Final Report

Project title (Acronym)
Study on the diversity of phytoplasmas detected in European forests (PhyFor)

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Contents

1. Research consortium partners

Coordinator – Partner 1	
Organisation	*Alma Mater Studiorum* – University of Bologna (UNIBO)
Name of Contact	Prof. Assunta Bertaccini
Job Title	Professor in Plant Pathology
Postal Address	Viale G. Fanin 40, 40127 Bologna, Italy
E-mail	assunta.bertaccini@unibo.it
Phone	+39 0512096723

Partner 2	
Organisation	Austrian Agency for Health and Food Safety (AGES)
Name of Contact	Msc. Helga Reisenzein & Gudrun Strauss
Job Title	Researcher
Postal Address	Spargelfeldstraße 191, 1220 Vienna, Austria
E-mail	helga.reisenzein@ages.at; gudrun.strauss@ages.at
Phone	+43 5055533340

Partner 3	
Organisation	Plant Health Laboratory of the French Agency for Food, Environmental and Occupational Health & Safety (ANSES)
Name of Contact	Dr Marianne Loiseau
Job Title	Deputy head team 'Virology/Phytoplasmology' in charge of phloem bacteria research.
Postal Address	Laboratoire de la santé des végétaux – 7 rue Jean Dixméras – 49044 ANGERS CEDEX 01 - FRANCE
E-mail	marianne.loiseau@anses.fr
Phone	+332 41207459

Partner 4	
Organisation	Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria – Centro di ricerca Difesa e Certificazione (CREA-DC) e Centro per la Ricerca in Viticoltura ed enologia (CREA-VE)
Name of Contact	Dr. Luca Ferretti, Dr. Sabrina Bertin, Dr. Elisa Angelini
Job Title	Researcher
Postal Address	Via C. G. Bertero 22, Rome, Via XXVIII Aprile 26 Conegliano (TV), Italy
E-mail	luca.ferretti@crea.gov.it; sabrina.bertin@crea.gov.it; elisa.angelini@crea.gov.it;
Phone	+39 0682070223, +39 0438439171

Partner 5	
Organisation	NIB National Institute of Biology (NIB)

Name of Contact	Prof. Marina Dermastia, Dr. Natasha Mehle
Job Title	Scientific counsellor, research councillor
Postal Address	Večna pot 111, 1000 Ljubljana, Slovenia
E-mail	Marina.dermastia@nib.si; Natasa.mehle@nib.si
Phone	+ 386 (0)59 232 805 (Marina); + 386 (0)59 232 808 (Natasa)

Partner 6	
Organisation	University of Hohenheim (UHOH)
Name of Contact	Prof. Dr. Michael Kube, Dr. Bernd Schneider
Job Title	Head of Department
Postal Address	University of Hohenheim, Garbenstr. 30, 70599 Stuttgart (former address: Thünen-Institute of Forest Genetics, Eberswalder Chaussee 3a, D-15377 Waldsieversdorf, Germany)
E-mail	michael.kube@uni-hohenheim.de
Phone	+ 49 (0) 711 459 24910

Partner 7	
Organisation	Institute of Pesticide and Environmental Protection (IPEP)
Name of Contact	Dr Bojan Duduk, Dr Emil Rekanovic
Job Title	Researcher
Postal Address	Banatska 31b, 11080 Belgrade-Zemun, Serbia
E-mail	bojan.duduk@pesting.org.rs; emil.rekanovic@pesting.org.rs
Phone	+38 1113076133

Partner 8	
Organisation	Department of Pharmacy, University of Salerno (DIFARMA)
Name of Contact	Prof. Carmine Marcone
Job Title	Associate Professor of Plant Pathology
Postal Address	Via Giovanni Paolo II, 132, 84084 Fisciano (Salerno), Italy
E-mail	cmarcone@unisa.it
Phone	+39 089968298

