Final Report

Project title (Acronym)
Epitrix (flea beetle) species, life cycles and detection methods (Epitrix II)

Project duration:

Start date:	2017-10-01
End date:	2020-12-31

Contents

1. Research consortium partners

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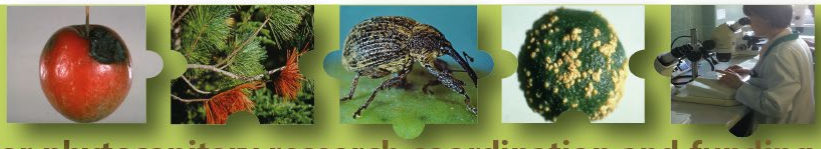

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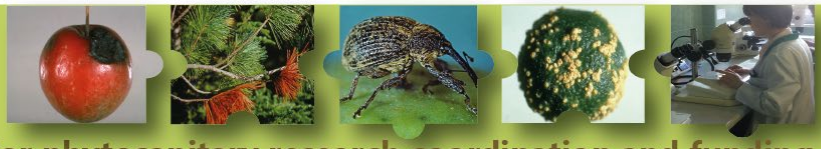


2. Short project report

2.1. Short executive summary

Epitrix Foudras 1860, is a genus of flea beetles (Coleoptera: Chrysomelidae: Alticinae) with nearly 180 species worldwide. Most species occur in South and Central America (neotropics), and only 12 and 10 species are known from North America and Europe, respectively. A few species of *Epitrix* are reported to feed on potato plants, the adults feeding on the leaves and the larvae on the roots and tubers. In the European Union (EU) emergency measures have been introduced to prevent the introduction of the EPPO A1 listed species *Epitrix tuberis* and *E. subcrinita* and the spread of the EPPO A2 species *E. cucumeris* and *E. papa*, accidentally introduced in Portugal and Spain. The three former species are native from North America and are known to cause damage to tubers, with *E. tuberis* being the most damaging one. *E. papa* is a newly described species with unknown geographic origin and known distribution limited to some regions of Portugal and Spain and causes damage to tubers. Other neotropical *Epitrix* species could also be of concern for European phytosanitary authorities, such as *E. yanazara*, which are known to feed on potato. Further research into the taxonomy and pest status of other potential *Epitrix* species of concern within the neotropics is required to understand these lesser-known species better and the impacts they may pose to other regions.

This project utilises the expertise of a network of scientists developed during Euphresco Epitrix I project to increase knowledge, understanding and preparedness for potential new outbreaks of *Epitrix* spp. affecting potato crops.

Epitrix species are uniform in their external morphology and difficult to distinguish in the field or in the laboratory even by specialists. Taxonomic experts from affected areas in Europe and North America prepared samples of accurately identified *Epitrix* beetles to help validate molecular detection methods for the benefit of the plant inspection services, particularly for regions where this expertise does not exist. Validated methods are now ready for interlaboratory comparative tests, which will be carried out in autumn 2021.

Many new potential pest *Epitrix* spp. were collected on solanaceous plants during a field expedition to Peru in 2020. This material will be used to help to bridge the knowledge gap of lesser-known *Epitrix* species within the neotropics.

Plant volatile organic compounds (VOCs) were investigated in EPITRIX I in the laboratory and in field experiments as potential attractants for detecting and monitoring *Epitrix*. Further research into the attractiveness of the VOC substances were studied, and although the results were encouraging, the attractiveness of the substances tested in the field was insufficient for reliable insect detection and monitoring. An additional study for testing new attractants (plant and insect volatiles) was therefore proposed but unfortunately not funded.

Available licensed insecticides and cultural management practices were reviewed to ensure that chemical control measures are still feasible, as several widely used pesticides have recently been removed from the market or are under review in the EU and the United Kingdom. The study showed that cultural practices (such as crop rotation, dates of planting, plantation of a trap crop, destruction of crop residues, and control of solanaceous weeds) may be used for efficiently disrupting the insect's biology and target control.

