

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-1.1: to improve knowledge on the biology, epidemiology and ecology of priority invasive and (re)emerging pests
- Objective 2017-R-1.2: to support taxonomic research for the unambiguous identification of pests
- Objective 2017-R-6.1: to test and validate methods for in situ detection and identification of pests

1.3. Topic title

Sampling and analysis of asymptomatic *Citrus* fruits and leaf litter to detect the infection of *Phyllosticta citricarpa*.

1.4. Description of the problem the research should solve

Inspection of *Citrus* fruits at the border for the detection of *Citrus Black Spot* (CBS) is mostly based on visual examination followed by laboratory testing of fruits exhibiting suspect symptoms. However, due to the long incubation period of *P. citricarpa*, CBS symptoms on fruits are visible only at maturity, several months after infection (EFSA PLH Panel, 2014).

Phyllosticta citricarpa has recently been detected in leaf litter in some EPPO citrus-growing countries although CBS symptoms were not observed on tree canopy (Guarnaccia *et al.*, 2017). Although surveys are being conducted in the European countries involved in the paper of Guarnaccia *et al.* (2017), more intensive surveys have to be conducted by NPPOs in EPPO citrus-producing countries to evaluate the pest status in their territory. EFSA is currently developing a survey card to provide guidance on how to conduct the surveys to determine the pest status in EU citrus-producing Member States and raised the need to include asymptomatic fruits in the survey. Moreover, a new *Phyllosticta* species, namely *P. paracitricarpa*, detected in citrus leaf litter in Greece, has been reported to cause "atypical" CBS symptoms on citrus fruits following artificial inoculation (Guarnaccia *et al.*, 2017). So far, this species has not been included in the EU quarantine list of harmful organisms.

In Argentina and Brazil (countries where CBS is present) exporters are performing an Ethephon treatment (Baldassari *et al.*, 2007) to enhance symptom expression on fruits in order to avoid that exported infected, though asymptomatic, fruits are rejected upon arrival in Europe. In order to consider asymptomatic fruits in the CBS survey, such treatment could be envisaged to be included as methodology in the CBS survey plan for fruits harvested in European orchards. However, concerns have been raised on the efficacy and practical feasibility of the proposed treatment and if it would also be extended to imported fruits.

In the framework of the proposed Euphresco project, an evaluation of the Ethephon treatment will be performed before considering including this methodology as an obligatory step in survey plans. Fruits collected in Italy and Greece where *P. citricarpa* or *P. paracitricarpa* have been respectively detected could also be included in the evaluation.

Molecular methods, in particular real-time PCR, are already available to detect and identify *P. citricarpa*; some of those methods are included in the EPPO Diagnostic Protocol PM 7/17(2) and others have recently been published. However, those methods have not been validated to detect the fungus on asymptomatic citrus tissues. In addition, a critical point is that so far these methods do not allow the distinction between *P. citricarpa* and *P. paracitricarpa* (Guarnaccia *et al.*, 2017).

Therefore, it is important to evaluate the currently available molecular methods and newly developed tests for their ability to detect and identify *P. citricarpa* on asymptomatic infected citrus tissues, and to identify the best sampling strategy to be used for this purpose.

1.5. Description of the expected results

A Diagnostic Protocol to detect *P. citricarpa* and *P. paracitricarpa* on asymptomatic citrus tissues that could include:

- Relevant sampling methodologies;
- Methods to stimulate early development of symptoms caused by *P. citricarpa* and *P. paracitricarpa*, including methods to stimulate the production of pycnidiospores on infected tissues;
- Validated molecular methods to identify *P. citricarpa* in asymptomatic fruit and leaf tissues.

1.6. Beneficiaries of this research product

- National Plant Protection Organisations (NPPOs)
- EPPO and International Plant Protection Organisations.
- Plant Health diagnostic labs
- Nurseries, farmers and others directly involved in the citrus industry.

