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-1.1: 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-2.2: to expand knowledge on transmission of disease and pathogens for healthy planting material
☒ 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-I-2.1: to support data exchange, data use and re-use for the benefit of plant health research activities
☒ Objective 2017-I-2.2: to contribute to databases for plant pests identification and diagnostics

1.3. Topic title

Xylophilus ampelinus presence and accurate detection in nurseries and vineyards

1.4. Description of the problem the research should solve

Xylophilus ampelinus (Panagopoulos 1969) is present in some countries of Africa, Asia and Europe (Sarejanni 1939; Erasmus 1974; Komatsu 2015; EPPO 2021a). In the EPPO region *X. ampelinus* is considered a quarantine pest, posing a risk for wine and grape producing countries, including the Mediterranean and Balkan areas and Russia and countries involved in the trade of propagation plant material. Severe outbreaks were described at the end of the last century and later reported in Slovenia (Dreo *et al*., 2005). Similarly to *Xylella fastidiosa*, detection is complicated by the fastidious growth and difficult isolation of the pathogen (Moralejo *et al*., 2020; EPPO, 2021). Moreover, disease symptoms in *Vitis vinifera* may be caused by other organisms such as *X. fastidiosa* or *Xanthomonas campestris* pv. *viticola (Rodrigues et al. 2011, DROPSA 2016, EPPO 2021b)*. The unique certification scheme for pathogen-tested material of grapevine is focused on the need for inspection of nuclear rootstocks (EPPO, 2008). Attention is also given to the inspection at places of production (EPPO, 2018) highlighting the spread of the bacterium through cutting instruments. Furthermore, there is lack of knowledge of the whole diversity of strains causing bacterial blight in vine plants, as well as, on the epidemiology of this organism in the EPPO region and in other countries.
The EPPO diagnostic protocol on *Xylophilus ampelinus* was adopted in 2009 (EPPO PM 7/96). Since then, laboratories have generated a significant amount of data and have gathered experience with different plant matrices. Based on anecdotal observations there are concerns that the tests currently in use may not be specific enough, in particular for latent testing of grapevine roots. The aim of the project is to gather experiences, including those arising from the EU project Valitest (GA 773139), in order to improve the diagnosis of the bacterium and to propose revisions of the EPPO diagnostic protocol if required.
Reference collections may be a source of strains for research and routine diagnostic activities but many isolates are not recent. Moreover, only few articles are available and over the last six years only three genomes have been sequenced (NCBI, 25062021).

There is an urgent need to better understand this bacterium and to deliver tools for diagnostics and control of *X. ampelinus* on *Vitis vinifera*.
This Euphresco project intends to address the gaps in the knowledge and:

- collect existing data on the use and performance of diagnostic tests especially on a) cross-reactions/discrepant results among the tests and b) latent testing (e.g. woody grafted plants, dormant cutting and roots)
- Build knowledge to allow design of new/improved tests leading to a more specific and reliable latent testing
- generate genome sequences of different strains of *X. ampelinus* and/or relevant other bacteria to enable the identification of novel diagnostic markers and allow the design of novel tests.

1.5. Description of the expected results

The project aims to:

- Gather data on the frequency and nature of discrepant results observed in routine testing either in past or on-going surveys. Data on the incidence/prevalence of positive cases will be analysed using biostatistical and epidemiological methods, if relevant
- List currently circulating *X. ampelinus* strains, particularly those that are not available in culture collections. This task aims to characterize a broader collection of strains to improve an accurate understanding of the epidemiology of *X. ampelinus*. Screening of virulence genes and pathogenicity associated genes previously identified and retrieved from genome information available at the NCBI may provide information on the population structure of the bacterium
- Obtain genome sequences of selected strains of *X. ampelinus* and/or other strains of interest through HTS. These data will be used to potentially identify novel genetic markers and design new tests. If relevant, these tests will be employed by the participants in their routine testing in parallel to commonly used test. Depending on the results, the novel tests will be further evaluated in a test performance study, with the molecular method of standard EPPO PM 7/96
- Surveys vineyards and nurseries of the main grapevine production areas determine the incidence and distribution of *X. ampelinus* in the EPPO region. These surveys will be implemented by the collection of samples and their characterization, using classical and biomolecular tests selected from the EPPO standard, as well as other available tests
- Identify the risk of spreading with trade of cuttings, rootstocks and grafted plants and define effective procedures based on laboratory analysis to detect potential latent infections on these propagating plant materials. Data on the incidence/prevalence of positive cases will be analysed using biostatistical and epidemiological methods
- Identify genomic markers specific for *X. ampelinus* diversity to be used for accurate and sensitive detection and identification. The selection of new and specific markers will enable the production of new molecular detection methods
- Make available reference materials and robust sequence data (through Q-bank and Genbank) for microbiological, molecular studies and diagnostic purposes.

1.6. Beneficiaries of this research product

The intended users/ stakeholders of the research results are researchers, diagnosticians from quarantine laboratories, phytosanitary inspectors: farmers, and companies.