Partner 9	
Organisation	University of Banjaluka, Faculty of Agriculture
Name of Contact	Prof Dr Duska Delic
Job Title	Professor
Postal Address	Bulevar vojvode Petra Bojovica 1A, 78000 Banjaluka, Bosnia & Erzegovina
E-mail	Duska.delic@agro.unibl.org
Phone	+38 766748888

2. Short project report

2.1. Short executive summary

The goal of the project was to monitor the European forests for the presence of decline associated with the presence of phytoplasmas and to monitor their insect vectors or putative vectors. The project developed a network for the application of common existing or newly developed protocols for the monitoring and identification of phytoplasmas in the European forests and in the insects collected there. The outcome of the project broadened the knowledge on phytoplasma epidemiology, provided useful information about phytoplasma monitoring and detection/identification in forests to associated decline syndromes with phytoplasma presence, with special focus on phytoplasmas and insect vectors of exotic origin or never identified in European forest before. Wild areas surrounding grapevine, fruit trees and other cultivated crops were investigated in Austria, Bosnia & Herzegovina, France, Germany, Italy, Serbia and Slovenia. The insects and plant samples collected produced a preliminary picture of the presence and diversity of phytoplasma in these agroecosystems.

Several diverse phytoplasmas were identified in *Scaphoideus titanus*, the insect vector of grapevine "flavescence dorée". Of these, only '*Candidatus* Phytoplasma asteris', was demonstrated to be transmitted to plant species, however the ability of *S. titanus* to transmit the other phytoplasmas should be studied more thoroughly. In Italy *Anaplotettix fuscovenosus*, *Arboridia ribauti*, *Euscelis incisus*, *Hyalestes obsoletus*, *Neoaliturus fenestratus*, *Orientus ishidae*, *Philaenus spumarius* and *Synophropsis lauri*, were shown to host a number of diverse phytoplasmas (16SrI, 16SrII, 16SrIII, 16SrV, 16SrVI, 16SrVI, 16SrIX and 16SrXII-A) in restricted areas near vineyards in northern Italy while in central Italy *Synophropsis* spp was shown to harbour 16SrIX-E and *E. incisus* 16SrI-R phytoplasmas, respectively. The ability of these insects to vector the carried phytoplasmas was not experimentally demonstrated.

Surveys conducted in various plant species collected in forest areas that surround cultivated fields provided information on the presence of phytoplasmas. In Germany about 5,000 elm trees of diverse species in forests were tested and 1/3 of the trees tested positive for the presence of '*Candidatus* Phytoplasma ulmi'. Phytoplasmas of the 16SrV group were identified in Italy, Germany, Slovenia, Serbia or Bosnia & Herzegovina in *Ailanthus altissima*, *Alnus glutinosa*, *Alnus incana*, *Clematis vitalba*, *Fraxinus* spp., *Salix* spp., *Ulmus glabra*, *Ulmus laevis*, and *Ulmus minor*. '*Ca*. P. solani' was detected in Italy in *Parthenocissus quinquefolia* and *Skimmia* spp; phytoplasmas of the 16SrX group were detected in *Convolvulus arvensis, Cirsium arvense* and *Celtis orientalis*; in the latter species also 16SrIX phytoplasmas were identified. Moreover, phytoplasmas in group 16SrI were identified in *Clematis vitalba*, *Morus* spp., *Rubus* spp. and *Sorghum halepense*, in one case in mixed infection with 16SrX group phytoplasmas. In some cases, such as in Slovenia and in Serbia phytoplasmas were detected in cultivated plantations of *Corylus avellana* and in sugarbeet fields showing severe decline, respectively. The plants did not always show symptoms typical of phytoplasma presence. In Slovenia, the presence of '*Ca*. P. fragariae' was confirmed in *Acer campestre*, *Carpinus betulus*, *Crataegus laevigata*, *Fraxinus ornus*, *Quercus petraea* and *C. avellana*. '*Ca.* P. fragariae' was also detected in France in ash samples during the ash dieback survey. Extensive gallery of symptomatic forest species and a protocol for sampling plants were also provided to the participants to the network.

