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

1.3. Topic title

Reliable detection of plant pathogens in soil.

1.4. Description of the problem the research should solve

Soil borne plant pathogens, whether it be fungal, bacterial or nematodes can cause plant diseases that are responsible for major crop yield reduction worldwide. Spreading of these pathogens by starting material may enhance these problems. Pre-screening soil on the presence of plant pathogenic organisms allows to prevent cultivation of starting material on infected soils. In some cases, this is the choice of the grower, on other cases this is governed by phytosanitary regulations.
In order to detect soil pathogens in various crops, bioassays and isolations by serial dilutions on more or less selective growth media are currently being used. Advantages of this method are that bioassays evaluate the presence of pathogens in relatively large volumes of soil and that this method can discriminate between viable and non-viable pathogens. The current bioassays are very impractical due to long incubation period (several weeks), while they are time-consuming in terms of labour demand, and a tedious evaluation is required for the presence of symptoms on the rinsed roots. Also, isolations on growth media are time consuming and not always reliable.
Molecular detection of plant pathogens directly in soil can be advantageous because a field can then be tested rapidly and shortly before planting. For several soil-borne pathogens, reliable and very specific real-time PCR tests have been developed - even at the formae speciales sublevel of *Fusarium oxysporum* - to identify cultures of harmful pathogens. However, it has been difficult to analyse representative soil samples from the field in the laboratory. Most available commercial kits only allow very small volumes of soil for total nucleic acid (TNA) extraction. Yield for TNA extraction is dependent on both amount of soil and type of soil (organic, clay, sand, *etc*.). As a consequence, it is very difficult to prepare representative samples for analysis of the field in question. In this topic, we want to explore the possibility of using large volume of soil (>100 g) for TNA extraction taking into account different type of soil, in order to obtain TNA is suitable to use in a PCR or barcoding approach. In addition, a systematic randomised soil sampling procedure has to be tested and put in place. Plant pathogens of particular interest are: *Phytophthora spp. (P. fragraria, P. cactorum, P cinnamomi), Fusarium oxysporum, Agrobacterium tumefaciens, Plasmodiophora brassicae Verticillium* spp. and several plant pathogenic nematodes (a.o. Globodera) and *Clavibacter michiganensis subsp michiganensis*.
Validity of the new protocols will be established based on the validation criteria described in the actual version of the EPPO standard PM 7/98 *Specific requirements for laboratories preparing accreditation for a plant pest diagnostic activity* and will include a comparison between the sensitivity of the bioassay versus real-time PCR based techniques.

1.5. Description of the expected results

Harmonised validated protocol for the extraction of total nuclear acid (TNA) from specific soil-borne pathogens from soil. This protocol allows the isolation of nucleic acids that can be used

in a molecular detection test like PCR or barcoding approaches. Sensitivity and specificity of this test will be comparable or better that the currently applied tests.

1.6. Beneficiaries of this research product

- National Plant Protection Services and government bodies (reference laboratories, inspectors, risk managers).
- Approved laboratories for official diagnostic tests.
- Commercial laboratories for diagnostic tests.
- Professional associations and Technical Institutes.

1.7. Research funders and research contribution/ distribution

Funding organisation	Research activity and researchers involved
1. Naktuinbouw, the Netherlands Marcel Toonen m.toonen@naktuinbouw.nl	-Project coordination; -Research on the extraction of total nucleic acid from soil samples for the detection of: *Phytophthora fragrariae*, *Fusarium oxysporum*, *Agrobacterium tumefaciens*, *Plasmodiophora brassicae* and several plant pathogenic nematodes; Contact person: Daniel Bakker E-mail address: d.bakker@naktuinbouw.nl Contact person: Ruud Barnhoorn E-mail address: r.barnhoorn@naktuinbouw.nl
2. Federal Ministry for Sustainability and Tourism, Austria Sylvia Bluemel sylvia.bluemel@ages.at	-Research on soil sampling procedure linked to the extraction of total nucleic acid from soil samples for the detection of: *Verticillium dahliae*, *V. albo atrum*, *Phytophthora fragariae*, *P. cactorum*, *Fusarium oxysporum* (f. sp. *fragariae*); -Comparison of established bioassays to newly developed molecular based procedures; Contact person: Ulrike Persen E-mail address: ulrike.persen@ages.at Contact person: Thomas Leichtfried E-mail address thomas.leichtfried@ages.at Contact person: Richard Gottsberger E-mail address richard.gottsberger@ages.at
3. Ministry of Agriculture, Rural Development and Environment, Cyprus Tefkros Iacovides tiacovides@da.moa.gov.cy	-Research on the extraction of total nucleic acid from soil samples for the detection of: *Globodera* spp., *Clavibacter michiganensis* subsp. *michiganensis*. Research will be conducted as part of our routine surveys when applicable;

