemed to pose the greatest potential risk for <i>R. solanacearum</i> infection are targeted and tested annually. Sampling intensity along the watercourse, depends on a number of risk factors, including previous presence of the pest and geography of the watercourse: a high-risk source will have multiple points targeted whereas a source considered to be a lower risk will have fewer points or a single point sampled and tested. For general annual surveillance, points are sampled one time a year, generally late summer/early autumn when water temperatures would be highest and taking ×3 replicate samples simultaneously from that point. For any new sources identified, the location of the irrigated crop is taken into consideration and point(s) will be sampled just downstream where possible. In the event of a suspect/positive finding then the intensity of points and sampling will increase.</p>
<p>In <b>England and Wales (GB)</b>, the number of watercourses sampled is risk and resource-based and proportionate to the area of potato grown in each region. Generally, watercourses which have not been recently sampled are targeted. However, in some cases, locations upstream of known contaminated rivers are sampled in order to determine whether demarcated zones should be increased. The target watercourses are usually sampled 4 times over a 4-week period, at two points along the water course.</p>



to begin testing the weeds before testing the watercourse itself; but this may be more labour-intensive.

Examples of strategies for the surveillance of surface watercourses are provided in Table A1.

## 2. Surveillance strategy for potato tubers

### 2.1. General surveillance

Surveillance is generally targeted based on risk analysis, and depends on resources. When establishing a surveillance programme, the following elements of risk should be considered:

- category of material (certified seed potatoes, ware, starch potatoes),
- activity of the stakeholder (producer vs. reseller vs. processing industry, quantity of lots handled)
- origin of the production (national vs. third countries)

In some countries, a certain proportion (up to 100%) of the seed potato lots are already systematically tested, with one sample of 200 tubers per lot of usually 2 ha and of maximum 6–10 ha. For ware potatoes, most samples are usually taken at random packers' premises.

### 2.2. Surveillance during investigations after a finding

In the case an outbreak is reported, sampling and testing is performed to determine the extent and primary source of the outbreak (see 2. *Determination of pest presence*). The sampling strategy should be established with the objective to not need to come back later to take more samples from the same lot(s).

When there is a suspicion for a particular lot (e.g. when the lot was grown on the same production site as an infected lot, when there is a clonal relationship or for other particular reasons), the highest sampling rate should be applied. In the Netherlands, one sample of 200 tubers is taken per 5 tonnes (with a maximum of 20 samples per lot). When the finding is at the end or during the sorting process and that different lots of the same variety have been mixed, it may not be possible to know in which crates the infected lot is stored. Sampling is generally performed once (200 tubers) per crate (1200 kg), or per tonne.

In the place of production where the outbreak was reported, all lots where there is no direct suspicion for infection are usually sampled with a lower sampling rate (e.g. sampled once (200 tubers) per 25 tonnes).