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

1.1. Topic area

Diagnostics, surveillance, phytoplasma

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-5.1: to understand the biological significance of a positive molecular diagnosis
☒ Objective 2017-R-6.1: to test and validate methods for in situ detection and identification of pests
☒ Objective 2017-I-2.2: to contribute to databases for plant pests identification and diagnostics
☒ Objective 2017-C-3.1: to favour knowledge exchange and support common initiatives with relevant players

1.3. Topic title

Development of efficient methods and identification of barcodes for discriminating Grapevine flavescence dorée *sensu-stricto* from other related phytoplasmas and investigation of potential correlation between taxonomic identity and grapevine, alders and hazelnut plant hosts

1.4. Description of the problem the research should solve

Several phytoplasmas from a taxonomic group 16SrV, subgroups -C and -D, are associated with grapevine yellows diseases. In Europe, the most important one is Grapevine flavescence dorée (GFD) phytoplasma, which is efficiently vectored among grapevines by the leafhopper *Scaphoideus titanus*. The resulting GFD disease can cause up to 100% loss during severe epidemics. Epidemic properties of GFD phytoplasmas are linked to adhesin-like variable membrane proteins (VMP), which sequences also determine Vectotypes II and III that are epidemic in European vineyards (Malembic-Maher *et al*., 2020).
Non-epidemic isolates of subgroup 16SrV-C are transmitted from alder trees to grapevine by *Oncopsis alni.* They were first described as Palatine grapevine yellows (PGY) in Germany, but have been found in other European regions (i.e., in French vineyards), too. Non-epidemic isolates correspond to the Vectotype I (Malembic-Maher *et al*., 2020). In addition, '*Candidatus* Phytoplasma ulmi' (16SrV-A) has been detected in some grapevine plants in Italy. (Angelini *et al*., 2018)
All diagnostic tests recommended in the EPPO standard PM7/079(2) on Grapewine flavescence dorée phytoplasma are targeting the 16SrV group as a whole and therefore lack specificity. Sequencing of PCR/nested PCR products can be performed to distinguish GFD phytoplasma *sensu stricto* from other phytoplasmas belonging to the same group (e.g., PGY and '*Ca*. P. ulmi') (Arnaud *et al*., 2007; Malembic-Maher *et al*., 2020). According to the current EPPO standard, this is recommended in particular for the first detection in an area and in non-grapevine samples. Sequencing of PCR products is time consuming and not reliable on samples with low concentration of phytoplasmas, which on the other hand can still be detected by real-time PCR. A VMP-based real-time PCR has been developed by INRAE to detect and distinguish vectotypes, but the current protocols need to be evaluated in different laboratories.
Phytoplasmas from group 16SrV have been isolated also from several other plant species (Malembic-Maher *et al*., 2020), recently also from the declining hazelnut trees (*Corylus avellana*) in several orchards in Slovenia (Mehle *et al*., 2019). Molecular characterisation and

phylogenetic analyses of partial 16S rRNA, secY, map and ribosomal protein genetic locus of hazelnut 16SrV phytoplasma isolates showed that they were identical to isolates associated with GFD disease. In addition, GFD-related phytoplasmas have also been detected in asymptomatic uncultivated hazelnut shrub samples from the forest close to a GFD-infected vineyard in Switzerland (Casati *et al*., 2017). As a possible vector of these phytoplasmas between hazelnut shrubs and grapevines, the introduced Asian leafhopper *Orientus ishidae* has been suggested, which has rapidly extended its range in recent years and is commonly found on both alder trees and hazelnut. Since only a small part of the genome of hazelnut 16SrV phytoplasma has been explored thus far and the information about the association of decay symptoms with these phytoplasmas in hazelnut plants in other countries are scarce, further studies are needed to confirm the identity of phytoplasmas in hazelnuts and grapevines.

1.5. Description of the expected results

The aim of the proposed project is to evaluate the possibility to develop a reliable test, to be used in routine analysis, to distinguish between GFD phytoplasma and other 16SrV phytoplasmas. In the framework of the proposed project, DNA and/or sequences of several grapevine isolates of 16SrV phytoplasma group will be collected and thus different grapevine phytoplasmas from the group 16SrV will be studied in depth. Collected DNA and/or sequences will be used for the development of molecular tests, which will be further validated. Results of the project might lead to the revision of the EPPO Diagnostic Protocol PM7/079.
The aim of suggested project is also to determine the occurrence and geographic distribution of hazelnut trees infected with GFD-related isolates. Isolates and sequences of GFD-related phytoplasmas infecting hazelnuts in different countries will be collected and compared with those found on grapevines. Additionally, evaluation of the potential vectors of this hazelnut phytoplasma isolates will be studied with the aim to define the epidemiological routes.

1.6. Beneficiaries of this research product

The beneficiaries of this research results would be grapevine and hazelnut growers, the plant health diagnostic laboratories, the National Plant Protection Organizations and the policy makers.

