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-2.1: to improve knowledge on emerging pathways of entry and means of spread for pests
☒ Objective 2017-R-2.2: to expand knowledge on transmission of disease and pathogens for healthy planting material
☒ Objective 2017-R-6.1: to test and validate methods for in situ detection and identification of pests

1.3. Topic title

Diagnosis of *Xylella fastidiosa*: detection on dormant plants, important for Mediterranean countries

1.4. Description of the problem the research should solve

Xylella fastidiosa is a gram-negative, xylem-limited, and slow-growing bacterium transmitted by some xylem-feeding vectors, and it is the causal agent of several plant diseases. The concentration of the bacterium in a plant depends upon environmental factors, strains and the host plant species or cultivars. This is particularly true for deciduous plant species. The EPPO Diagnostic Protocol PM 7/24 gives some recommendations for sampling according to host plants, seasons and locations. According to Hopkins (1981), sampling should preferably be performed during the period of active growth of the plant to maximize the likelihood of detection. Recent experiments conducted in the framework of EU projects POnTE and XF-ACTORS have shown that in Mediterranean countries *Xylella fastidiosa* can be detected in plants (such as almond and cherry) all over the year and especially during the asymptomatic phases or the dormancy, the period with the lowest bacterial concentration. Although detecting *Xylella fastidiosa* in dormant plants was shown possible in some cases, the performance of the tests is dependent on the plant species and the geographical locations. The current EPPO Diagnostic Protocol PM 7/24 mentions: 'Experience in temperate areas shows that in grapevine or deciduous trees, e.g. cherry and almond, that have been infected for some time, the bacterium is not detected into the new season's growth until the middle of summer, when symptoms may also become visible. For example, the most suitable time for searching for symptoms in grapevine is late summer to early autumn when weather conditions are predominantly hot and dry or when grape plants are exposed to drought stress (Galvez et al., 2010).' In this context, this Euphresco project aims at evaluating the distribution dynamics of *Xylella fastidiosa* within dormant Mediterranean plants and matrices (such as almond, cherry, grapevine) that are commercially important throughout the year and during dormancy on woody stems.

1.5. Description of the expected results

The project will:

- **WP1 Inventory**

1.1. Make an inventory of dormant plants that could be infected by *Xylella fastidiosa*, focusing on Mediterranean plants and evaluate if infected dormant plants are available for sampling during the year;

1.2. Make an inventory of methods (sampling, DNA extraction, diagnostic tests) used by the different laboratories for the detection of *Xylella fastidiosa* in dormant plants

- **WP2 Evaluation of methods on spiked samples**

2.1. Collect and share dormant plant material important for the Mediterranean region
2.2. Evaluate diagnostic tests selected by participants for the diagnosis of *Xylella fastidiosa* (sub)species in spiked samples of different dormant plant species (i.e. olive, grape) at low bacterial concentrations (test performance study)
2.3 Evaluate the influence of the plant matrix collected at different period of the year on the diagnosis of *Xylella fastidiosa* (sub)species in spiked samples of dormant plant species at low bacterial concentrations.

- **WP3 Evaluation of methods on naturally infected samples**

3.1. Collect and share dormant plant material naturally infected by *Xylella fastidiosa*
3.2. Evaluate diagnostic tests selected by participants for the detection of *Xylella fastidiosa* in dormant plants naturally infected and validate protocols (sampling, DNA extraction, PCR)
3.3. If material is available, evaluate the distribution dynamics of *Xylella fastidiosa* within naturally infected woody stems throughout the year (including during dormancy)

1.6. Beneficiaries of this research product

The project will benefit:

- Official laboratories responsible for the diagnosis of *Xylella fastidiosa*
- National and international policy makers
- National and Regional Plant Protection Organisations, including risk managers and diagnosticians
- Growers and nurseries

1.7. Contribution/ distribution

In the framework of the CIHEAM-Euphresco initiative on the Plant Health research priorities for the Mediterranean region[1], the following organizations have preliminarily expressed an interest to be involved in this research project:

Funding organisation	Research activity and researchers involved
1. French Agency for Food, Environmental and Occupational Health and Safety, Plant health laboratory, France Géraldine Anthoine geraldine.anthoine@anses.fr	-Project coordination; -Sharing of protocols; -Sharing of plant material, if available; -Test validation; -Xf diagnostic test evaluation on dormant plants; Contact person: Anne-Laure Boutigny E.mail address: anne-laure.boutigny@anses.fr
2. Department of Agriculture, Water and the Environment, Australia Keira Beattie PHSgovernancegroups@agriculture.gov.au	-Sharing of protocols; -Assistance in protocol validation; -Participation in the test performance study; Contact person: Toni Chapman E.mail address:

[1] See Supplement 1 : Compendium on the plant health research priorities for the Mediterranean region https://zenodo.org/record/6805519#.YtbDZ3ZBzct

	toni.chapman@dpi.nsw.gov.au Contact person: Fiona Constable E.mail address: fiona.constable@agriculture.vic.gov.au
3. Flanders Research Institute for Agriculture, Fisheries and Food, Belgium Kris de Jonghe Kris.DeJonghe@ilvo.vlaanderen.be	-Contribution to be detailed; Contact person: Jolien Venneman E.mail address: Jolien.Venneman@ilvo.vlaanderen.be
4. Central Administration of Plant Quarantine, and Plant Pathology Research Institute, Ministry of Agriculture and Land Reclamation, Egypt Ahmed Kamal El-Attar ippc@capq.gov.eg	-Contribution to protocols validation (sampling, DNA extraction, PCR); -Participation in the evaluation of the distribution dynamics of *Xylella fastidiosa* throughout the year; -Sharing of protocols; -Sharing of plant materials to be used in ring test; Contact person: Kamel Elhalag E.mail address: kamel_moon_82@yahoo.com Contact person: Nevein Messiha E.mail address: nevein_messiha@yahoo.com
5. Department for Environment Food and Rural Affairs, United Kingdom Jasmine Burr-Hersey Jasmine.Burr-Hersey@defra.gov.uk	Contribution to be detailed: Contact person: E.mail address:
6. Science and Advice for Scottish Agriculture, United Kingdom David Kenyon david.kenyon@sasa.gov.scot	-Inventory of methods (sampling, DNA extraction, PCR) used by the different laboratories; -Validation of protocols (sampling, DNA extraction, PCR); Contact person: David Kenyon E.mail address: david.kenyon@sasa.gov.scot
7. Ministry of Agriculture, Plant Biosecurity, Plant Protection and Inspection Services, Israel Yael Meller Harel YaelM@moag.gov.il	-Detection of *Xylella fastidiosa* in dormant grapevine; -Study of *Xylella fastidiosa* infection in grapevine throughout the year; -Contributing to protocols validation (sampling, DNA extraction, PCR); Contact person: Yael Meller Harel E.mail address: yaelm@moag.gov.il
8. Department of Agriculture, Food and Marine, Ireland Maria Destefanis Maria.Destefanis@agriculture.gov.ie	-Share diagnostic protocols; -Validation of protocols (sampling, DNA extraction, PCR); Contact person: Thuy Do E.mail address: Thuy.Do@agriculture.gov.ie

