1. Content of the 'Topic Description' document

1.1. Topic area

Diagnostics, field detection, surveillance.

1.2. Topic title

Early detection of *Cryphonectria parasitica* in planting material.

1.3. Description of the problem the research should solve

An early, sensitive and fast detection procedure for *Cryphonectria parasitica* in planting material for intensive chestnut orchards shall be developed. The aim is to improve the control of the pathogen and the quality of planting material (*Castanea* spp.) within possible certification schemes to avoid further spread into pest free areas.

1.4. Description of the expected results

Due to the regulated status and damaging nature of *C. parasitica* the ultimate aim of the project would be to ensure that consignments of plants for planting can be tested and shown to be free from the pathogen.

Plants for planting were highlighted as the highest risk pathway for entry of the pathogen into protected zones (Jeger *et al*., 2016). There is evidence of a long latent period on infected plants for planting (Anderson *et al*., 2013), therefore a robust, sensitive detection method to screen for latent infections of planting material is required to support plant health inspection and potential certification regimes.

The project would aim to produce a validated protocol for the real-time PCR for the detection of *C. parasitica* in plants for planting. To support the diagnostic method, the project would also produce a protocol for sampling from production units and/or orchards, as well as a specific protocol covering how to subsample from the material for laboratory analysis. To achieve these outcomes, it would be necessary to establish the optimum time period for sampling, the age of material required and the proportion of crop to be sampled.

Expected outcomes from the project:

- Sampling protocol to support screening for latent infections in plants for planting.
- Sub-sampling protocol to identify optimum (laboratory) sub-sampling to detect latent infection.
- Diagnostic protocol for the detection of *C. parasitica* in planting material.

To achieve these final outcomes the following intermediate outcomes would be required:

- Assess published real time PCR assays for suitabilty for use on latent material.
- Determine the best time of the year to carry out sampling.
- Determination of proportion of lot to be sampled.
- Inter-laboratory proficiency test for detection of *C. parasitica* on plant material by real-time PCR.

1.5. Beneficiaries of this research product

The beneficiaries of this project would be stakeholders such as plant health inspectors, particularly those inspectors for NPPOs of protected zones. Importers, nurseries and orchard owners would benefit from any future certification schemes.

1.6. Research funders and research contribution/ distribution

Funding organisation	Research activity and researchers involved
1. Department for Environment Food and Rural Affairs, United Kingdom	-Project coordination

Belinda Phillipson belinda.phillipson@defra.gsi.gov.uk	Contact person: Ann Barnes E.mail address: ann.barnes@fera.co.uk
2. Austrian Agency for Health and Food Safety, Austria Sylvia Bluemel sbluemel@ages.at	-Establish selected real-time PCR(s) and participate in the test performance study or inter laboratory proficiency test. -Monitoring of *Castanea* spp. plant material in trade, plantations and natural landscape for *C. parasitica*. Contact person: Richard Gottsberger E.mail address: richard.gottsberger@ages.at; Contact person: Ulrike Persen E.mail address: ulrike.persen@ages.at
3. Institute for Agricultural and Fisheries Research, Belgium Martine Maes martine.maes@ilvo.vlaanderen.be	-Contribution to be detailed Contact persons: tbd E.mail address: tbd
4. Federal Ministry of Food and Agriculture, Germany Bettina Beerbaum Bettina.Beerbaum@bmel.bund.de Silke Steinmöller silke.steinmoeller@julius-kuehn.de	-Examination of asymptomatic material (or latent material) from infected areas in Germany. -Support on the work for a protocol for sampling. -Assist in the validated protocol for the real-time PCR. -Implementing early detection and control strategies of *C. parasitica*. Contact person: Clovis Douanla-Meli E.mail address: clovis.douanla-meli@julius-kuehn.de
5. Consiglio per la ricerca e l'economia in agricoltura, Italy Sauro Simoni sauro.simoni@crea.gov.it	-To provide material with different origins and ages. Contact person: Sauro Simoni E.mail address: sauro.simoni@crea.gov.it
6. National Institute for Agricultural and Veterinarian Research, Portugal Leonor Cruz leonor.cruz@iniav.pt	-Contribution to be detailed Contact person: tbc E.mail address: tbc

1.7. 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 to advertise the topic outside the Euphresco network

Information to sharpen the profile of sought partners could be useful (but not mandatory): country/region (if there are preferences), skills/expertise required, etc.