2.2. Project aims

The main aim of this project was to bring together scientists from different areas and specialisms to work collectively on developing a better understanding of *Epitrix* spp.. The project focussed on two main areas of work:

- Detection and identification
  - Assess the potential of plant and insect volatiles as lures for the detection and monitoring of *Epitrix* spp.
  - Validate generic and specific real-time PCR tests to ensure accurate identification of *Epitrix* spp.
  - Undertake the morphological and molecular characterisation of Epitrix South American species
- Biology and control
  - Review current control methods, taking into account the reduction of licensed plant protection products available and potential of using management practices

2.3. Descriptions of the main activities and results

2.3.1. Validation of molecular methods

(i) Facilitate the exchange of insect collection material

SASA (GB) coordinated the exchange of insect specimens that each partner/institute could provide. INIAV (PT) provided *Epitrix papa* and *Epitrix cucumeris* specimens; ANSES (FR) provided *Epitrix atropae* specimens; and SASA (GB) provided non-*Epitrix* flea beetles including *Neocrepidodera spp.*, *Longitarsus spp.*, *Phyllotreta undulata, Chaetocnema spp.*, *Psylliodes spp.*, and *Altica spp.*. Clemson University (US) were also able to provide *Epitrix fuscula*, *Epitrix fasciata* and *Epitrix brevis* (the exchange for these specimens is still ongoing and will continue after this project). Batches of the supplied specimens were sent to SASA (GB) to be distributed to those partners that required specimens and/or extracted DNA. A Material Transfer Agreement (MTA) was created for the exchange of material between partners for this project. A blank copy of the MTA is available in Appendix 1 for subsequent material transfers in future or ongoing Euphresco projects.

(ii) Validation of molecular methods – Generic Epitrix real-time PCR test

Based on the results from the previous work in the EUPHRESCO project 'Epitrix (flea beetle) species, life cycles and detection methods (EPITRIX I)' in which a generic real-time PCR test based on TaqMan technology was developed, the EPITRIX II consortium further tested and improved the test.

The test was validated on the following species: *Epitrix similaris, E. hirtipennis, E. subcrinita, E. cucumeris, E. tuberis, E. fasciata.* As non-target organisms the following species were tested: *Phyllioides chrysocephala, Podagrica decolorate, Chaetocnema tibialis, C. confinis, Phyllotreta nemorum, P. undulata, Aphthona lutescens.* All *Epitrix* species gave positive results, whereas non-target organisms gave negative results, with the exception of *A. lutescens.* The false positive results were improved by increasing the annealing temperature in the real-time PCR from 60 ºC to 62 ºC. This resulted in significantly higher Ct values from *A. lutescens* DNA around Ct 31 (>10 Ct values higher than DNA from *Epitrix*), compared to approximately 6 Ct values higher when the tests were run at 60 ºC.

The consortium considers the results satisfactory and thus recommends the test developed in EPITRIX I to be performed with an annealing temperature of 62 ºC, and that Ct values above 30 should not be considered positive (generally, results show that *Epitrix* species are positive at Ct 18-22).

(iii) Validation of molecular methods – validation of species-specific real-time PCR tests with new/fresh material

Due to limited availability of *Epitrix* specimens only two of the nine species-specific real-time tests developed in the previous project EPITRIX I were validated; those for *E. papa* and *E. cucumeris.*

To counter the lack of material available for validation purposes, plasmids containing the *COI* gene region for target and non-target *Epitrix* spp. were synthesised (Eurofins). COI sequences were obtained from sequences generated in the EPITRIX I project, the identities of which were confirmed on NCBI (BLAST).

Destructive and non-destructive extractions were carried out using the Qiagen DNeasy Blood & Tissue kit. Average DNA concentrations were low for *E. papa* and *E. cucumeris* specimens (~2 ng/µl) compared to the bigger *E. tuberis* specimens (40-50 ng/µl). The concentration of DNA and subsequent performance in downstream molecular applications i.e. sequencing and real-time PCR, was similar in destructive vs non-destructive extractions (compared to the same species of similar size). Non-destructive extraction was favoured as it enables the specimen to be kept and used for subsequent morphological analysis if required.