2.2. Project aims

1– <u>Coordination of the activities of the project and dissemination of results</u>.

The project aimed to update the presence and distribution of phytoplasmas in the European forests. Therefore a network was developed for the application of common protocols to monitor and identify phytoplasmas. A strong activity coordination was done to share protocols and results achieved together with focused kick off (2018, Wien) and intermediate meetings that were held in person (2019, Valencia) as well as a final virtual meeting (2021). Minutes of these meetings were prepared and disseminated among partners together with the published information. A virtual presentation of the activity to EPPO was done in 2022 and the publication of a success story is present in the EPPO web page since the beginning of 2023.

2- <u>Study the insect vectors and potential vectors occurring in European forests and elucidate their potential role in phytoplasma disease spread, both in forests and neighbouring crops.</u>

Surveys for identification of specific symptomatology in forest plant species and presence of potential insect vectors were carried out together with the molecular verification of phytoplasma presence in collected materials. Selected PCR protocols were used as common tools for phytoplasma detection, whereas DNA barcoding was employed for their identification together with identity confirmation on full 16S rRNA gene by RFLP or sequence analyses. Areas where the problems related to phytoplasma presence are more common (*i.e.,* near cultivated crops such as grapevine, apple or other crops) were surveyed in particular together with areas in which alien weeds or potential alien insect vectors were reported.

<u>3- Information on the presence and diversity of phytoplasmas in forest trees for evaluation of the potential risk linked to their presence in areas surrounding relevant crops.</u>

National surveys focussing on phytoplasmas in forest areas were implemented in France, Germany, Slovenia, Austria and Italy. Data on distribution on phytoplasmas in European forests were collected and circulated. Field surveys were preferably carried out in the same forest areas covered by monitoring of insect vector populations to correlate possible phytoplasma infections with the occurrence of identified insect vectors. Both symptomatic and/or asymptomatic plant samples were collected from suspicious species and when possible, type and severity of symptoms were recorded and documented.

<u>4- Combined analysis of the risk factors determining the impact of phytoplasma diseases in forests.</u>

Data obtained were processed and combined to evaluate the potential risk connected to the presence of plant species and insects acting as reservoir and vectors of phytoplasmas in forests. Maps reporting the presence and distribution of phytoplasmas and phytoplasmas strains as well as insect vectors in the investigated forest areas are under preparation for verification of possible epidemiological impact of phytoplasmas on the health of forests and neighbouring crops.

2.3. Description of the main activities

Partner 1 (UNIBO) coordinated the project and organized three meetings (kick-off, mid-term, and final). Due to the Covid 2 pandemic the last meeting was online. The kick-off meeting was held in Vienna on 7-8 June 2018, the second meeting in Valencia, on 8 September 2019 and the virtual meeting on 9 June 2021. The meetings were organized between WP leaders and the other participants to the project to guarantee the flow of information and results, to update on the progress of work and discuss future tasks. Partners 3 (ANSES) and 4 (CREA-VE) helped preparing the minutes of the Valencia and virtual meetings. In September 2019 some of the project results were presented to a wide audience during a PhyFor dedicated session inside the fourth IPWG meeting. Partners 3 (ANSES) and 6 (UHOH) served as chairpersons for the Euphresco PhyFor session during this meeting. There were 5 oral presentations and a large dissemination among the meeting participants. The results of the group on *Fraxinus* survey in France were presented during the IPWG PhyFor in Valencia and published as extended abstract in Phytopathogenic Mollicutes journal together with other reports from Germany (Partner 6, UHOH), Slovenia (Partner 5, NIB), Serbia (Partner 7, IPEP) and Italy (Partner 1, UNIBO). The Partner 5 (NIB) presented the results obtained in the PhyFor project at the 14th Slovenian Conference on Plant Protection.

