

## 1. Content of the 'Topic Description' document

### 1.1. Topic area

Diagnostics, field detection, surveillance.

### 1.2. Topic title

Development, validation and verification of a diagnostic tool for detection and identification of *Ralstonia solanacearum* and *Clavibacter Michiganensis* subspecies *sepedonicus* directly on plant tissue.

### 1.3. Description of the problem the research should solve

*Ralstonia solanacearum* causes severe economic damage in potato and several other economically important crops. The bacterium is therefore regulated within the EPPO region and many other countries. It has a wide host range which includes several ornamentals and other horticultural plants besides potato. Control is largely dependent on prevention and exclusion, for which reliable detection and identification methods are indispensable.

Current diagnostic methods are described in EU Directive 2006/63/CE (brown rot) and EU Directive 2006/56/CE (ring rot), which contain detailed information for detection and confirmation of the two plant pathogens. These methods require a high level of expertise, require high level quarantine premises, and are time consuming as pathogen isolation and bio-assays are obligatory for diagnostic confirmation according to these EU control directives. This causes high operational costs for National Plant Protection Organisations and affects potato industry and trade as well. To reduce this burden alternative methods will have to be developed and validated by cooperation between Member States. For acceptance of these tests within the EU Control directives for brown rot and ring rot, approval of the EU Standing Committee on Plant Health will be needed.

*R. solanacearum* is a highly variable species and is subdivided into genetically distinct races, biovars, phylotypes and sequevars. Recently this overlapping classification has been resolved by using a polyphasic approach to revise the *R. solanacearum* species complex into three species: *R. solanacearum*, *R. pseudosolanacearum* and *R. syzygii*. Development and validation of a direct PCR-based method requires a test panel of strains that covers the genetic variation of the bacterium.

### 1.4. Description of the expected results

The project is expected to deliver:

- Lists of potential target genes coding for virulence factors (for brown rot and ringrot, respectively)
- Collections of strains to be used for the identification of virulence factors and for the test performance study
- Validated and EU-verified diagnostic tools for detection and confirmation of respectively *Ralstonia solanacearum* and *Clavibacter michiganensis* subsp. *sepedonicus* directly on plant tissues using specific virulence determinants as alternative for the bio-assay.
- Protocols for the two above mentioned tools

### 1.5. Beneficiaries of this research product

- National and Regional Plant Protection Organisations
- Public and private testing laboratories and to the potato industry
- Potato industry and trade
- Ornamental growers (e.g. rose, Anthurium)
- Vegetable growers (e.g. tomato, eggplant)



## 1.6. Research funders and research contribution/ distribution

Funding organisation	Research activity and researchers involved
<p>1. Netherlands Food and Consumer Product Safety Authority, the Netherlands</p> <p>Martijn Schenk <a href="mailto:M.Schenk1@nvwa.nl">M.Schenk1@nvwa.nl</a></p>	<p>-Project coordination</p> <p>Contact person: Leon Tjou-Tam-Sin <a href="mailto:n.tjou-tam-sin@nvwa.nl">n.tjou-tam-sin@nvwa.nl</a></p>
<p>2. Institute for Agricultural and Fisheries Research, Belgium</p> <p>Martine Maes <a href="mailto:martine.maes@ilvo.vlaanderen.be">martine.maes@ilvo.vlaanderen.be</a></p>	<p>-Provide strains of bacteria for testing. -Provide infected plant material. -Participation in the test performance studies.</p> <p>Contact person: Johan Van Vaerenbergh <a href="mailto:johan.vanvaerenbergh@ilvo.vlaanderen.be">johan.vanvaerenbergh@ilvo.vlaanderen.be</a></p>
<p>3. Canadian Food Inspection Agency, Canada</p> <p>Cheryl Dollard <a href="mailto:Cheryl.Dollard@inspection.gc.ca">Cheryl.Dollard@inspection.gc.ca</a></p>	<p>-Based on comparative genomics, multiple qPCR assays will be optimized for detecting and identifying <i>Ralstonia solanacearum</i> race 3 biovar 2 stains. Assays for other races and biovars of the <i>Ralstonia solanacearum</i> species complex will be explored.</p> <p>Contact Person: Sean Li <a href="mailto:sean.li@inspection.gc.ca">sean.li@inspection.gc.ca</a></p>
<p>4. Central Institute for Supervising and Testing in Agriculture, Czech Republic</p> <p>Michal Hnizdil <a href="mailto:Michal.Hnizdil@ukzuz.cz">Michal.Hnizdil@ukzuz.cz</a></p>	<p>- Provide different strains of bacteria for testing. - Participation in the verification of proposed tests through test performance studies.</p> <p>Contact person: Roman Zavadil <a href="mailto:roman.zavadil@ukzuz.cz">roman.zavadil@ukzuz.cz</a></p>
<p>5. Federal Ministry of Food and Agriculture, Germany</p> <p>Bettina Beerbaum <a href="mailto:bettina.beerbaum@bmel.bund.de">bettina.beerbaum@bmel.bund.de</a></p>	<p>-Take part in the test performance studies of the proposed test and original bioassay.</p> <p>Contact person: Eva Fornefeld <a href="mailto:eva.fornefeld@julius-kuehn.de">eva.fornefeld@julius-kuehn.de</a></p>
<p>6. Ministry of Agriculture, Hungary</p> <p>George Melika <a href="mailto:melikag@nebih.gov.hu">melikag@nebih.gov.hu</a></p>	<p>-Contribution to be detailed</p>
<p>7. International Center for Advanced Mediterranean Agronomic Studies, Italy</p> <p>Anna Maria D'Onghia <a href="mailto:donghia@iamb.it">donghia@iamb.it</a></p>	<p>-Validation of the real-time LAMP assay for quick and efficient detection of <i>Ralstonia solanacearum</i> in host plants. -Validation of this method for on site detection (field, quarantine stations etc.). -Evaluation of specificity and sensitivity of the real-time LAMP assay as compared to PCR and real-time qPCR assays. -Development of technical protocol of real time LAMP. -Ring test among EU and Mediterranean NPPO's laboratories for the performance of the real time LAMP.</p>