E. papa and *E. cucumeris* specimens collected using sticky traps and those stored in 70% ethanol produced poorer quality DNA and the majority failed to give exploitable sequence data. Specimens collected on sticky traps did amplify in real-time PCR tests, with Ct values similar to samples that were collected using mouth aspirators, however it was noted that cross reaction was observed for these samples. It is therefore advised that specimens should ideally be caught using mouth aspirators and then stored either by freezing or in ≥95% ethanol where possible. Storage of specimens in 70% ethanol is not advised as DNA degradation can occur.

In EPITRIX I, diagnostic tests were developed for *E. cucumeri*s and *E. papa* using targets within the COI gene region. Due to cross reaction observed in both tests from non-target species, cut off Ct values have been calculated (33.0 and 34.0 for the *E. cucumeris* and *E. papa* tests, respectively). It is unclear whether cross reactions observed were due to potential contamination issues and/or the poor DNA quality of samples. Therefore, it is recommended that samples should be tested against both tests for reliable identification of species. It is also advised to use an endogenous positive control (e.g. Eukaryotic 18S rRNA Endogenous Control, Life Technologies). The three triplicates should be below the recommended Ct cut-off values and have positive amplification for the 18S endogenous control to be considered true positives.

Both tests were validated against the following non-target genomic DNA: *Epitrix tuberis*, *E. atropae*, *Psylliodes affinis*, *P. chrysocephalus*, *Chaetocnema concinna*, *Neocrepidodera transversa*, *N. ferruginea*, *Longitarsus curtus*, *Phyllotreta undulata* and *Altica lythri*. Multiple *E. papa* samples were also tested against the *E. cucumeris* test and vice versa. Finally, both tests

were tested against COI plasmid DNA from *E. similaris*, *E. subcrinita*, *E. hirtipennis*, *E.cf.hirtipennis*, *E. brevis*, *E. pubescens*, *E. tuberis*, *E. cucumeris* and *E. papa*. No *E. atropae*, *E. tuberis* or non-*Epitrix* flea beetle genomic DNA were amplified by either test, however some cross reactions at CT values of >33 were found with *E. cucumeris* and *E. papa*.

For both tests, the limit of detection was calculated and the same experiment was repeated three times between two different QuantStudio 6 Flex machines, with no major differences observed in Ct values. An alternative real-time PCR master mix, qPCRBIO Probe Mix (PCR Biosystems), was also tested as a comparison to the JumpStart Taq Ready Mix (Sigma-Aldrich). Similar Ct values were observed for genomic DNA samples for both tests, although statistical analysis will be required to confirm this.
The experiment was repeated again using a different real-time PCR system, the CFX96 Touch Real-Time PCR machine (BioRad). Later Ct values were observed using the Bio-Rad instrument, although it is important to note that there was substantial background noise present in both runs, and the threshold had to be adjusted manually. It will therefore be necessary to re-run both tests on this machine again before statistical analyses can be performed.
In the upcoming months, additional non-target *Epitrix* species will be tested and further optimisation of the tests will be carried out to attempt to reduce the cross reaction observed. SASA (GB) will also be taking part in an interlaboratory comparison with INIAV, Portugal and AU, Denmark for testing *E. cucumeris* and *E. papa* samples.

(iv) Validation of molecular methods – validation of screening and identification duplex real-time PCR method for E. papa *and* E. cucumeris *based in SybrGreen and Tmelting*

In a previous project funded by the Portuguese Foundation for Science and Technology (FCT), a duplex SybrGreen- real-time PCR test was developed for the identification of *E. papa* and *E. cucumeris* using primers within the *COI* gene region. The test was in-house pre-validated for specificity. Inclusivity was checked on DNA from *Epitrix papa* and *E. cucumeris,* and exclusivity was tested on DNA from *Phylotretta atra* and *Chaetocnema concinna* which are frequently present in potato fields during the surveys. Out of all the tested species, only *E. papa* and *E. cucumeris* gave positive results, as expected.