1.7. Research funders and research contribution/ distribution

Funding organisation	Research activity and researchers involved
1. Agricultural Research and analysis of the Economy Council, Italy Luca Riccioni luca.riccioni@crea.gov.it	-Development and validation of a protocol to detect <i>P. citricarpa</i> on asymptomatic citrus tissues; -Evaluation of ethephon treatment on fruits, including samples collected in the Italian site where <i>P. citricarpa</i> has been reported E-mail address; -Evaluation of molecular methods to analyse asymptomatic fruit and leaves for <i>P. citricarpa</i> ; Contact person: Luca Riccioni E-mail address: luca.riccioni@crea.gov.it
2. French Agency for Food, Environmental and Occupational Health & Safety, France Géraldine Anthoine geraldine.anthoine@anses.fr	-Validation of a protocol to detect <i>P. citricarpa</i> on asymptomatic tissues; Contact person: Renaud Ioos E-mail address: renaud.ioos@anses.fr
3. Benaki Phytopathological Institute, Greece Irene Vloutoglou i.vloutoglou@bpi.gr	-Development and validation of a protocol to detect <i>P. citricarpa</i> on asymptomatic citrus tissues through the evaluation of Ethephon treatment on fruit, including samples collected in the Greek site where <i>P. paracitricarpa</i> has been reported; -Molecular methods to analyse asymptomatic fruit and leaves for <i>P. citricarpa</i> ; Contact person: Irene Vloutoglou E-mail address: i.vloutoglou@bpi.gr

	<p>Contact person: Eleni Kalogeropoulou E-mail address: e.kalogeropoulou@bpi.gr</p> <p>Contact person: Dimitrios Tsirogiannis E-mail address: d.tsirogiannis@bpi.gr</p>
<p>4. Centro de Citricultura Sylvio Moreira, Brazil</p> <p>Helvecio Della Coletta-Filho hdcoletta@ccsm.br</p>	<p>-Evaluation of protocols in asymptomatic fruits under Brazilian field conditions; -Prevalence of mating type of <i>P. citricarpa</i> in CBS lesions;</p> <p>Contact person: Helvecio Della Coletta-Filho E-mail address: hdcoletta@ccsm.br</p>
<p>5. European and Mediterranean Plant Protection Organization, International</p> <p>Françoise Petter fp@eppo.fr</p>	<p>-Revision of the EPPO Diagnostic Protocol on <i>P. citricarpa</i>;</p> <p>Contact person: Françoise Petter E-mail address: fp@eppo.fr</p>
<p>6. Ministry of Agriculture, Tunisia</p> <p>Mohamed Lahbib Ben Jamaâ benjamaaml@gmail.com</p>	<p>-Provision of asymptomatic fruits; -Development of a protocol for <i>P. citricarpa</i> detection on asymptomatic citrus tissues under Tunisian conditions; -Molecular tools to detect the pathogen from asymptomatic fruits and leaves;</p> <p>Contact person: Naima Boughalleb E-mail address: n.boughalleb2017@gmail.com</p>

1.8. Research project partnership outside Euphresco

Partners from Argentina and Tunisia will be invited, as well as research groups from other European countries like Spain, Malta and Portugal. The collaboration/involvement of EFSA is also considered important.

1.9. Any other relevant information on content

References :

- Braga Baldassari, R., Brandimarte, I., Gustavo de Andrade, A., Cestari Gonçalves de Souza, D., Moretto, C., and Goes, A. 2007. Induction of the precece expression of *Guignardia citricarpa* symptoms in fruits of pera-rio sweet orange. Revista Brasileira de Fruticultura 29:269-275.
- EFSA Panel on Plant Health. 2014. "Scientific Opinion on the risk of *Phyllosticta citricarpa* (*Guignardia citricarpa*) for the EU territory with identification and evaluation of risk reduction options." EFSA Journal 12(2): 3557.
- Guarnaccia, V., Groenewald, J. Z., Li, H., Glienke, C., Carstens, E., Hattingh, V., Fourie, P. H., and Crous, P. W. 2017. First report of *Phyllosticta citricarpa* and description of two new species, *P. paracapitalensis* and *P. paracitricarpa*, from citrus in Europe. Stud. Mycol. 87:161-185.

2. Euphresco management aspects of the project

2.1. Indication of the topic budget

Funding organisation ^a	Mechanism ^b	Total Budget ^c
1. CREA (IT)	NC	€
2. ANSES (FR)	NC	€
3. BPI (GR)	NC	€
4. CCSM (BR)	NC	€
5. EPPO (International)	NC	€
6. MoA (TN)	NC	€
total		€

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

24 months (1-3-2020 – 28-2-2021).

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

If a project coordinator is not identified by 2019-11, the project will not be initiated.

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

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

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