

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

### 1.1. Topic area

Diagnostics, (field) detection, surveillance

### 1.2. Links to the Euphresco Strategic Research Agenda

The topic addresses the following objective(s) of the 2017-2022 Euphresco Strategic Research Agenda :

- Objective 2017-R-1.2: to support taxonomic research for the unambiguous identification of pests
- Objective 2017-R-5.1: to understand the biological significance of a positive molecular diagnosis
- Objective 2017-R-5.4: to test and validate the use NGS (e.g. whole genome sequencing, metagenomics, deep sequencing, typing by sequencing) for routine diagnostics
- Objective 2017-R-6.1: to test and validate methods for in situ detection and identification of pests
- Objective 2017-I-1.2: to improve access to collections of phytosanitary importance
- Objective 2017-I-2.2: to contribute to databases for plant pests identification and diagnostics

### 1.3. Topic title

*Curtobacterium flaccumfaciens* on bean and soybean: engaging the old enemy

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

Bacterial wilt of common bean (*Phaseolus vulgaris*) and bacterial tan spot of soybean (*Glycine max*) are caused by *Curtobacterium flaccumfaciens* pv. *flaccumfaciens* (*Cff*). The current status of the bacterium is 'not known to occur in the EU'. Yet in the past, the pathogen was sporadically recorded from symptomatic plants, i.e. in 2005 on field-grown bean cv. Donna in the coast of Granada, Spain and in 2011 on soybean cv. Capnor in breeding trials in Thüringen, Germany. It was also isolated from bean seeds in Spain in 2001. Nevertheless, it is present outside the EU and emerging in certain areas, i.e. in eastern Europe and the near East (e.g. Turkey, Iran and southern Russia), in several states of the United States of America and in South America (e.g. Brazil).

The seed-borne nature of the pathogen contributes to the bacterium spread around the globe. The use of healthy seeds is the most effective control measure against it. The role of other pathways (e.g. irrigation water and infected residues) for dissemination is uncertain, yet many crops and weeds are, or may be, hosts or reservoirs. Although the identity of the bacterium is well established and identification methods are available, its diversity in terms of phenotype (e.g. pigmented variants), genotype (e.g. virulence repertoires) and pathogenicity (differences in aggressiveness) is exceptional. Analysis demonstrated that *Curtobacterium flaccumfaciens* (*Cf*) strains that are pathogenic on bean are polyphyletic and scattered throughout the taxon, irrespective of their biological or geographical origin. Pathogenic and environmental strains cluster together.

The altered EU quarantine status of *Cff* is being challenged with this ambiguous situation. The bacterium can be introduced, it may establish, spread and have an impact on its host crops. The proposed research project should resolve the difficulties in identification of *Cf* strains pathogenic on bean and soybean and its detection on seeds, eventually complexed with detection of other bean associated bacterial pathogens. The results of the project should also contribute to a revision of the current classification in the genus *Curtobacterium*.

### 1.5. Description of the expected results

The first result is a collection of strains and isolates representing the biological and geographical diversity of *Cf*. The collection should cover all pathogens isolated from their (normal) host but also from non-host plants and from environmental samples. This collection is essential for the outcome of any research activity done in this project.

Further expected results are:

- Protocols for identification by phenotype characterization, DNA barcode(s), MALDI-TOF MS and whole genome sequencing
- Design and validation of TaqMan real-time PCR for detection
- Design and validation of a diagnostic LAMP test
- Virulence profile of benchmark strains by vascular infiltration and leaf spraying of bean and soybean cultivars
- A standardized and TPS validated detection test for analysis of seeds based on selective plating and molecular tests
- Underpinning data for classification of strains in *Curtobacterium*.

### 1.6. Beneficiaries of this research product

The results of the project can be used and implemented by plant health policy makers, seed companies, diagnostic plant health labs and the scientific community.

### 1.7. Research funders and research contribution/ distribution in the Euphresco network

Funding organisation	Research activity and researchers involved
1. Belgium: Flanders Research Institute for Agriculture, Fisheries and Food (ILVO), Unit Plant Sciences  Kris De Jonghe <a href="mailto:kris.dejonghe@ilvo.vlaanderen.be">kris.dejonghe@ilvo.vlaanderen.be</a>	-Project coordination; -Provide cultures for strain collection identification barcodes based on virulence; -Validation data of TaqMan and LAMP tests; -Organization and participation in TPS or PT on detection in bean seeds; -WGS and classification;  Contact person: Johan Van Vaerenbergh E.mail address: <a href="mailto:johan.vanvaerenbergh@ilvo.vlaanderen.be">johan.vanvaerenbergh@ilvo.vlaanderen.be</a>
2. French Agency for Food, Environmental and Occupational Health & Safety, France  Géraldine Antoine <a href="mailto:geraldine.anthoine@anses.fr">geraldine.anthoine@anses.fr</a>	-Strain collection (mainly non targets); -Instruction for identification by phenotype; - Characterization (including media for plating), DNA barcode(s), MLSA (Goncalves et al., 2019); -Validation of TaqMan real-time PCR for detection (performance data); -Participation in the TPS (selective plating and molecular tests);  Contact person: Amandine Cunty E.mail address: <a href="mailto:amandine.cunty@anses.fr">amandine.cunty@anses.fr</a>
3. Council for agronomic research and economic analysis, Italy	-Strain collection (non target strains) ; -Validation of TaqMan real-time PCR for



