

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

### 1.1. Topic area

A: 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

### 1.3. Topic title

Use of new diagnostic tools for the detection of *Pantoea stewartii* subsp. *stewartii* from plant and seeds

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

*Pantoea stewartii* subsp. *stewartii* (Pss) is indigenous to the Americas and has been introduced to other parts of the world (mainly Asia) probably by trade with maize seeds. It causes a devastating disease called Stewart's wilt and as a result, quarantine restrictions are in place to prevent spread of this pathogen. The principal host is *Zea mays* (maize), especially sweet corn, but dent, flint, flour and popcorn cultivars can also be infected. In the Americas, *Chaetocnema pulicaria* Melsheimer (Coleoptera: Chrysomelidae) is the only insect known as efficient vector and the main overwintering host of the bacterium. It subsequently introduces the pathogen into feeding wounds via its feces when the crop emerges in spring (Roper, 2011; Correa *et al.*, 2012). Pss subsequently colonizes the interstitial spaces in maize leaf tissues, where it causes the development of water-soaked lesions, and subsequently colonizes the xylem, resulting in systemic spread and wilting. The pathogen is then acquired by the last seasonal generation of the corn flea beetle in which it again overwinters (Roper, 2011). Pss can be transmitted through seeds, and has occasionally been found to be able to overwinter in soil, manure or maize stalks too.

In contrast, the other member of the species *P. stewartii*, *Pantoea stewartii* subsp. *indologenes* (Psi), is avirulent on maize and was long considered as non-pathogenic. However, it has been linked to symptom development on pearl and foxtail millet, *Eucalyptus*, cluster bean and rot of pineapple fruit, suggesting that unlike the Pss this subspecies has a broader host range (Mergaert *et al.*, 1993; Coutinho *et al.*, 2002). It is important to notice that Psi can be found on corn seeds.

In Europe, outbreaks have been occasionally described (Austria, 1992; Ukraine, 2014). In Italy within the framework of the surveillance of the territory focussing on corn seed crops, two fields with symptoms of *Pantoea stewartii* subsp. *stewartii* confirmed by laboratory analyses, were detected in 2015 and 2016. In June 2017, symptomatic leaves were sampled from maize plants in Friuli-Venezia Giulia region. In August 2017, the identity of the bacterium was confirmed. In the infected area (7 ha) and its surroundings, maize is grown for animal feed only. The source of this outbreak is still unknown, but investigations are currently carried out to clarify the origin of the seeds used. In conclusion, this bacterium constitutes a high risk of emergence for Europe.

The American vector is not present in Europe. The possibility of transmission by other potential European insect(s) is a current question.

EPPO published a new version of the protocol PM7/60 in 2016. This improved protocol allows using molecular tools for detecting Pss in plants, seeds and on pure cultures. As Pss and Psi are phylogenetically closely related, the current molecular tools are not accurate enough to

distinguish between them (Tambong *et al.*, 2008; Euphresco report 2011<sup>1</sup>). The risk of false positive result is high when Psi is present in corn seeds.

Moreover, the available tools do not allow distinguishing among strains of Pss and therefore do not allow to trace the geographic origin of the strains for example.

Most of the published diagnostic tests are intended for use on pure cultures. Producing data on the respective limits of detection of these tests would be relevant and particularly when analyses are performed directly on seed samples. DNA extraction may be needed and can also affect the limit of detection.

Seed-to-seedling transmission of Pss may occur at low rates in seed lots. For testing seed lots, the recommended sample size is 400 seeds per lot. This size of sample is questionable for maize. The project will provide a platform to discuss the issue.

### 1.5. Description of the expected results

The proposal aims to compare and validate detection/identification molecular tests and to improve the diagnostic protocol using DNA sequencing in order to differentiate *P. stewartii* subsp. *stewartii* strains and contribute to identify their geographic origin.

The tests considered will be those based on specific primers (Thapa *et al.*, 2012; Gehring, 2014; new CREA primers), and those based on a housekeeping gene scheme (Brady *et al.*, 2008; Wensing *et al.*, 2010; De Maayer, 2017). The abovementioned tests will be adapted to be used directly on seed samples (including DNA extraction step).

