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-5.2: to develop and validate high-throughput DNA extraction methods

1.3. Topic title

Inventory and validation of quality control procedures for the extraction of nucleic acids for real-time PCR used for the diagnosis of pests

1.4. Description of the problem the research should solve

The titer of micro-organisms in infected tissues can sometimes be very low, such as bacteria, phytoplasmas or viruses that infect seed lots, dormant tubers or lignified tissues. As the micro-organisms' concentration is close to the detection limit of diagnostic tests, the diagnosis may be challenging. Moreover, the sensitivity of the test can be further reduced by the presence of inhibitors. Control reactions are intended to measure deviations in test performance and ensure that negative diagnosis is delivered on a sound and standardized basis. When real-time PCR is used as a diagnostic method, the control samples must provide Cq values within a predetermined range. To date, the different control procedures for the extraction steps are not generally applicable and when they are, they are rarely validated and formalised in the form of recommended procedures and threshold values. The aim of this project is to take stock of the extraction procedures used in the participating laboratories and in the literature. These procedures will be tested and compared on a range of plant matrixes infected with pathogens of interest in order to formulate recommendations for diagnostic laboratories.

1.5. Description of the expected results

The project aims will promote exchange of know-how and practices on the use of controls for molecular tests. It will provide a list of recommendations for the set-up of control experiments that can be used to validate the quality of DNA or RNA extracts to be used for real-time based diagnostic protocols.

Practices used in the participating laboratories or published (such as spiking techniques or use of constitutive reference genes) will be identified and evaluated in order to develop recommendations for defining the extraction efficiency within which the diagnosis can be confirmed with a certain level of confidence. These recommendations concern the sequence of primers that can be used for a selection of matrices, the type of spiked material when its use is recommended, the acceptable Cq values for these control operations.

1.6. Beneficiaries of this research product

The project will benefit to National Plant Protection Organizations and diagnostic laboratories.

1.7. Research funders and research contribution/ distribution

Funding organisation	Research activity and researchers involved
1. Agroscope, Switzerland Olivier Schumpp olivier.schumpp@agroscope.admin.ch	-Project coordination; -Depending on the needs and objectives defined at the start of the project with all the participants, organisation of the

	interlaboratory test, reference material preparation, participation and analysis; Contact person: Olivier Schumpp E.mail address: olivier.schumpp@agroscope.admin.ch
2. Federal Public Service of Health, Food Chain Safety and Environment, Belgium Ria Nouwen ria.nouwen@health.fgov.be	Researchers involved: to be confirmed after national VP-selection Potential research activities: to be confirmed after national VP-selection & peer review. -Compilation of an inventory of extraction procedures used in the partner's laboratory. Focus on procedures for RNA and DNA viruses and phloem or xylem bacteria in various complex plant matrices, woody plants, tuber crops, nematodes and insect vectors; -(Contribution to) literature screening of available extraction procedures, and drafting of a side by side comparison of literature and partners' protocols; -(Contribution to) selection of potential protocols for RNA and DNA pathogens in various matrices for further evaluation, optimisation and validation in the context of classical (e.g. PCR) and novel (e.g. high-throughput sequencing) downstream diagnostics; -(Contribution to) the establishment of standardised nucleic acid extraction protocols; -(Contribution to) communication and dissemination activities;
3. National Research Institute for Agriculture, Food and Environment, France Jean-Pierre Rossi Jean-Pierre.Rossi@inra.fr	-Contribution to literature screening of available extraction procedures with a focus on bacterial DNA from various complex plant matrices; -Selection of potential protocols for bacterial DNA extraction from different matrices for further validation; -Participation in the interlaboratory test; -Participation in the drafting of the protocol/ guidelines; Contact person: Sophie Cesbron E-Mail address: sophie.cesbron@inrae.fr
4. Federal Ministry of Food and Agriculture, Germany Bettina Beerbaum bettina.beerbaum@bmel.bund.de	-Contribution to be detailed; Contact person: Heiko Ziebell E-Mail address: Heiko.ziebell@julius-kuehn.de