<p>Luca Riccioni  <a href="mailto:luca.riccioni@crea.gov.it">luca.riccioni@crea.gov.it</a></p>	<p>detection;          -Participation in TPS;</p> <p>Contact person: Stefania Loreti          E. mail address: <a href="mailto:stefania.loreti@crea.gov.it">stefania.loreti@crea.gov.it</a></p>
<p>4. 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>-Identification by MALDI-TOF mass spectrometry;          -Validation of TaqMan real-time PCRs;          -Participation in TPS;          -Virulence testing;</p> <p>Contact person: Chiel Pel          E. mail address: <a href="mailto:m.j.c.pel@nvwa.nl">m.j.c.pel@nvwa.nl</a></p>
<p>5. Ministry of Agriculture, Forestry and Food, Slovenia</p> <p>Erika Oresek  <a href="mailto:erika.oresek@gov.si">erika.oresek@gov.si</a></p>	<p>-Strain or DNA collection;          -Real-time PCR and LAMP verification;          -Identification of <i>Curtobacterium</i> strains isolated from plants with barcoding and MALDI-TOF MS;          -Participation in TPS;</p> <p>Contact person: Manca Pirc          E. mail address: <a href="mailto:manca.pirc@nib.si">manca.pirc@nib.si</a></p> <p>Contact person: Spela Alic          E. mail address: <a href="mailto:spela.alic@nib.si">spela.alic@nib.si</a></p>
<p>6. Department for Environment, Food and Rural Affairs, United Kingdom</p> <p>Iain Dummett (Mr)  <a href="mailto:Iain.Dummett@defra.gov.uk">Iain.Dummett@defra.gov.uk</a></p>	<p>-Strain collection, in particular isolates of unknown virulence and from countries where the disease is present;          -WGS and identification of virulence markers participation in TPS;</p> <p>Contact person: Edward Haynes          E. mail address: <a href="mailto:Edward.Haynes@fera.co.uk">Edward.Haynes@fera.co.uk</a></p> <p>Contact person: Joana Vicente          E. mail address: <a href="mailto:joana.vicente@fera.co.uk">joana.vicente@fera.co.uk</a></p>
<p>7. Agroscope, Switzerland</p> <p>Christophe Debonneville  <a href="mailto:christophe.debonneville@agroscope.admin.ch">christophe.debonneville@agroscope.admin.ch</a></p>	<p>-Strain virulence in plants;          -Development/validation of molecular tests;</p> <p>Contact person: Christophe Debonneville          E. mail address: <a href="mailto:christophe.debonneville@agroscope.admin.ch">christophe.debonneville@agroscope.admin.ch</a></p>
<p>8. University of Florence, Italy</p> <p>Stefania Tegli  <a href="mailto:stefania.tegli@unifi.it">stefania.tegli@unifi.it</a></p>	<p>The Molecular Plant Pathology Laboratory has a wide collections of <i>Cff</i> strains having different geographical origin, containment laboratories and greenhouses for <i>in planta</i> tests, to contribute to:</p> <p>-Design and validation of PCR-based diagnostic tests and of selective/semi-selective culture media for <i>Cff</i>.          -Identification and validation of biochemical traits and virulence/pathogenicity</p>