	<p>Contact person: Thaer Yaseen <a href="mailto:y.thaer@iamb.it">y.thaer@iamb.it</a></p>
<p>8. Ministry of Economic Affairs, Department of Agroknowledge, the Netherlands</p> <p>Annet Zweep <a href="mailto:a.t.zweep@minez.nl">a.t.zweep@minez.nl</a></p>	<p>-Based on relevant RNA expression and mapping of the expression of the genotypic virulence factors, specific target loci will be selected for the design of an assay on virulence factors of <i>R. solanacearum</i>. Oligonucleotides will be designed used for implementation of a quantitative RNA-based reverse transcriptase TaqMan assay. The diagnostic tool will be validated in a validation research, providing detailed data on the accuracy, sensitivity, specificity, repeatability and reproducibility. Additionally the tool will be verified among NPPO's from EU member states, leading in potato production and research through a test performance study.</p> <p>Contact person: Jan van der Wolf <a href="mailto:jan.vanderwolf@wur.nl">jan.vanderwolf@wur.nl</a></p>
<p>9. National Institute for Agricultural and Veterinarian Research, Portugal</p> <p>Maria Leonor Cruz <a href="mailto:leonor.cruz@iniav.pt">leonor.cruz@iniav.pt</a></p>	<p>-Contribution to be detailed</p>
<p>10. Science and Advice for Scottish Agriculture, United Kingdom</p> <p>David Kenyon <a href="mailto:David.Kenyon@sasa.gsi.gov.uk">David.Kenyon@sasa.gsi.gov.uk</a></p>	<p>-Participate in test performance studies.</p> <p>Contact person: Karen Fraser <a href="mailto:Karen.Fraser@sasa.gsi.gov.uk">Karen.Fraser@sasa.gsi.gov.uk</a></p>
<p>11. US Department of Agriculture, Animal and Plant Health Inspection Service, United States of America</p> <p>Laurene Levy <a href="mailto:laurene.levy@aphis.usda.gov">laurene.levy@aphis.usda.gov</a></p>	<p>-Participate in ring test evaluations for detection of <i>R. solanacearum</i> and <i>R. pseudosolanacearum</i>; potentially for <i>C. michigananensis</i> as well</p> <p>Contact Person: John Rascoe <a href="mailto:john.rascoe@aphis.usda.gov">john.rascoe@aphis.usda.gov</a></p>
<p>12. Agricultural University of Tirana, Albania</p> <p>Magdalena Cara <a href="mailto:mcara@ubt.edu.al">mcara@ubt.edu.al</a></p>	<p>-Contribution to be detailed</p> <p>Contact person: Magdalena Cara <a href="mailto:mcara@ubt.edu.al">mcara@ubt.edu.al</a></p>
<p>13. International Potato Center, Peru</p> <p>Jan Kreuze <a href="mailto:j.kreuze@cgiar.org">j.kreuze@cgiar.org</a></p>	<p>-Nucleic acid extractions from worldwide bacterial wilt collection to be used for the identification of virulence factors and for the test performance study.</p> <p>-Participation in the validation process of the EU-verified diagnostic tool for detection of <i>R. solanacearum</i> by testing a diverse set of <i>R. solanacearum</i> isolates selected from our collection.</p> <p>-Participation in any training course and / or</p>