Silke Steinmöller silke.steinmoeller@julius-kuehn.de	
5. Department of Agriculture, Food and Marine, Ireland Destefanis, Maria Maria.Destefanis@agriculture.gov.ie	-Participation in interlaboratory tests; -Participation in the draft of procedures; Contact person: Manuel Lopez Vernaza E-mail address: manuel.lopezvernaza@agriculture.gov.ie Contact person: Rebecca Ham Email address: Rebecca.ham@agriculture.gov.ie
6. Ministry of Agriculture, Plant Biosecurity, Plant Protection and Inspection Services, Israel Abed Gera AbedG@moag.gov.il Yael Meller Harel YaelM@moag.gov.il	-Development and sharing of protocols for the diagnosis of whitefly and aphid trasnmitted DNA (begomoviruses) and RNA (poleroviruses, criniviruses, etc.); -Extraction of DNA from plants, single insects and their organs, legs, *etc*. Use of real-time PCR and real-time RT-PCR protocols for diagnosis; -Development and sharing of protocols for the diagnosis of psyllid-transmitted bacterial pathogens of the genus *Liberibacter* (Liberibacter solanacearum, L. asiaticus); -Extraction of DNA from plants, single insects and organs for diagnosis purposes. Use of real-time PCR protocols for diagnosis; Contact person: Murad Ghanim E.mail address: ghanim@volcani.agri.gov.il
7. Ministry of Primary Industries, New Zealand Aurelie Castinel Aurelie.Castinel@mpi.govt.nz	-Sharing of protocols for PCR internal controls for seed testing; - Participation in interlaboratory test Contact person: Zoila Perez E-mail: zoila.perez@mpi.govt.nz
8. National Institute for Agricultural and Veterinarian Research, Portugal Leonor Cruz leonor.cruz@iniav.pt	-Preparation of DNA and RNA extracts of matrixes of interest for an interlaboratory validation; -Participation in the TPS; -Participation in the work-flow design for a validation or verification exercise; Contact person: Eugénia de Andrade E.mail address: eugenia.andrade@iniav.pt
9. Ministry of Agriculture Forestry and Food, Slovenia Erika Oresek erika.oresek@gov.si	-Participation in the interlaboratory test -Participation in the drafting of the protocol/ guidelines; Contact person: Natasa Mehle E.mail address: natasa.mehle@nib.si

	Contact person: Irena Mavrič Pleško E.mail address: irena.mavric@kis.si
10. Ministry of Food Agriculture and Forestry, General Directorate of Food and Control, Turkey Yunus Bayram yunusbayram@tarimorman.gov.tr	-Participation in the interlaboratory test; -Participation in the drafting of the protocol/ guidelines; -Isolation of RNA and DNA in hazelnut plant (especially Apple mosaic virus), diagnosis of harmful organisms by real-time PCR and real-time RT-PCR; -DNA isolation of *Xylella fastidiosa* (especially from *Lavandula* spp.), DNA isolation of '*Candidatus* Liberibacter solanacearum' (especially from carrot seeds), DNA isolation of '*Candidatus* phytoplasma solani' Contact person: Hamza Şenyurt E.mail address: hamza.senyurt@tarimorman.gov.tr
11. University of Forestry Sofia, Bulgaria Rumen Tomov rtomov@yahoo.com	-Contribution to be detailed; Contact person: Zhelyu Avramov E.mail address: zhelu.avramov@gmail.com
12. GEVES, France Valerie Grimault valerie.grimault@geves.fr	-Contribution to be detailed; Contact person: Valerie Grimault E.mail: valerie.grimault@geves.fr Contact person: Thomas Baldwin E.mail: Thomas.baldwin@geves.fr
13. Dienstleistungszentrum ländlicher raum rheinpfalz, Germany Thierry Wetzel thierry.wetzel@dlr.rlp.de	-Contribution to be detailed; Contact person: Thierry Wetzel E.mail address: thierry.wetzel@dlr.rlp.de
14. International Seed Federation, International Rose Souza Richards R.SouzaRichards@worldseed.org	-Contribution to be detailed; Contact person: Rose Souza Richards E.mail address: R.SouzaRichards@worldseed.org
15. Teagasc, Crop Science Department, Oak Park, Ireland Louise.McNamara Louise.McNamara@teagasc.ie	-Interests include understanding the incidence and diversity of viral (B/CY/DV) strains in cereals, and enhancing the sensitivity and specificity of molecular-based B/CYDV detection in plants (and aphids); -Participation in interlaboratory tests evaluating practices to confirm nucleic acid extraction efficiency during diagnostics; Contact person: Louise.McNamara