1.8. Any other relevant information on content

The project would look to tie into the epidemiological information produced through the Euphresco project CERACRY.
The research group would also approach the European Mycological Network for laboratories to take part in any proficiency tests.
Additionally, the Australian Quarantine and Inspection Service (AQIS) currently have a two year post-entry requirement on *Castanea* species for the presence of *C. parasitica* with testing carried out by direct sequencing of suspect lesions. Approaches should be made to look to capitalise on this work by collaborating with AQIS to test the new protocols in parallel.

It is anticipated that the project would be managed through a number of work packages as detailed below:

WP1 Project Management
Initial set up meeting.
Projects are reviewed on a regular basis (monthly to quarterly) with a comprehensive review of progress both scientifically and financially.
Wrap up meeting.

WP2 Validation of published real time PCR for use on latent planting material.
The objective of this work package is to assess and validate published real-time PCR methods for use on latent planting material to detect early infection before symptom expression. This would involve verifying the specificity and sensitivity of the primers. Evaluating the extraction methods and would eventually include an inter-laboratory proficiency test.

WP3 Sampling protocols developed for both the crop/production site and for the planting material for PCR
The objective of this work package would be to produce statistically significant sampling protocols for sampling from a crop/production site/batch of plants. This would involve working with a statistician. A protocol for where to take test material from on the plants for extraction would also be needed and would involve assessing whether infection points such as wounds are enough or whether natural opening/fissures would need to be included.

WP4 Dissemination
Presentation at the European Mycological Network annual conference.
A paper submitted to the EPPO bulletin.
Look to linking into inspector training programmes to raise awareness.
Outreach to industry to raise awareness of any future certification schemes.

References:

- Anderson et al. (2013) http://www.fera.defra.gov.uk/plants/plantHealth/pestsDiseases/documents/rapidAssessmentCryphonectriaParasitica.pdf
- Euphresco 09/2016-08/2018 CERACRY – Identification and early detection of *Cryphonectira parasitica* and *Ceratocystis platani* occurring on trees in Europe

- *Cryphonectria parasitica*, the causal agent of chestnut blight: invasion history, population biology and disease control. Daniel Rigling* and Simone Prospero Version of Record online: 24 APR 2017DOI: 10.1111/mpp.12542
- Cunnington JH, Pascoe IG (2003) Post entry quarantine interception of chestnut blight in Victoria. Australasian Plant Pathology 32: 569-570
- Bryner, S. F., Sotirovski, K., Akilli, S., Risteski, M., Perlerou, C. and Rigling, D. (2013), Informative value of canker morphology on the presence or absence of virus infection in chestnut blight cankers. For. Path., 43: 496–504. doi:10.1111/efp.12063
- Risk assessment and reduction options for *Cryphonectria parasitica* in the EU. Michael Jeger, et al EFSA Journal Dec 2016, Volume 14, Number 12
- Rubio, S., Barnes, A., Webb, K. and Hodgetts, J. (2017), A real-time PCR assay for improved rapid, specific detection of *Cryphonectria parasitica*. Ann. Appl. Biol., 171: 52–61. doi:10.1111/aab.12354

2. *Euphresco management aspects of the project*

2.1 Indication of the topic budget

Funding organisation [a]	Mechanism [b]	Total Budget [c]
1. DEFRA (GB)	NC	€ 58 644
2. ILVO (BE)	NC	€ 20 000
3. AGES (AT)	NC	€ 31 990
4. BMEL (DE)	NC	€ 33 000
5. CREA (IT)	NC	€ tbc
6. INIAV (PT)	NC	€ 15 000
total		€

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

12-24 months, expected start date between 1st February and 1st April, 2018.

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.