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

☒ Objective 2017-R-3.1: to identify and evaluate (horizontal) risk reduction options (effectiveness, feasibility and cost)
☒ Objective 2017-R-6.1: to test and validate methods for in situ detection and identification of pests
☒ Objective 2017-C-1.1: to address plant health challenges through integrative approaches and support collaboration among disciplines

1.3. Topic title

Diagnostic protocols for the detection of viruses in *Xiphinema* species of quarantine concern.

1.4. Description of the problem the research should solve

Nematodes of the genus *Xiphinema* (dagger nematodes) are migratory root ectoparasites with a broad host range. There are more than 260 species, of which approximately 60 belong to the *Xiphinema americanum* group (*X. americanum sensu lato*). The non-European populations of this group have a quarantine status as some of the species can transmit certain nepo- and cheraviruses. The identification up to species level is quite difficult based on morphological and morphometric data. In most cases, identification is impossible because only few specimens or no appropriate stadia are present in the sample. In addition, to date, there is not enough molecular information available to develop reliable tools such as PCR or barcoding for all species. Detecting the viruses in nematodes could be an alternative to determining *Xiphinema* species, as the viruses are of major quarantine concern. In case of doubt about the *Xiphinema* species identification, the presence of viruses in the nematode can determine which phytosanitary measures need to be applied.

1.5. Description of the expected results

Several benefits can be gained by working in a transnational network of researchers targeting this topic. In the first place, it will bring together all current knowledge on virus detection within nematodes. Several research groups might have built up experience which is not visible as there is a lack of recent publications. Additionally, the plant virus research community recently invested a lot of time and means in the standardization of HTS techniques in plant virus diagnostics. The focus up to now was on standardizing the bioinformatics pathways, starting mainly with infected plant material. When expanding the experience to other matrices, such as nematodes, a transnational project will again benefit from the experience that several laboratories built up in the use of HTS over the years.
A generic nepovirus detection method for nematodes of the genus *Xiphinema* will be optimized and validated Ultimately, a test performance study, (to open to interested laboratories), will be organized.
The input of several laboratories is required to deliver viruliferous *Xiphinema* nematodes: the availability of these specimens is the main bottleneck of the project. The cooperation of countries where these nematodes are naturally found or reared is therefore crucial. These laboratories can benefit from the collaboration by participating in the test performance study.
Several subgroups of nepoviruses exist, each with their own specific vector species. To improve diagnostic procedures, and increase our knowledge on which *Xiphinema* species are specifically capable of acquiring certain nepoviruses, high-throughput sequencing (Illumina) will be used as an untargeted approach to detect and identify viruses inside populations of different *Xiphinema* spp. Besides indicating the targeted viruses, high-throughput sequencing

can reveal new viruses, i.e. previously not reported to being associated with *Xiphinema*. This will open doors for further research. Additionally, an explorative assay using nanopore sequencing will be conducted to assess the benefits and limitations of this very promising 'on-site' technique to quickly detect and identify viruses inside nematode vectors.
The project shall aim to address the following aspects of the proposed research:

- Screening of the current methods to detect nepoviruses in plants and nematodes and selection of the generic methods for the detection of the different nepovirus subgroups that perform the best. Collect *Xiphinema* spp. and establish cultures (WP1).
- Optimization and validation of selected method(s) for virus detection inside the nematodes. Establishing a standardized protocol covering nematode extraction, RNA extraction, virus detection and virus identification. Emphasis will be on specificity, sensitivity and robustness of the method (WP2).
- Organizing a test performance study for the validated methods of virus detection in nematodes from WP2. Through the test performance study, other parameters such as repeatability and reproducibility will also be assessed (WP3).
- Assessing the feasibility to use nanopore sequencing (MinIon strategy) as a fast, reliable method to identify nepoviruses in nematode samples (WP4).
- Disseminating results. The validated methods will be communicated to the Plant Health community in general and to national reference laboratories in particular (WP5).

1.6. Beneficiaries of this research product

National Plant Protection Organisations and diagnostic laboratories will benefit from project's outputs. The approach could later be applied to other nematode genera transmitting viruses. Ultimately, growers and traders in plants will benefit from a fast and reliable diagnosis.