The sampling activities related to insect vectors were focused on the three main taxonomic groups of hemipterans known to transmit phytoplasmas: leafhoppers (Auchenorrhyncha: Cicadellidae), planthoppers (Auchenorrhyncha: Fulgoromorpha), and psyllids (Sternorrhyncha: Psyllidae). The insect collection was performed mainly during the growing season (*i.e.,* springs and summers) when adults were present. Surveys were conducted in forests and natural habitats surrounding crops on forest trees, deciduous wood and shrubs. Insects were collected through sticky traps positioned in each sampling area and/or by sweep netting. For the collected sample's geographic location (GPS coordinates), presence of phytoplasma symptoms, presence of barriers between the natural habitats and crops were recorded. The selection of the areas of interest, the sampling procedures and the insect storage methods were discussed and defined by partners during the kick-off meeting. The identification of phytoplasma vector species was mainly relying on morphological characters of adults. The collected specimens were examined with stereomicroscope and the external morphological features were used to determine family and genus. For species identification, further characters (mainly pertaining to the male genitalia) were considered after specimen dissection. If the identification of these morphological features did not allow the distinction of closely related species, the taxonomy of the specimens was solved by amplifying the mitochondrial gene encoding cytochrome-c oxidase I (COI). These molecular analyses included: insect DNA extraction, COI amplification by PCR using universal primers and sequencing, and sequence upload in databases such as GenBank and Barcode of Life Data System (BOLD) to retrieve the species. Moreover, molecular taxonomic keys based on COI-RFLP assays available for some vector species belonging to the families Cixiidae and Psyllidae, were also applied. The collected insect specimens were then tested for the presence of phytoplasmas. The same DNA extracted for species identification was used as template in PCR assays for phytoplasma detection. Preliminary analyses performed using universal primers were carried out to identify the phytoplasma positive samples. On these samples, strain-specific diagnostic assays were performed, as described for the analyses of plant samples below.