During the EPITRIX II project, sample preparation and storage, DNA extraction and the real-time PCR test were further studied.

The preservation of specimens in ethanol 70% and dried/frozen was evaluated. In both cases, preservation was successfully observed, but the latter in many situations hampers DNA extraction. For DNA extraction of specimens conserved in ethanol 70%, it is necessary to let them completely dry overnight inside a fumehood with ventilation. The sample size was set at 1-10 specimens, irrespective of the species and the matrix (adults or larvae).

DNA can be extracted with the Quick-gDNA™ MiniPrep (Zymo Research, USA) or Qiagen DNeasy Blood & Tissue kit without difference on sensitivity.

Test sensitivity was validated by preparing several samples composed of different proportions of both *Epitrix* species and the non-related species. The detection/identification of 1 specimen, in a mixture of 10, was always successfully achieved.

Analytical sensitivity was not deeply studied. When extracting DNA out of 10 specimens, it is recommendable to dilute the DNA extract 1:20 prior to the PCR reaction.
A full technical report will be prepared and published by SASA/AU/INIAV colleagues on completion of the interlaboratory test performance study.

2.3.2. Morphological and molecular characterisation of South American *Epitrix* spp.

In the framework of a collaboration with the “Universidad Nacional Agraria La Molina” in Lima (PE), a collecting trip was organised in January 2020 in order to establish an inventory of *Epitrix* species in several potato-growing areas in Peru. Several sampling localities were chosen in the provinces of Lima (district La Molina), Cajamarca (districts Llacanora, Namora, Baños del Inca, Cajamarca), Anta (districts Cachimayo, Pucyura, Ancahuasi, Huarcondo), Cusco (district San Jeronimo), Paucartambo (district Caicay) and Quispicanchi (districts Oyopesa, Andahuaylillas, Urcos).
As a result, over 2 000 specimens were collected from 30 localities, with altitudes ranging from 0 to 4 000m. The collection of insects was carried out on the basis of observation of pest damage on potato foliage (adults of *Epitrix* feeding on the upper part of the plant cause multiple small holes in the leaves). Insects were caught with insect aspirators and preserved in 95% ethanol. At the time of writing this report, the *Epitrix* specimens are still in Peru waiting for exportation permits and formalisation of a research agreement between the different partners. The next steps consist in identifying and building a reference collection (including adult specimens, genitalia of males and females, and DNA barcodes) of the different *Epitrix* species found during the collects, thus contributing to the development of knowledge of *Epitrix* potato pests in South America.

2.3.3. Control Methods

A literature review of current control measures was conducted, with input from colleagues in affected countries. This was considered against current IPM practices in the United Kingdom and the potential loss of plant protection products currently under review. It was concluded that current aphid control practices in the United Kingdom may limit the damage to crops and management practices could be adapted to further mitigate risk. However, it is acknowledged that establishment of this pest would be highly detrimental to trade of a high value commodity. This report has been shared with project partners and will be submitted to Scottish and UK plant health policy. A summary is presented in Appendix 1.

2.3.4. Pheromone trapping

In order to identify plant volatile organic compounds (VOCs) attractive to *Epitrix* spp. adults, field and laboratory experiments were carried out in sequence during Epitrix I, with traps baited with Z3-6:Ac/Linalool (1:1) attracting significantly more *E. papa* and *E. cucumeris* adults than the control. Since then, further research was carried out to find synergist substances to improve the attractiveness of this mixture. The choice of individual *E. papa* adults between this mixture alone or combined with several other plant VOCs was sequentially tested in a Y-tube olfactometer. Adding (E)-β-ocimene to Z3-6:Ac/Linalool (1:3) increased the attractiveness of

the mixture and the result was verified in two field experiments (Boavida *et al* 2018). Although these preliminary results were promising and encouraged more research, an additional study testing different plant and insect volatiles was unfortunately not funded.