A collaborative test performance study will be organised, to verify the robustness and the reproducibility of the data in condition of different laboratories. The TPS will be organised on pure cultures and on seeds.

Recommendations on increasing the sample size for testing seed lots will be developed.

### 1.6. Beneficiaries of this research product

The project will benefit to National and EU policy makers, National Plant Protection Organisations, including risk managers and diagnosticians, EPPO and its members (validated diagnostic protocols; information contributing to EPPO PRAs), maize industry and other stakeholders testing laboratories including National Reference Laboratories.

### 1.7. Research funders and research contribution/ distribution

Funding organisation	Research activity and researchers involved
1. Anses, French Agency for Food, Environmental and Occupational Health & Safety, France  Geraldine Anthoine <a href="mailto:geraldine.anthoine@anses.fr">geraldine.anthoine@anses.fr</a>	<ul style="list-style-type: none"> <li>- Bibliography on molecular tools and protocols available on Pss detection;</li> <li>- Coordination of the comparison of some specific detection tools;</li> <li>- Evaluation of relevant primers to be used directly on seed samples;</li> <li>- Organization and participation to a test performance study;</li> <li>- Coordination on discussion on sample size;</li> </ul> Contact person: Valerie Olivier E.mail: <a href="mailto:valerie.olivier@anses.fr">valerie.olivier@anses.fr</a>
2. Austrian Agency for Health and Food Safety, Austria  Sylvia Blümel	<ul style="list-style-type: none"> <li>- Participation in actions 1 and 2: comparison of some specific detection tools;</li> <li>- Evaluation of relevant primers to be used directly on seed samples;</li> </ul>

<sup>1</sup> [https://www.euphresco.net/media/project\\_reports/pantoea\\_final\\_report.pdf](https://www.euphresco.net/media/project_reports/pantoea_final_report.pdf)



<p><a href="mailto:sylvia.bluemel@ages.at">sylvia.bluemel@ages.at</a></p>	<p>- participation to a test performance study;</p> <p>Contact person: Richard Gottsberger E.mail: <a href="mailto:richard.gottsberger@ages.at">richard.gottsberger@ages.at</a></p>
<p>3. Agricultural Research and analysis of the Economy Council, Italy</p> <p>Luca Riccioni <a href="mailto:luca.riccioni@crea.gov.it">luca.riccioni@crea.gov.it</a></p>	<p>- Design new primers based on Italian strains, if possible; - Sequence the whole genome of Pss and write a pipeline;</p> <p>Contact person: Valeria Scala E.mail: <a href="mailto:valeria.scala@crea.gov.it">valeria.scala@crea.gov.it</a></p>
<p>4. All-Russian Plant Quarantine Centre, Russia</p> <p>Natalia Sherokolava <a href="mailto:natalia_sh@mail.ru">natalia_sh@mail.ru</a></p>	<p>-Contribution to be detailed</p> <p>Contact person: Konstantin Kornev Email address: <a href="mailto:konstantin.kornev@gmail.com">konstantin.kornev@gmail.com</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>- Methods for distinguishing among <i>Pantoea</i> spp. and <i>Pantoea</i> subspecies; - Characterization of <i>Pantoea</i> spp. isolated from maize; - Sharing of previously obtained data on performance of the methods;</p> <p>Contact person: Tanja Dreo E.mail: <a href="mailto:Tanja.dreo@nib.si">Tanja.dreo@nib.si</a></p>

**a. 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

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

**b. Any other relevant information on content**

The work produced among all laboratories will be contribute to the next version of the EPPO protocol PM7/60 and could be valorised through communication to international congress. In addition, publication in a scientific journal would be considered according to results.

## 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. Anses (FR)	NC	€ 46 100
2. AGES (AT)	NC	€ 14 526.60
3. CREA (IT)	NC	€ 5 000
4. FGBU-VNIKR (RU)	NC	€ 10 000
5. NIB (SI)	NC	€ 8 000
total		€ 83 626.6

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

30 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

<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.