	<p>determinants useful as a marker for <i>in situ</i> detection of <i>Cff</i>, by combining transcriptomic and phenotype microarray approaches with spectroscopic analysis;</p> <p>Contact person: Stefania Tegli E.mail address: <a href="mailto:stefania.tegli@unifi.it">stefania.tegli@unifi.it</a></p>
<p>9. Naktuinbouw, the Netherlands</p> <p>Harrie Koenraadt <a href="mailto:h.koenraadt@naktuinbouw.nl">h.koenraadt@naktuinbouw.nl</a></p>	<p>-Strain collection &amp; WGS; -Validation of TaqMan real-time PCR; -Seed test; -Participation in TPS;</p> <p>Contact person: Harrie Koenraadt E.mail address: <a href="mailto:h.koenraadt@naktuinbouw.nl">h.koenraadt@naktuinbouw.nl</a></p>
<p>10. Nigeria Agricultural Quarantine Service, Post-Entry Quarantine and Diagnostic Station, Nigeria</p> <p>Kazeem Shakiru Adewale E.mail address: <a href="mailto:shakiru.kazeem@naqs.gov.ng">shakiru.kazeem@naqs.gov.ng</a></p>	<p>-Participation in the TPS; -Other contributions to be detailed;</p> <p>Contact person: Kazeem Shakiru Adewale E.mail address: <a href="mailto:shakiru.kazeem@naqs.gov.ng">shakiru.kazeem@naqs.gov.ng</a></p>
<p>11. Shiraz University, Iran</p> <p>Ebrahim Osdaghi <a href="mailto:eosdaghi@shirazu.ac.ir">eosdaghi@shirazu.ac.ir</a></p>	<p>-The group holds over 50 strains isolated in Iran (including pathogenic and non-pathogenic strains), greenhouse facility for <i>in planta</i> tests and naturally infected bean seeds for the validation of seed tests involving plating, real-time PCR and LAMP tests; -Virulence mechanisms and pathogenicity of <i>C. flaccumfaciens</i>. LAMP primers developed in the laboratory will be validated in the project;</p> <p>Contact person: Ebrahim Osdaghi E.mail address: <a href="mailto:eosdaghi@shirazu.ac.ir">eosdaghi@shirazu.ac.ir</a></p>
<p>12. National Institute of Biosecurity and Microbial Forensics-Oklahoma State University, United States of America</p> <p>Kitty Cardwell <a href="mailto:kitty.cardwell@okstate.edu">kitty.cardwell@okstate.edu</a></p>	<p>-Contribution to be detailed;</p> <p>Contact person: Kitty Cardwell E.mail address: <a href="mailto:kitty.cardwell@okstate.edu">kitty.cardwell@okstate.edu</a></p> <p>Contact person: Andres Espindola Camacho E.mail address: <a href="mailto:andres.espindola@okstate.edu">andres.espindola@okstate.edu</a></p> <p>Contact person: Francisco Ochoa-Corona E.mail address: <a href="mailto:ochoaco@okstate.edu">ochoaco@okstate.edu</a></p>

### 1.8. 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 that the topic is advertised outside the Euphresco network

### 1.9. Any other relevant information on content

Curators of EPPO Q-bank for Bacteria will be involved for the relevant tasks on collections, strains and identification barcodes.

Relevant papers used for elaboration of the topic description:

- Osdaghi E, Young AJ & Harveson RM (2020). Pathogen Profile. Bacterial wilt of dry beans caused by *Curtobacterium flaccumfaciens* pv. *flaccumfaciens*: a new threat from an old enemy. *Molecular Plant Pathology* DOI: 10.1111/mpp.12926.
- Osdaghi E, Taghavi SM, Calamai S, Biancalani C, Cerboneschi M, Tegli S & Harveson RM (2018). Phenotypic and molecular-phylogenetic analysis provide novel insights into the diversity of *Curtobacterium flaccumfaciens*. *Phytopathology* 108:1154-1164. DOI: 10.1094/PHYTO-12-17-0420-R.
- Gonçalves RM, Balbi-Peña MI, Soman JM, Maringoni AC, Taghouti G, Fischer-Le Saux M & Portier P (2019). Genetic diversity of *Curtobacterium flaccumfaciens* revealed by multilocus sequence analysis. *European Journal of Plant Pathology* 154:189–202. DOI: 10.1007/s10658-018-01648-0.
- Harveson RM, Schwartz HF, Urrea CA & Yonts CD (2015). Bacterial wilt of dry-edible beans in the Central High Plains of the U.S.: past, present and future. *Plant Disease* 99:1665-1677. DOI: 10.1094/PDIS-03-15-0299-FE.
- Tegli S, Sereni A, Surico G (2002). PCR-based assay for the detection of *Curtobacterium flaccumfaciens* pv. *flaccumfaciens* in bean seeds. *Letters in Applied Microbiology* 35: 331-337.
- Jeger M, *et al* (2018). Pest categorisation of *Curtobacterium flaccumfaciens* pv. *flaccumfaciens*. *EFSA Journal* 16(5):5299.

## 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. ILVO (BE)	NC	€
2. ANSES (FR)	NC	€
3. CREA (IT)	NC	€
4. NVWA (NL)	NC	€
5. MAFF (SI)	NC	€
6. Defra (GB)	NC	€
7. Agroscope (CH)	NC	€
8. UNIFI (IT)	NC	€
9. Naktuinbouw (NL)	NC	€
10. NAQS (NG)	NC	€
11. Shiraz (IR)	NC	€
12. OkState (US)	NC	€
total		€

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

36 months

### 2.3. Identification of project coordinator

Has the research project coordinator been identified?

Yes

No

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

None.

<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.