2.4. Final conclusions and recommendations to policy makers

2.4.1. Detection and identification

Molecular work

The TaqMan-based real-time PCR test for the generic detection of *Epitrix* and the test for the species-specific identification of *E. cucumeris* and *E. papa* have undergone further validation with promising results. The validation and screening for the SybrGreen duplex-test for the detection and identification of *E. cucumeris* and *E. papa* has been completed, the results from which will be published in due course. These tests allow a quick and reliable identification of pests. Limited availability of *Epitrix* and other non-*Epitrix* flea beetle specimens has impacted on the validation work of these tests, although additional *Epitrix* species from Clemson University (USA) which are in the process of being distributed will greatly help. SASA (GB), INIAV (PT) and AU (DK) will also be taking part in a ring test later during the autumn 2021 testing *E. cucumeris* and *E. papa* samples against each institutions' real-time PCR tests, which should further aid in strengthening each test.

It was noted that the quality and concentration of DNA when adopting the non-destructive extraction method was similar to using the destructive method, with the additional benefit of non-destructive extractions enabling the specimen or "voucher" to be kept as a useful reference specimen. It was also noted that specimens caught on sticky traps or stored in 70% ethanol produced poorer quality DNA and caused greater cross reaction in the TaqMan-based species-specific real-time PCR tests. It is therefore advised that for future molecular work on *Epitrix*, specimens should ideally be caught using mouth aspirators and then stored either by freezing or in ≥95% ethanol. This may also be useful to note when considering methods used for surveillance and detection programmes.

South American *Epitrix* spp. collections

The *Epitrix* specimens collected from several potato-growing areas in Peru will greatly contribute to the knowledge of *Epitrix* potato pests within South America, which up until now have been poorly documented on. This work is vital in understanding new potential *Epitrix* species that could be pose a threat to countries, and most importantly will help to update *Epitrix* morphological and molecular reference collections.

Pheromone trapping

Since EPITRIX I, additional research into VOCs as potential attractants for detecting and monitoring *Epitrix* have had encouraging preliminary results. However, the substances tested were insufficiently attractive for reliable *Epitrix* detection and monitoring in the field. Due to an additional study testing new attractants not being funded, visual inspection remains the only method of detection for *Epitrix* spp. Further research using insect pheromones as lures for *Epitrix* spp. need to be funded in the future.

2.4.2. Biology and control

Control methods
The literature review of current *Epitrix* control methods and approved insecticides will help guide future plant health policies tackling new or local outbreaks of *Epitrix* spp. A summary is presented in Appendix 1.

2.5. Benefits from trans-national cooperation

The involvement of partners from a range of European and further afield countries (Canada and the USA) has been extremely beneficial for the exchange of knowledge and expertise on *Epitrix* biology, ecology and molecular diagnostic methods. Transnational cooperation also allowed native specimens or DNA to be exchanged and distributed to partners from countries that otherwise would not be able to obtain them. In total, *Epitrix* and other non-*Epitrix* flea beetles were exchanged between 7 countries (both European and non-European) during this project. The specimens (or DNA) exchanged were vital to the research work carried out within this project and will continue to aid the partners in their *Epitrix* research going forward.
Exchanging material has not been without its challenges and the Nagoya protocol has meant that extra consideration was necessary when exchanging biological material between different countries. Partners from the *Epitrix* projects have worked together to develop a simple yet thorough Material Transfer Agreement (MTA) document, allowing the exchanges of specimens and DNA to continue for method development by all partners whilst ownership is retained within the country of origin. A template of the MTA is available in Appendix 2 for subsequent material transfers in future or ongoing Euphresco projects.

3. Publications

3.1. Article(s) for publication in the EPPO Bulletin

None.

3.2. Article for publication in the EPPO Reporting Service

None.