Partner 3 (ANSES) shared with the different partners the data sheet and the survey protocol produced during the project. Survey for specific symptomatology and presence of phytoplasmas were carried out in forest tree species including conifers, deciduous wood and shrubs. Forest areas close to cultivated crops (fruit tree orchards and grapevines) were also inspected. Field surveys were preferably carried out in the same forest areas covered by monitoring of insect vector populations in order to correlate possible phytoplasma infections with the occurrence of identified insect vectors. Symptomatic and/or asymptomatic plant samples were collected according with the diverse geographic area surveyed. Partner 7 (IPEP) worked in **Serbia** where *Alnus glutinosa* and *A. viridis* asymptomatic trees were randomly collected among forest trees for verification of phytoplasma presence. Partner 8 (DIFARMA) in **Southern Italy** performed visual symptom assessment and PCR assays using universal and group-specific primer pairs directed to rDNA sequences were used to survey the occurrence and incidence of elm yellows and alder yellows diseases in several areas of Basilicata and Campania. The elm trees examined were *Ulmus minor* (syn.: *U. carpinifolia*), *U. glabra*, *U. laevis* and hybrids whereas the alder trees examined consisted mostly of *A. glutinosa* and to a lesser extent of *A. cordata*. Partner 4 (CREA-DC) in Latium region (**Central Italy**) surveyed both alternative host plant species and (putative) insect vectors. In 2019 in Piglio (Frosinone province) the insect collection was done in and around a vineyard infected by 16SrXII-A and 16SrI group phytoplasmas. Samples were collected from the surrounding plants of *Fraxinus ornus*, *Carpinus betulus*, *Acer campestre*, *Pistacia terebinthus* and *Quercus* spp.. A further sampling in 2021 was done in Viterbo province on wild hazelnut (*Corylus avellana*) and chestnut (*Castanea sativa*) trees bordering a hazelnut orchard infected by '*Ca*. P. fragariae' (16SrXII-E). Partner 4 (CREA-VE) in **Northern Italy** in Veneto, analysed 240 trees, grouped in 63 samples. The species belonged to *Acer campestre* and *A. negundo*, *Ailanthus altissima, Alnus glutinosa, Corylus avellana*, *Fraxinus* spp., *Morus alba, Salix alba*, *Ulmus* spp. Partner 1 (UNIBO) in Emilia-Romagna, Veneto (Treviso province) and Alto Adige samples sampled mainly asymptomatic *Clematis vitalba*, *Convolvolus arvensis*, *Morus* sp., *Partenocissus quinquefolia*, *Ranunculus* spp., *Robinia pseudoacacia*, *Rubus* spp., *Skimmia* sp., *Sorghum halepense*, *Urtica dioica*, *Zea mais* and (putative) insect vectors were collected. Partner 6 (UHOH) in **Germany** a countrywide survey was performed to verify the occurrence of elm yellows phytoplasmas in white elm, wych elm and the field elm species native to Germany for a total of more than 5,000 samplings. Disease symptoms as witches' broom for wych elm and yellowing and small leaves for field elm were only occasionally visible. During an ash survey in **France** Partner 3 (ANSES) performed a study to verify phytoplasma presence in DNA extracts from 260 ash tree displaying dieback symptoms. In **Bosnia and Herzegovina** Partner 9 performed a small-scale survey in the last week of July 2019 from three parks in Banjaluka city and from two locations in Kostajnica and 16 plant genera were sampled(*Populus*, *Platanus*, *Paulownia*, *Ulmus*, *Morus*, *Betula*, *Fraxinus*, *Quercus*, *Acer*, *Salix*, *Robinia*, *Carpinus*, *Sambucus*, *Tilia*, *Alnus* and *Ostrya*). In **Austria** and **Slovenia**, locations mainly flanking relevant agricultural areas were surveyed and samples were collected from Partners 2 (AGES) and 5 (NIB), respectively.

Plant samples collected during field surveys from forests and/or neighbouring crops were molecularly analysed by conventional or quantitative PCR employing universal or group-specific primers and/or probes after extraction of total DNA. Selected strains were further characterized by molecular approaches including DNA barcoding, qPCR, MLST analyses, and sequencing. In particular for the main grapevine yellows associated phytoplasmas the TaqMan qPCR with universal primers for 16S rRNA gene, and TaqMan qPCR with primers for 16SrV and -XII phytoplasmas were used; these phytoplasmas were identified by RFLP analyses on 16S rRNA gene, and when possible, the *map* gen was amplified and sequenced.