1.7. Research funders and research contribution/ distribution

Funding organisation	Research activity and researchers involved
1. Federal Public Service Health, Food Chain Safety and Environment, Belgium Ria Nouwen ria.nouwen@gezondheid.belgie.be	Potential research activities: to be confirmed after national VP-selection & peer review. -Project coordination; -Participation in WP1-WP5; Contact person: to be confirmed after national VP-selection
2. Agri-Food and Bioscience Institute, Ireland Deborah Cox Deborah.cox@afbini.gov.uk	-Contribution to be detailed; Contact person: Deborah Cox E-Mail address: Deborah.cox@afbini.gov.uk Contact person: Thomas Fleming E-Mail address: Thomas.fleming@afbini.gov.uk
3. All Russian Plant Quarantine Center, Russian Federation Natalia Sherokolova natalia_sh@mail.ru Oxana Dobrovolskaya oxana-d@yandex.ru	- Sampling and analysis of soil samples in the regions of Russia; - Participation in the interlaboratory test; Contact person: Yuri Shneyder E-mail address: yury.shneyder@mail.ru Contact person: Abrosimova Svetlana

	E-mail address: svetlana.eremina@mail.ru Contact person: Maria Kopina E-mail address: kopinamaria645@gmail.com
4. Ministry of Agriculture Forestry and Food, Slovenia Erika Oresek erika.oresek@gov.si	-Sharing the lab experience on virus detection in nematodes (WP1 and WP2); -Participation in interlaboratory test (WP3); -Provision of material (WP4); Contact person: Irena Mavrix Plesko E-mail address: irena.mavric@kis.si
5. National Institute for Agricultural Research and Food Technology, Spain Elena Rodriguez rodriguez.elena@inia.es	-Participation in suggested WP 1, 2 and 3. -Provision of material (WP4) Contact person: Lee Robertson E-mail address: robertson.lee@inia.es
6. Department for Environment Food and Rural Affairs, United Kingdom Elspeth Steel Elspeth.Steel@defra.gov.uk	-Provision of *Xiphinema americanum*-group specimens for molecular research; -Participation in interlaboratory testing; -Dissemination of results; Contact person: Thomas Prior E-mail address: Thomas.Prior@fera.co.uk
7. University of Cukurova, Turkey Halil Elekcioglu halile@cu.edu.tr	-Identification of nematodes and viruses; -Carry out virus transmission studies, also on bait plants; -Participation in all project WPs; Contact person: Halil Elekcioglu E-mail address: halile@cu.edu.tr ; halilelekcioglu@gmail.com
8. Tekirdağ Viticulture Research Institute, Aydoğdu, Turkey Lerzan Öztürk lerzanozturk@gmail.com	-Identification of nematodes and viruses; -Supply of *Xiphinema* and *Longidorus* populations. Supply of virus infected grapevine material; -Carry out virus transmission studies, also on bait plants; -Meristem culture to produce virus free grapevine plants and use them in transmission experiments. -Participation in all project WPs; Contact person: Lerzan Öztürk E-mail address: lerzanozturk@gmail.com
9. The James Hutton Institute, United Kingdom Roy Nelson roy.neilson@hutton.ac.uk	-Provision of *X. americanum* populations from North and South America, South Africa and potentially Oceania though for commercial reasons the latter may be a problem. Also provision of native non-*X. americanum* virus-vector populations to act as a control (WP1); -Participation in interlaboratory ring testing of methods developed in WP2 (WP3);

	-Collaboration, if required, on the use of MinIon for nematodes (WP4); -Knowledge exchange with national policymakers, industry and practitioners to highlight outcomes of the project (WP5); -If required, access to infrastructure, such as MiSeq (also access to HiSeq), MinIon and Sanger sequencing facilities (WP1-WP4); Contact person: Roy Neilson E-mail address: roy.neilson@hutton.ac.uk Contact person: David Roberts E-mail address: david.roberts@hutton.ac.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.
Partners in the USA would be welcome as *Xiphinema americanum* is present there, as well as the viruses they transmit.

1.9. Any other relevant information on content

A major challenge for the project will be the availability of specimens of *Xiphinema americanum sensu latu* carrying viruses. They are difficult to culture (long cycle) and to infect with viruses. Unless one of the partners has such specimens or cultures available, the consortium will have to work with alternative nematode species carrying viruses to test the proof-of-concept.
There nematodes species of interest are: *X. americanum s.s., Xiphinema californicum*, *Xiphinema rivesi*, *Xiphinema bricolense*, *Xiphinema tarjanense*, *Xiphinema intermedium*, *Xiphinema inaequale.* Viruses of interest are: *Cherry rasp leaf virus* (CRLV) (Cheravirus), *Tobacco ringspot virus* (TRSV) (Nepovirus), *Tomato ringspot virus* (ToRSV) (Nepovirus), and *Strawberry latent ringspot virus* (unassigned member of the Secoviridae).

2. Euphresco management aspects of the project

2.1. Indication of the topic budget

Funding organisation [a]	Mechanism [b]	Total Budget [c]
1. FPS (BE)	VP	€
2. AFBINI (IE)	NC	€
3. VNIIKR (RU)	NC	€
4. MKGP (SI)	TBD	€
5. INIA (ES)	NC	€
6. DEFRA (GB)	TBC	€
7. JHI (GB)	TBD	€
8. UCukurova (TR)	TBD	€
9. TVRI (TR)	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

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.