3.3. Article(s) for publication in other journals

- Boavida C, Santos M & Naves P (2019). Biological traits of *Epitrix papa* (Coleoptera: Chrysomelidae: Alticinae), a new potato pest in Europe, and implications for pest management. *Agricultural and Forest Entomology* **21**, 379-387. DOI: 10.1111/afe.12344.
- Conceição Boavida, Márcia Santos, Antje de Bruin, Roland Mumm, Gonçalo Costa and Kees Booij (2018). Searching attractants for the detection of potato Epitrix species. *Rev. Ciênc. Agr.*, vol. 41, n. spe., p. 125-132 http://dx.doi.org/10.19084/RCA.17077 .
- Mouttet R, Ginez A, Germain JF, Streito JC (2017). Présence en France d'*Epitrix hirtipennis* (Melsheimer, 1847) (Coleoptera, Chrysomelidae, Alticinae). *Bulletin de la Société entomologique de France*, 122(4), 451-454.

4. Open Euphresco data

Partner ANSES has provided some barcode sequences to the Q-bank curators for some *Epitrix* species that were still missing in the Q-bank database: the regulated species *Epitrix subcrinita* and the European species *E. intermedia* and *E. atropae*.

Appendix 1 Summary of the report 'Updated control methods for Epitrix species affecting potato-how could we manage an incursion of this pest in Scotland?'

Epitrix species are not present in the UK; aphids are the main insect pest of potato, requiring approximately 5.9 (seed) and 2.3 (ware) treatments per crop in Scotland. The most frequently used insecticide products on Scottish potato crops are pyrethroids and neonicotinoids, which would be effective against *Epitrix*. However, label guidance currently requires a 5M 'non-target insect' spray buffer for pyrethroids, which may reduce efficacy against this pest. Several pyrethroids are also currently under review, therefore their future is uncertain and neonicotinoids are unlikely to remain an option for long. If this pest were to establish, adapting current IPM practices would allow targeted chemical control, potentially reducing the need for pesticides against *Epitrix* spp. by up to 90% as observed with *E. tuberis* control in BC, Canada. Disruption to life cycles and populations using monitoring, management practices and modelling may reduce the impact of this pest even further. Biological limitations of *E. papa* under Scottish climate conditions may also mitigate risk of crop damage and potential of establishment. However, the impact on Scotland's pest free status for trade would be significantly affected unless eradication in both agriculture and wild hosts could be fully confirmed.

Integrated pest management guidelines for several plants that *Epitrix* and other flea beetles can target in California, USA. This information was obtained from The University of California Statewide Integrated Pest Management Program website.

Plant	***Epitrix* spp. known to be associated with and cause damage to the plant**	**Treatment options available (cultural)**	**Treatment options (chemicals)**	**Publication reference**
Eggplant	*Epitrix fuscula* *Epitrix hirtipennis*	Trap crops, row covers, white or yellow sticky traps, and good field sanitation.	SPINOSAD (Organically accepted) CLOTHIANIDIN THIAMETHOXAM PERMETHRIN ESFENVALERATE PYRETHRIN	*UC IPM Pest Management Guidelines: Eggplant* UC ANR Publication 3475
Peppers	*Epitrix cucumeris* *Epitrix subcrinita*	Remove weeds along field margins and plant residue after harvest. Pay particular attention to cruciferous (Brassicaceae) weeds and crops. Transplanting peppers usually avoids the problem.	ESFENVALERATE DINOTEFURAN PERMETHRIN CARBARYL BIFENTHRIN PYRETHRIN	*UC IPM Pest Management Guidelines: Peppers* UC ANR Publication 3460
Corn	*Epitrix cucumeris*	Keep fields weed-free, particularly of field bindweed and mustard. Heavily damaged fields should be replanted.	CARBARYL	*UC IPM Pest Management Guidelines: Corn* UC ANR Publication 3443