2.4. Main results

Phytoplasma presence in forest plants

In **Serbia** all examined asymptomatic *Alnus glutinosa* trees are infected with alder yellows phytoplasmas (16SrV-C). However, none of the examined *Alnus viridis* bushes tested positive. In **Southern Italy** most of the foliar and crown symptoms associated with alder yellows disease are similar to those of phytophthora root and collar rot caused by *Phytophthora alni* and to those of alder dieback associated either with *Phomopsis alnea* or *Erwinia alni*, special attention was given to visual symptoms assessment to exclude the involvement of fungal and/or bacterial infections for the symptoms observed. The genetic variability of phytoplasmas associated with elm yellows and alder yellows was elucidated through multi-locus sequence typing and sequencing; phylogenetic analyses showed a considerable variability within these phytoplasmas detected in southern Italy at *rpsV* (*rpl22*), *rpsC* (*rps3*) and *secY*-map locus (*map* gene) gene level. During the survey in **Central Italy** all the plant samples collected in both years tested negative for phytoplasmas. In **Northern Italy** in Veneto two *S. alba* plants were infected by 16SrV-C phytoplasmas, two elms by 16SrV-A phytoplasmas, while five *A. glutinosa* trees were infected with 16SrV-C subgroup phytoplasmas of the FD2- and FD3 *map* clusters. In Emilia-Romagna, Veneto (Treviso province) and Trentino-Alto Adige *P. quinquefolia* from two diverse areas, *Skimmia* sp. and *C. vitalba* were positive for 16SrXII-A and 16SrV-C phytoplasmas, respectively. In *Clematis* spp., *Rubus* spp., mulberry and sorghum 16SrI phytoplasmas were present, in one case sorghum was double infected by phytoplasmas of the 16SrI and 16SrX groups. In **Southern Italy,** a high percentage of elm and alder trees were infected reaching more than 80% in some areas. More than half of alder trees examined were latently infected and none of the alder trees that scored phytoplasma-positive showed typical symptoms. All positive trees were infected by 16SrV-A and 16SrV-C phytoplasmas. Some 16SrV-C strains proved to be closely related and/or identical to either "flavescence dorée" or Palatinate grapevine yellows (PGY) phytoplasma strains. In **France** '*Ca*. P. fragariae' was detected in 5% of the tested ash samples. Phytoplasmas detected in **Slovenia** were '*Ca*. P. ulmi' in the leaves and roots of *U. minor*, *U. glabra*, and *U. laevis*. FD-related phytoplasma strains in asymptomatic *Ailanthus altissima*, *A. glutinosa*, and *A. incana* and also in cultivated *Corylus avellana*, showing decline symptoms. '*Ca*. P. fragariae' was detected in symptomatic *Acer campestre*, *Carpinus betulus*, *Crataegus laevigata*, *Fraxinus ornus*, *Quercus petraea* and in cultivated *C. avellana*. In **Bosnia and Hercegovina** the analyses of samples by PCR showed suitable products in 6 out of 42 tested samples from *Populus nigra* cv. italica; *Platanus acerifolia*; *Ulmus* sp.; *A. glutinosa* and *Acer pseudoplatanus*. RFLP analyses identified the presence of 16SrI and 16SrV phytoplasma groups in five samples and sequencing confirmed the presence of '*Ca*. P. asteris' in *P. nigra* cv. Italica and *P. acerifolia; Ulmus* sp. and *A. glutinosa* sequences showed 100% identity to '*Ca*. P. ulmi' and "flavescence dorée" phytoplasmas, respectively.

Collection and identification of insects as putative vectors of phytoplasmas in forests.

In Latium region (**Central Italy**) in 2019, 6 planthopper specimens belonging to *Reptalus cuspidatus* and *Issus* spp. and 7 leafhoppers belonging to *Synophropsis* spp., and *Euscelis incisus* species were identified. Among these samples, one specimen of *Synophropsis* spp. collected on *C. betulus* resulted infected by a 16SrIX group phytoplasma while one specimen of *E. incisus* in vineyard edge resulted positive for a 16SrI phytoplasma. Identification of insects collected in 2021 on wild hazelnut and chestnut trees, revealed the presence of several leafhoppers belonging to the species *Euscelis lineolatus*, *Psammotettix confinis*, *Macropsis fuscula* and *Acericerus* spp. The same species together with two other leafhoppers (*Aphrodes makarovi* and *Anaceratagallia* spp.), and *Laodelphax striatellus* and *Hyalesthes obsoletus*, collected on adjacent weeds, were also identified. None of these insect samples tested positive for phytoplasmas. In Veneto, **Northern Italy** in 2021 yellow sticky traps were placed in the borders of forests and edges close to 7 vineyards highly infected by “flavescence dorée” phytoplasmas and changed every 7 or 20 days from July to September. A total of 7,557 insects, belonging to 31 species, were found, the most abundant being *Orientus ishidae*, with 2,237 individuals. A total of 131 insects, belonging to 4 species (*O. ishidae, Phlogotettix cyclops, Euscelidius variegatus* and *Japananus hyalinus*). The majority of the samples were negative for phytoplasmas (126). At this site, 60 *O. ishidae* were collected, and 5 of them tested positive (8,3%) for 16SrV-C phytoplasmas of the map6 and map50 genotypes. No phytoplasma was detected in the insects collected in the other 6 sites, and neither in the samples of the other 3 species. In Emilia-Romagna and Veneto (Treviso province) a total of 211 insects of 8 species (*S. titanus*, *H. obsoletus*, *Orientus ishidae*, *Hishimonus hamatus*, *Philaenus spumarius*., *Zygina rhamni*, *Psammotettix striatus*, *Neoaliturus fenestratus*) were tested and phytoplasmas were identified in all species except for *H. hamatus*, *Z. rhamni* and *P. striatus*. In the Modena province phytoplasmas belonging to group 16SrX were detected in *P. spumarius*, and 16SrI and 16SrXII-A phytoplasmas were found in *N. fenestratus*. In the Treviso areas 16SrV-C and 16SrXII-A phytoplasmas were detected in *S. titanus* and *O. ishidae*, andin *S. titanus*, *O. ishidae* and *H. obsoletus, respectively*. 16SrX-B and 16SrI-B group phytoplasmas were identified in *S. titanus*, while 16SrI-B group phytoplasmas were found in *O. ishidae* and *H. obsoletus*. 16SrVII-A group phytoplasmas were identified in *S. titanus* and *O. ishidae*.