	<p>networking meeting.</p> <p>Contact person: Jan Kreuze <a href="mailto:j.kreuze@cgiar.org">j.kreuze@cgiar.org</a></p>
<p>14. General Directorate of Agricultural Research, Turkey Alev Burçak <a href="mailto:aburcak@tagem.gov.tr">aburcak@tagem.gov.tr</a></p> <p>Biological Control Research Institute (BCRI)</p> <p>Plant Protection Central Research Institute, Ankara (PPCRI)</p> <p>Plant Protection Research Institute, Bornova (PPRI)</p>	<p>-Participation in ring test evaluations for detection of <i>R. solanacearum</i> and <i>C. michiganensis</i> and verification of proposed tests through test performance studies.</p> <p>-To provide different strains of bacteria for testing.</p> <p>-Participation in training courses.</p> <p>-Determination of species of the strains previously identified as <i>Ralstonia solanacearum</i> in the view of new classification of the <i>Ralstonia solanacearum</i> complex into three species as <i>R. solanacearum</i>, <i>R. pseudosolanacearum</i> and <i>R. syzygii</i>; determination of genetic variability of <i>Ralstonia solanacearum</i> and <i>Clavibacter michiganensis</i> subsp. <i>sepedonicus</i> strains.</p> <p>Contact Person: H.Nilufer Yildiz <a href="mailto:yildiz_hn@hotmail.com">yildiz_hn@hotmail.com</a></p> <p>Contact Person: Raziye Ç.Yildiz <a href="mailto:raziye.yildiz@tarim.gov.tr">raziye.yildiz@tarim.gov.tr</a></p> <p>Contact Person: Aynur Karahan <a href="mailto:aynur.karahan@tarim.gov.tr">aynur.karahan@tarim.gov.tr</a></p> <p>Contact Person: Nursen Ustun <a href="mailto:nursen.ustun@tarim.gov.tr">nursen.ustun@tarim.gov.tr</a></p> <p>Contact Person: Neziha Arslan <a href="mailto:neziha.arslan@tarim.gov.tr">neziha.arslan@tarim.gov.tr</a></p>

### 1.7. Research project partnership outside Euphresco

Euphresco funding ensures a certain level of transnational collaboration among Euphresco member countries. It is possible, if the funding consortium is interested, to contact funding organisations or research groups outside the geographical area covered by Euphresco members. The Euphresco coordinator could advertise the research topic in order to have an enlarged collaboration. If funders are interested in this possibility, please check the case below:

The funding consortium of the topic mentioned in section 1.2 requires to advertise the topic outside the Euphresco network

Information to sharpen the profile of sought partners could be useful (but not mandatory): country/region (if there are preferences), skills/expertise required, etc.

### 1.8. Any other relevant information on content

The coordinating organisation would like to use the same approach for *Ralstonia solanacearum* and *Clavibacter Michiganensis* subspecies *sepedonicus* but the budget (providing the research is granted) will only allow to develop one test.

- The EU Directive 2006/63/CE; on the control of potato Brown rot. Obligatory for control and diagnostic procedures within EU member states.
- The EU Directive 2006/56/CE; on the control of potato ring rot. Obligatory for control and diagnostic procedures within EU member states.
- The EPPO Diagnostic Protocol PM 7/21(1) on *Ralstonia solanacearum* is currently under revision.
- The knowledge and data produced within the project could be used to support the revision of the Standard and control directives.



## 2. Euphresco management aspects of the project

### 2.1. Indication of the topic budget

Funding organisation <sup>a</sup>	Mechanism <sup>b</sup>	Total Budget <sup>c</sup>
1. NVWA (NL)	NC	€ 13 000
2. ILVO (BE)	NC	€ 2 000
3. CFIA (CA)	NC	€ 15 000
4. CISTA (CZ)	NC	€ 1 000
5. BMEL (DE)	NC	€ 5 000
6. NFKSO (HU)	NC	€ 2 500
7. EZ-DAK (NL)	NC	In-kind
8. CIHEAM (IT)	NC	€ 10 000
9. INIAV (PT)	NC	€ 20 589
10. SASA (GB)	NC	€ 5 500
11. APHIS (USA)	NC	€ 4 840
12. AUT (AL)	NC	€ 1 000
13. CIP (PE)	NC	€ 5 000
14. TAGEM (TR)	NC	€ 5 325
total		€

### 2.2. Expected duration of the project (only for non-competitive topics)

36 months.

### 2.3. Any other relevant information on topic organisation and management

<sup>a</sup> First member is project coordinator. A minimum of two partners are necessary for each proposal. Add lines as needed.

<sup>b</sup> Please indicate the preferred mechanism (e.g. real pot RP; virtual pot VP; non-competitive NC), or several mechanisms if there is flexibility.

<sup>c</sup> Optional, as this amount can still change in the next phase. In-kind contribution should also be indicated in this column.