	E.mail address: Louise.McNamara@teagasc.ie Contact person: Stephen Byrne E.mail address: stephen.byrne@teagasc.ie
16. National Research Council, Italy Marzachi Cristina cristina.marzachi@ipsp.cnr.it	-Contribution to be detailed; Contact person: Marzachi Cristina E.mail address: cristina.marzachi@ipsp.cnr.it
17. University of Catania, Italy Santa Olga Cacciola olga.cacciola@unict.it	-Validation of extraction methods with oomycetes and fungal pathogens, including quarantine pathogens; -Analysis of results and sharing oomycetes and eumycetes isolates; Contact person: Santa Olga Cacciola E.mail address: olga.cacciola@unict.it Contact person: Federico La Spada E.mail address:federico.laspada@unict.it
18. University of Modena & Reggio Emilia, Italy Emilio Stefani emilio.stefani@unimore.it	-Preparation and delivery of reference plant material for the detection of regulated bacteria; -Delivery of DNA extraction and purification protocols for the detection and identification of phytopathogenic bacteria; -Validation of DNA extraction methods; -Participation in test performance studies; Pathogens of interest are: *Ca*. Liberibacter solanacearum, *Ralstonia solanacearum* species complex, *Acidovorax citrulli*, *Xanthomonas* spp. (tomato and pepper), *Clavibacter michiganensis* subsp. *michiganensis*, *Clavibacter insidiosus*, *Pantoea stewartii*. Contact person: Emilio Stefani E.mail address: emilio.stefani@unimore.it
19. University of Tuscia, Italy Anna Maria Vettraino E.mail address: vettrain@unitus.it	-Material preparation, participation and analysis; Contact person: Anna Maria Vettraino E.mail address: vettrain@unitus.it
20. Cleardetection, the Netherlands Marta Santos marta.santos@cleardetections.com	-Contribution to be detailed; Contact person: Marta Santos E.mail address: marta.santos@cleardetections.com
21. Plant and Food Research, New Zealand Simon Bulman Simon.Bulman@plantandfood.co.nz	-Preparation of material: bacterial infections of woody and herbaceous (host plants of special interest are kiwifruit and stonefruit; pathogens are *Pseudomonas*, *Xanthomonas* and *Xylella*);

	-Validation of DNA extraction procedures from different matrices; -Benchmarking of real-time PCR and high throughput sequencing protocols from DNA prepared from infected plant material; Contact person: Simon Bulman E.mail address: Simon.Bulman@plantandfood.co.nz Contact person: Sandra Visnovsky E.mail address: Sandra.Visnovsky@plantandfood.co.nz
22. Bioreba AG, Switzerland Marco Kaiser kaiser@bioreba.ch	-Contribution to be detailed; Contact person: Marco Kaiser E.mail: kaiser@bioreba.ch
23. Eskisehir Osmangazi University, Turkey Refik Bozbuga refik.bozbuga@ogu.edu.tr	-Laboratory test of molecular diagnostics of virus and viroids using real-time PCR; Contact person: Refik Bozbuga E.mail address: refik.bozbuga@ogu.edu.tr Contact person: Elen Ince E.mail address: elen.ince@tarimorman.gov.tr Contact person: Pakize Gokguler E.mail address:pakize.gokguler@tarimorman.gov.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

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 [a]	Mechanism [b]	Total Budget [c]
1. Agroscope (CH)	tbd	€
2. FPS (BE)	NC/VP	€
3. BMEL (DE)	tbd	€
4. INRAE (FR)	tbd	€
5. DAFM (IE)	tbd	€
6. MoA (IL)	tbd	€
7. MPI (NZ)	tbd	€
8. INIAV (PT)	tbc	€
9. MAFF (SI)	tbd	€
10. TARIMORMAN (TR)	tbd	€
11. UoF (BG)	tbd	€
12. GEVES (FR)	tbd	€
13. DLR (DE)	tbd	€
14. ISF (Int)	tbd	€
15. TEAGASC (IE)	tbd	€
16. CNR (IT)	tbd	€
17. UNICT (IT)	tbd	€
18. UNIMORE (IT)	tbd	€
19. UNITUSCIA (IT)	tbd	€
20. Cleardetection (NL)	tbd	€
21. PLANTANDFOOD (NZ)	tbd	€
22. Bioreba AG (CH)	tbd	€
23. EOU (TR)	tbd	€

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.

[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.