<u>Evaluation of the potential risk due to the occurrence of phytoplasmas and insect vectors in forest areas</u>

Data obtained were processed and combined to evaluate the potential risk connected to the presence of plant species and insects acting as reservoir and vectors of phytoplasmas in forests. Maps reporting the presence and distribution of phytoplasmas and phytoplasmas strains as well as insect vectors in the investigated forest areas will be generated to assess the epidemiological impact of phytoplasmas on the health of forests and neighbouring crops.

2.5. Conclusions and recommendations to policy makers

The surveys conducted showed a multifaceted situation according to the different agroecosystems which were inspected.

The results from Serbia, Slovenia, Bosnia and Hercegovina and Southern Italy showed the presence of phytoplasmas of the 16SrV-C subgroup in all the examined *A. glutinosa* trees and also in all elm species, often not exhibiting symptoms, especially in the northern European areas. This result agrees with literature data from Germany, Hungary and France and shows that this phytoplasma is common/abundant in the investigated forest areas. Although the diseases examined were known in southern Italy, results of this project indicate that 16SrV-A and 16SrV-C phytoplasmas are more widespread than previously thought and are of considerable ecological and epidemiological significance. Also, the genetic variability of the associated phytoplasmas had previously not been investigated and suggests that the pathogens are endemic also in the areas where the symptomatology is not present. The presence of these phytoplasmas could contribute to increase the damages due to the presence of other pathogen such as other bacteria, fungi or oomycetes. Therefore great attention is needed with continuous monitoring. The survey carried out in Germany demonstrated a wide occurrence of '*Ca*. P. ulmi' with infection rates over 90% at some sites examined. Despite this high infection rate -specific disease symptoms were rarely observed, regardless of the tree age. However, experimental inoculation trials with European white elm seedlings grafted with accessions from infected wych elms induced witches' broom formation, indicating a host tolerance or a low strain virulence in natural habitats. The pathogen was not uniformly distributed in Germany and hot spots of the disease were identified. The reason for this uneven distribution in Germany is not clear and might only reflect the current state of the spread of this insect-vectored pathogen.

In Northern Italy, a number of plants and one insect species were infected with *'Ca.* P. asteris' strains indicating the potential of spreading of this phytoplasma in natural environments. Phytoplasmas enclosed in groups 16SrVI and 16SrVII were detected in *S. titanus* and *O. ishide;* considering that these phytoplasmas were also detected in the same vineyards, further studies are necessary to investigate the role of these two insects as phytoplasma vectors to crops in the studied areas.