1.7. Research funders and research contribution/ distribution

Funding organisation	Research activity and researchers involved
1. Ministry of Agriculture Forestry and Food, Slovenia Erika Oresek erika.oresek@gov.si	-Project coordination; -Collecting and comparing the sequences of different 16SrV isolates with a focus on those that infects grapevine and hazelnut plants; -Evaluation of different tests for efficient discrimination of GFD phytoplasma from other 16SrV phytoplasmas; Contact person: Nataša Mehle E.mail address: natasa.mehle@nib.si
2. French Agency for Food, Environmental and Occupational Health & Safety, France Géraldine Anthoine geraldine.anthoine@anses.fr	-Co-organization with INRAE of Bordeaux of a validation ringtest for available tests to distinguish between GFD phytoplasma and other 16SrV phytoplasmas; -Survey on the occurrence of 16SrV phytoplasmas on hazelnut in France;

	- Participation in the training organized by OkState University; Contact person: Marianne Loiseau E.mail address: marianne.loiseau@anses.fr
3. INRAE -department of Plant Health and Environment Jean-Pierre Rossi Jean-Pierre.Rossi@inra.fr	-Collecting and comparing the sequences of different 16SrV isolates with the focus to those infecting grapevine, hazelnut plants and alders grown for wood products; - Co-organization with ANSES of a validation ringtest for available methods to distinguish between GFD phytoplasma and 16SrV phytoplasmas-related phytoplasmas Map and VmpA-R1 barcodes; - Robustness and sensitivity improvement of a reverse transcription real-time PCR test to specifically detect and distinguish Vectotypes (see Malembic-Maher *et al.*, 2020); -Improvement of MLSA and HTS-based genotyping for 16SrV-C and D phytoplasmas and for phytoplasmas in wild and cultivated hazelnut trees in the vineyard environment and associated potential vectors in NE and SW France; -Setting-up and deposition of a specific database for corresponding barcodes (including precise protocols) at the European Nucleotide Archive; -Participation to the training organized by partner OkState Univeirsity; Contact persons: Xavier Foissac E.mail address: xavier.foissac@inrae.fr Contact persons: Sylvie Malembic-Maher E.mail address: Sylvie.malembic-maher@inrae.fr
4. Federal Ministry of Food and Agriculture, Germany Silke Steinmöller silke.steinmoeller@julius-kuehn.de	-Collect sequences of 16SrV phytoplasmas from grapevine and leafhoppers; -Evaluation of the infection status of hazelnut in Germany; -Survey of leafhoppers associated with hazelnut and their infection status; Contact person: Michael Maixner E.mail address: michael.maixner@julius-kuehn.de
5. Ministry of Agriculture, Plant Biosecurity, Plant Protection and Inspection Services, Israel Yael Meller Harel YaelM@moag.gov.il	-Research involvement will be decided at a later stage; Contact person: E.mail address: Tomerg@moag.gov.il

6. Council for Agricultural Research and Economics, Italy Luca Riccioni luca.riccioni@crea.gov.it	-Survey on the occurrence of phytoplasmas on hazelnut in Italy and molecular identification and characterization of the infecting phytoplasmas eventually detected; -Obtention of sequence data from 16SrV phytoplasmas eventually identified on hazelnut and comparisons with those of GFDP; -Survey on leafhoppers associated with hazelnut in Italy and evaluation of their infection status; Contact person: Luca Ferretti E.mail address: luca.ferretti@crea.gov.it
7. National Institute for Agricultural and Veterinarian Research, Portugal Leonor Cruz leonor.cruz@iniav.pt	-Collect and compare the sequences of different 16SrV isolates; -Participation in a validation ringtest for available tests to distinguish between strains of GFDP as well as from other 16SrV phytoplasmas; -Evaluation of the infection status (virus and phytoplasmas) of a portuguese hazelnut collection with national and international varieties; Contact person: Esmeraldina Sousa E.mail address: esmeraldina.sousa@iniav.pt
8. US Department of Agriculture, Animal and Plant Health Inspection Service, United States of America David Schimmelpfennig david.schimmelpfennig@usda.gov	-Validation of HTS as a diagnostic tool using software system termed Microbe Finder (MiFi) that uses small highly-curated (specific) DNA databases called e-probes to detect pathogens in sequence data; - Develop e-probes data set for phytoplasmas and work with Euphresco researchers on a quick and portable diagnostic workflow; - Train researchers and diagnosticians on how to develop e-probes and/or use the MiFi diagnostic system; Contact person: Kitty Cardwell E.mail address: kitty.cardwell@okstate.edu Contact person: Andres Espindola E.mail address: andres.espindola@okstate.edu Contact person: Francisco Ochoa Corona E.mail address: ochoaco@okstate.edu
9. Service Centers in rural area, Germany Thierry Wetzel Thierry.wetzel@dlr.rlp.de	- Evaluation of the possibility to develop an efficient test to distinguish between GFD phytoplasma and other 16SrV phytoplasmas;

	Contact person: Thierry Wetzel E.mail address: Thierry.wetzel@dlr.rlp.de
10. Ondokuz Mayıs University, Plant Health Department, Turkey Hasan Murat Aksoy hmaksoy@omu.edu.tr	- Evaluation of the infection status of hazelnut in Turkey, and molecular identification and characterization of the phytoplasmas eventually detected; - Survey on leafhoppers associated with hazelnut in Turkey and evaluation of their infection status; Contact person: Hasan Murat Aksoy E.mail address: hmaksoy@omu.edu.tr

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

2. *Euphresco management aspects of the project*

2.1 Indication of the topic budget

Funding organisation [a]	Mechanism [b]	Total Budget [c]
1. MAFF (SI)	NC	TBD
2. ANSES (FR)	NC	TBD
3. INRAE (FR)	NC	TBD
4. JKI (DE)	NC	TBD
5. MOAG (IL)	NC	TBD
6. CREA (IT)	NC	TBD
7. INIAV (PT)	NC	TBD
8. APHIS (US)	NC	TBD
9. DLR (DE)	NC	TBD
10. OMU (TK)	NC	TBD
total		€

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

[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.