	Contact person: Despina Philippou E-mail address: dphilippou@da.moa.gov.cy
4. French Agency for Food, Environmental and Occupational Health & Safety, France Géraldine Anthoine geraldine.anthoine@anses.fr	-Research on the extraction of total nucleic acid from soil samples for the detection of fungi and oomycetes (*Fusarium* and *Phytophthora* spp.); Contact person: Jaime Aguayo E-mail address: jaime.aguayo@anses.fr
5. Department of Agriculture, Forestry and Marine, Ireland Maria Laura Destefanis Maria.Destefanis@agriculture.gov.ie	-Contribution to be detailed; Contact person: Maria Laura Destefanis E-mail address: Maria.Destefanis@agriculture.gov.ie
6. Ministry of Agriculture, Plant Biosecurity, Plant Protection and Inspection Services, Israel Yael Meller YaelM@moag.gov.il	-Research on the extraction of total nucleic acid from soil samples for the detection of *Fusarium oxysporum* and in particular *Fusarium oxysporum* f. sp. *cubense* TR4; Contact person: Yael Meller E-mail address: YaelM@moag.gov.il
7. Council for Agronomic Research and the Bioeconomy analysis, Italy Luca Riccioni luca.riccioni@crea.gov.it	-Research on the extraction of total nucleic acid from soil samples for the detection of: *Phytophthora* spp., *Phytophthora cinnamomi*, *Fusarium oxysporum*; Contact person: Anita Haegi E-mail address: anita.haegi@crea.gov.it
8. Ministry of Agriculture, Forestry and Food, Slovenia Erika Oresek erika.oresek@gov.si	-Research on the extraction of total nucleic acid from soil samples for the detection of selected plant pathogens; Contact person: Janja Lamovsek E-mail address: janja.lamovsek@kis.si Contact person: Hans-Josef Schroers, E-mail address: hans.schroers@kis.si
9. University of Guelph, Canada Bob Hanner rhanner@uoguelph.ca	-Contribution to be detailed; Contact person: Bob Hanner E-mail address: rhanner@uoguelph.ca
10. The National Federation of seed potato growers, France Yves Le Hingrat yves.lehingrat@fnpppt.fr Virginie Gobert virginie.gobert@fnpppt.fr	- Research on the extraction of total nucleic acid from soil samples for the detection of potato pathogens (nematodes, fungi, bacteria ..) Contact person: Anne-Claire Le Roux (nematodes, bacteria) E-Mail address: anneclaire.leroux@fnpppt.fr Contact person: Karima Bouchek (fungi, oomycetes)

	E-Mail address: karima.bouchek@fnpppt.fr
11. National University of Ireland, Galway, Ireland Alexandre de Meneze ademenez@gmail.com	-Contribution to be detailed; Contact person: Alexandre de Meneze E-mail address: ademenez@gmail.com
12. University College Dublin, Ireland Fiona Doohan fiona.doohan@ucd.ie	-Contribution to be detailed; Contact person: Fiona Doohan E-mail address: fiona.doohan@ucd.ie
13. ClearDetections, the Netherlands Marta Santos Paiva marta.santos@cleardetections.com	-Contribution to be detailed; Contact person: Marta Santos Paiva E-mail address: marta.santos@cleardetections.com
14. National Academy of Agrarian Sciences of Ukraine, Ukraine Liliya Janse liliya.janse@gmail.com	-Research on the extraction of total nucleic acid from soil samples for the detection of selected plant pathogens (*Globodera* spp., *Synchytrium endobioticum*). Research will be conducted as part of our routine surveys when applicable; Contact person: Liliya Janse E-mail address: liliya.janse@gmail.com
15. Agri-Food and Biosciences Institute Norther Ireland, United Kingdom Thomas Fleming Thomas.fleming@afbini.gov.uk	-Contribution to be detailed; Contact person: Thomas Fleming E-mail address: Thomas.fleming@afbini.gov.uk Contact person: Deborah Cox E-mail address: Deborah.cox@afbini.gov.uk

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 [a]	Mechanism [b]	Total Budget [c]
1. Naktuinbouw (NL)	NC	€
2. BMNT (AT)	NC	€
3. MoA (CY)	NC	€
4. ANSES (FR)	NC	€
5. DAFM (IE)	NC	€
6. MOAG (IL)	NC	€
7. CREA (IT)	NC	€
8. MKGP (SI)	NC	€
9. UoG (CA)	NC	€
10. FN3PT (FR)	NC	€
11. NUI (IE)	NC	€
12. UCD (IE)	NC	€
13. ClearDetections (NL)	NC	€
14. NAAS (UA)	NC	€
15. AFBINI (GB)	NC	€
total		€

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

24 months (from 2020-01-01 until 20121-12-31).

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

Collaboration will be established with topic 2019-C-326: Pathogen survival in soil.

[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.