In France '*Ca*. P. fragariae' was detected, with a frequency of 5%, in samples of ash trees showing dieback symptoms. This phytoplasma is poorly studied and its distribution and range of host species are not well known, however it was also detected in diverse forest species near to hazelnut plantations in Slovenia. It has previously been associated with diseased *Cornus sanguinea* and *Sambucus nigra* in Italy, potato disease in China, and hazelnut dieback during an epidemic in the United Kingdom and most recently in Slovenia.

The leafhopper *Scaphoideus titanus* transmits "flavescence dorée" phytoplasmas from grapevine to grapevine, and although it is oligophagous, it lives only on grapevine in Europe. It is not known to transmit "flavescence dorée" strains among alternative host plants, and although it can feed on a very few other host species, the possibility that it can acquire 16SrV phytoplasmas from these hosts cannot be ignored. Moreover, transmission of the 16SrV group phytoplasmas to grapevine by *O. ishidae* has been demonstrated. An abundant population of *O. ishidae* was reported as prevalent at the borders of an FD-infected vineyard and within a wood in which hazel plants were the most present and were shown to be infected by 16SrV strains, although these hazelnut shrubs were asymptomatic.

The recent introduction of several alien species of both possible phytoplasma insect vectors and weeds increased the risk of spreading new phytoplasmas or new strains that could affect the sanitary condition of the forests surrounding agricultural crops. The main recommendation resulting from the PhyFor project is that a continuous monitoring should be performed especially in the forest areas surrounding crops for the early detection of possible sources of phytoplasma infection and insect vectors taking especial attention to the new/alien species that are introduced accidentally in the forest as "new" crops considering the increasing impact of climate change that is obliging farmers to modify the species that are traditionally grown in the diverse areas and that were well settled in the diverse environments. The latent phytoplasma infection present in several areas might not be immediately dangerous but the accidental arrival of a new plant host or a new insect vector could trigger new epidemic that is possibly difficult to recognise in time if the knowledge and the awareness about phytoplasma associated disease risks is not considered.

2.6. Benefits from trans-national cooperation

Euphresco projects are a suitable tool to build-up transnational cooperation in agriculture. In the field of forest- and environmental phytoplasma research it is particularly difficult since scientists devoted to this branch of plant pathology are few, their financial support is little, and they are usually focused on local problems (epidemics) on economically important crops such as grapevine and fruit trees. However, within the PhyFor project a consortium of experienced researchers could be assembled which established an extended knowledge- and database comprising the distribution and incidence of phytoplasmas in forest trees and their associated symptoms. In addition, new putative insect vectors and alternative phytoplasma plant hosts were identified. Beside scientific results, protocols were harmonized, aligning standards between the project partners and which will make results more comparable in the future. Scientific results were published in national and international journals, some jointly, and presented at various meetings. The established network between the research groups will persist beyond the project period, and this is particularly important as the climate change will most likely aggravate negative effects of phytoplasma infection to trees, making transborder actions mandatory.

The transnational cooperation for studying and managing the phytoplasma associated disease is one of the best tools to speed up the knowledge about these diseases since only by field-based shared observation it is possible to acquire the appropriate knowledge about epidemics and their management. The climate change is now a transnational phenomenon that is enhancing the dangerous effects of these insect borne diseases traditionally impacting very severely the tropical and subtropical areas of the world. In this project the comparison of the phytoplasma presence, symptomatology and possibility to infect new and alternative host and insect species in diverse geographic areas of Europe started to delineate and clarify the possible relevant impact of these diseases in the natural European environments and also confirmed the impact on crops and the risk of introducing new crops into the diverse agricultural areas.

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

- Babaei, G., Esmaeilzadeh-Hosseini, S.A., Zandian, M., Bertaccini, A. 2020. Occurrence and identification of a phytoplasma associated with *Pinus brutia* witches' broom disease in Isfahan, Iran. *Australasian Plant Pathology*, 49, 655–660.
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4. Open Euphresco data

None.