Sugarbeet	*Epitrix cucumeris* *Epitrix tuberis*	Keep fields weed-free, particularly of field bindweed and mustard. Replant heavily damaged fields.	METHOMYL CARBARYL	*UC IPM Pest Management Guidelines: Sugarbeet* UC ANR Publication 3469
Cucurbits	*Epitrix* spp.	Eliminate plant stress from insufficient moisture and powdery mildew.	ACETAMIPRID CLOTHIANIDIN CARBARYL LAMBDA CYHALOTHRIN METHOMYL CRYOLITE PYRETHRIN	*UC IPM Pest Management Guidelines: Cucurbits* UC ANR Publication 3445
Tomatoes	*Epitrix hirtipennis*	If possible, rotate tomatoes with a nonhost crop. In fields not previously planted to tomatoes, flea beetle infestations are usually located at field borders. Replanting rows near borders that have been heavily damaged is an option.	DINOTEFURAN CLOTHIANIDIN THIAMETHOXAM ESFENVALERATE LAMBDA-CYHALOTHRIN PYRETHRIN CARBARYL ENDOSULFAN	*UC IPM Pest Management Guidelines: Tomato* UC ANR Publication 3470
Potatoes	*Epitrix* spp.	Systemic insecticides and foliar sprays applied for green peach aphid usually keep flea beetles below economically damaging levels.	DINOTEFURAN IMIDACLOPRID LAMBDA-CYHALOTHRIN CARBARYL	*UC IPM Pest Management Guidelines: Potato* UC ANR Publication 3463

Appendix 2 Material Transfer Agreement (MTA)

This Agreement applies to the use, handling, and distribution of biological material supplied by the provider.

Material Transfer Agreement between:	
Provider:	[Name of organisation]
	[Address of organisation]
Represented by:	[Name of representative]
Add the recipient(s):	
First Recipient:	[Name of organisation]
	[Address of organisation]
Represented by:	[Name of representative]
Please add more recipients here if applicable	

The term MATERIALS in this agreement refers to the biological materials being transferred. The term INFORMATION in this Agreement shall encompass all confidential information (verbal or written) relating to the MATERIALS.

SCOPE OF AGREEMENT

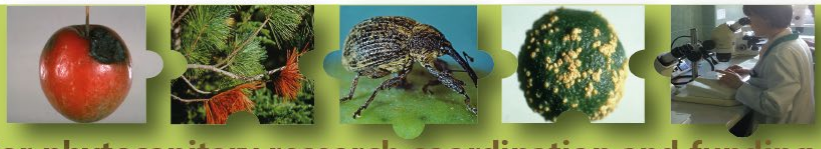
1.1 The MATERIALS, and the relevant INFORMATION in its possession belong to the provider. The MATERIALS are comprised of: [.....].

1.2 The recipients are interested in the MATERIALS for the e.g. Euphresco research project EPITRIX II of which all recipients and the provider are members of [change as appropriate]

1.3 The provider is acknowledged to be the sole owner of the MATERIALS and of the INFORMATION provided to the recipients and of the intellectual property rights related thereto. The recipients may not obtain any right, title deed or licence to the MATERIALS and INFORMATION provided by the provider. The recipients agree that information collected from the MATERIALS, i.e. research analysis results, are shared with the provider.

1.4 The provider agrees to provide the first recipient [name of first recipient] with the MATERIALS. The first recipient agrees to then distribute a sample of the MATERIALS to the second and third recipients [name of second and third recipients respectively - delete sentence if not applicable]. [The first recipient] agrees to keep accurate records of the distribution of the MATERIALS [delete sentence if not applicable].

SIGNATURES



I agree to comply with the conditions above

Provider:

Organisation:			
Address:			
Country:			
Name of representative:			
Email:			
Signed:		Date:	

Recipient (First):

Organisation:			
Address:			
Country:			
Name of representative:			
Email:			
Signed:		Date:	

Recipient (Second): - if applicable

Organisation:			
Address:			
Country:			
Name of representative:			
Email:			
Signed:		Date:	

Recipient (Third): - if applicable

Organisation:			
Address:			
Country:			
Name of representative:			
Email:			
Signed:		Date:	