9. Council for agronomic research and economic analysis, Italy Pio Federico Roversi piofederico.roversi@crea.gov.it	-Inventory of methods (sampling, DNA extraction, PCR) used by the different laboratories; -Development of a multiplex PCR based on the amplification of two/seven housekeeping genes to be used for Nanopore amplicon sequencing based on Faino *et al.* (2021); -Validation of protocols (sampling, DNA extraction, PCR); -Comparison among real-time PCR (Harper *et al*., 2010) and/or digital droplet PCR (Dupas *et al*., 2019) with Nanopore amplicon sequencing (Faino *et al*., 2021) in spiked samples, of different plant species (i.e. olive, grape) at low bacterial concentrations; -Evaluation of the influence of the plant matrix collected in different period of the year in the detection/identification of *Xylella fastidiosa* in spiked samples at low bacterial concentration; -Verification of the possible influence of inhibitors on the following tests: real-time PCR (Harper *et al*., 2010) and tetraplex real-time PCR (Dupas *et al*., 2019); -If material is available, evaluation of the distribution dynamics of *Xylella fastidiosa* throughout the year and during dormancy on woody stems; -Sampling of host plants in the area adjacent to the recent discovery of *Xylella fastidiosa* infected almond tree in Lazio region (Italy) and/or in the infected area of Monte Argentario, Tuscany region (Italy); Contact person: Stefania Loreti E.mail address: stefania.loreti@crea.gov.it
10. National Research Council, Italy Maria Saponari maria.saponari@ipsp.cnr.it	-Collection and sharing of plant material from outbreak area in Apulia (infected and not infected almond/cherry plant material) that could be used to prepare the samples for the ring test; -Participation in the test performance study; Contact person: Maria Saponari E.mail address: maria.saponari@ipsp.cnr.it; Contact person: Giuliana Loconsole E.mail address: giuliana.loconsole@ipsp.cnr.it
11. Institute for Agricultural Research, Morocco Faouzi Bekkaoui faouzi.bekkaoui@inra.ma	-Inventory of methods (sampling, DNA extraction, PCR) used by the different laboratories for testing plants and vectors; -Participation in the test performance study; Contact person: Samir Fakhour E.mail address: samir.fakhour@inra.ma

	Contact person: Zineb Belabess E.mail address: zineb.belabess@inra.ma Contact person: Khaoula Habbadi E.mail address: khaoula.habbadi@inra.ma Contact person: Fouad Mokrini E.mail address: fouad.mokrini@inra.ma
12. Netherlands Food and Consumer Products Safety Authority, Netherlands Martijn Schenk M.Schenk1@nvwa.nl	-Evaluate, compare and validate different DNA isolation and different detection methods; Contact person: M.J.Chiel Pel E.mail address: m.j.c.pel@nvwa.nl
13. Ministry of Agriculture, Forestry and Food, Slovenia Erika Oresek erika.oresek@gov.si	-Participation in the experiments for the detection in dormant plants with different molecular tests; -Quantify the concentration of *Xylella fastidiosa* in dormant plants with digital real time PCR; Contact person: Manca Pirc E.mail address: manca.pirc@nib.si
14. Valencian Institute of Agronomic Research, Spain Ester Marco Noales marco_est@gva.es	-Participation in the test performance study; - Potentially, evaluation of the distribution dynamics of *Xylella fastidiosa* throughout the year and during dormancy on woody stems; Contact person: Ester Marco Noales E.mail address: marco_est@gva.es
15. Ministry of Food Agriculture and Forestry, General Directorate of Food and Control, Turkey Suat Kaymak suatkaymak@tarimorman.gov.tr	-Surveillance study for ealy detection (the pathogen is not present in Turkey); -Participation in protocol validation and in the test performance study; Contact person: Nursen Üstün E.mail address: nursen.ustun@tarimorman.gov.tr
16. US Department of Agriculture, Animal and Plant Health Inspection Service, United States of America Heike Meissner heike.e.meissner@usda.gov	-Sharing of protocols; -Assistance in protocol validation; -Participation in the test performance study; Contact person: Jarred Yasuhara-Bell E.mail address: jarred.yasuhara-bell@usda.gov

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

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

1.9. Any other relevant information on content

None.

2. *Euphresco management aspects of the project*

2.1. Indication of the topic budget

Funding organisation	Mechanism	Total Budget
1. Anses (FR)	NC	€
2. DAWE (AU)	NC	€
3. ILVO (BE)	NC	€
4. CAPQ (EG)	NC	€
5. Defra (GB)	NC	€
6. SASA (GB)	NC	€
7. MOAG (IL)	NC	€
8. DAFM (IE)	NC	€
9. CREA (IT)	NC	€
10. CNR (IT)	NC	€
11. INRA (MA)	NC	€
12. NVWA (NL)	NC	€
13. MAFF (SI)	NC	€
14. IVIA (ES)	NC	€
15. TARIMORMAN (TR)	NC	€
16. APHIS (US)	NC	€

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

24 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.