1.7. Research funders and research contribution/ distribution

Funding organisation	Research activity and researchers involved
1. National Institute for Agricultural and Veterinarian Research, Portugal	-Project coordination; -Isolation and molecular characterization of

Leonor Cruz leonor.cruz@iniav.pt	strains and lookalikes collected from vineyards and rootstocks from distinct varieties throughout Portugal; -Gathering data on discrepant results and supporting the analysis; -Selection of species and strains-specific DNA markers -Iidentification of genetic determinants of pathogenicity and virulence; Contact person: Leonor Cruz E-mail address: leonor.cruz@iniav.pt Contact person: Camila Fernandes E-mail address: camila.fernandes@iniav.pt
2. French Agency for Food, Environmental and Occupation Health and Safety, France Géraldine Anthoine geraldine.anthoine@anses.fr Philippe Reignault philippe.reignault@anses.fr	-Gathering data on discrepant results and supporting the analysis; -Genome sequencing of selected isolates with potential to identify novel diagnostic markers; -Design and evaluation of real-time PCR tests; -Gathering data on available diagnostic protocols and NCBI available sequences; -To participate in the TPS if organised; Contact person: Bruno Legendre E-mail address: bruno.legendre@anses.fr Contact person: Amandine Cunty E-mail address: amandine.cunty@anses.fr
3. National Research Institute for Agriculture, Food and Environment, France Jean-Pierre Rossi Jean-Pierre.Rossi@inrae.fr	-Import new strains of *Xylophilus ampelinus* from Europe and beyond in the CIRM-CFBP collection; -Acquire MLSA and MALDI-TOF data on the new strains; -Sequence the complete genome of up to 10 strains; Contact person: Perrine Portier E-Mail address: perrine.portier@inrae.fr
4. Council for Agrocnomic Research and Bioeconomy, Italy Elisa Angelini elisa.angelini@crea.gov.it	-Carry out surveys in vineyards and nurseries in the North of Italy (main areas of production); -Make available reference material if the pathogen is found; -Evaluation of diagnostic procedures; -Perform molecular characterization using the genes identified by the Consortium; -Participation in the TPS if organised; Contact person: Elisa Angelini E-mail address: elisa.angelini@crea.gov.it

	Contact person: Luisa Filippin E-mail address: luisa.filippin@crea.gov.it
5. Ministry of Agriculture, Forestry and Food, Slovenia Erika Orešek erika.oresek@gov.si	-Gathering data on discrepant results and supporting the analysis; -Genome sequencing of selected isolates with potential to identify novel diagnostic markers; -Design and evaluation of real-time PCR tests; Contact person: Tanja Dreo E-mail address: tanja.dreo@nib.si
6. Agroscope, Switzerland Christophe Debonneville christophe.debonneville@agroscope.admin.ch	-Survey of swiss vineyards; -Participation to the high-throughput sequencing analysis; Contact person: Christophe Debonneville E-mail address: christophe.debonneville@agroscope.admin.ch
7. Friuli Venezia Giulia Phytosanitary Service, Italy Gian Luca Bianchi gianluca.bianchi@ersa.fvg.it	-Gathering data on discrepant results and supporting the analysis; -Monitoring grapevine nurseries of Friuli Venezia Giulia region; -Design and evaluation of additional real-time PCR tests; -Isolation trials on samples collected from grapevine nurseries and other plants; -Participation in the TPS if organised; Contact person: Gian Luca Bianchi E-mail address: gianluca.bianchi@ersa.fvg.it Contact person: Francesca De Amicis E-mail address: francesca.deamicis@ersa.fvg.it
8. National Research Council of Italy, Italy Slavica Matic slavica.matic@ipsp.cnr.it	-Gathering data on available diagnostic protocols and NCBI available sequences; -Performance of selected detection methods; -Search for possible new isolates in the field; Contact person: Slavica Matic E-mail address: slavica.matic@ipsp.cnr.it Contact person: Emanuela Noris E-mail address: emanuela.noris@ipsp.cnr.it
9. University of Naples Federico II, Italy Antonio Evidente evidente@unina.it	-Contribution to be detailed; Contact person: Antonio Evidente E-mail address: evidente@unina.it

	Contact person: Alessio Cimmino E-mail address: alessio.cimmino@unina.it Contact person: Marco Masi E-mail address: marco.masi@unina.it
10. Biological Control Research Institute, Turkey Nilufer Yildiz yildiz_hn@hotmail.com	-Surveys vineyards and nurseries in the Eastern Meditarranean region of Turkey (main areas of production); -Characterization of suspected strains with molecular methods e.g. real-time PCR; -Participation in the TPS if organised; Contact person: Nilufer Yildiz, E-mail address: yildiz_hn@hotmail.com Contact person: Raziye Ç.Yildiz E-mail address: yildizcr@gmail.com

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

New stakeholders and contributors may be identified until commissioning of the project.

1.9. Any other relevant information on content

This Topic has not been proposed for funding before so there is no risk of duplication from close collaboration with other projects in the field is envisioned. NPPO and curators of Q-bank will be asked to be involved.

2. *Euphresco management aspects of the project*

2.1. Indication of the topic budget

Funding organisation [a]	Mechanism [b]	Total Budget [c]
1. INIAV	NC	€
2. ANSES (FR)	NC	€
3. INRAE (FR)	NC	€
4. CREA (IT)	NC	€
5. MAFF (SI)	NC	€
6. Agroscope (CH)	NC	€
7. ERSA (IT)	NC	€
8. CNR (IT)	NC	€
9. UNINA (IT)	NC	€
10. BCRI (TR)	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

INIAV will be project coordinator but re-evaluation may be possible until final definition of the Consortium.

[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.