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SUSTAINABLE FOOD SECURITY
H2020-SFS-2014-2 SUB CALL OF: H2020-SFS-2014-2015
SFS-03A-2014: NATIVE AND ALIEN PESTS IN AGRICULTURE AND FORESTRY
GRANT AGREEMENT : 635646

POnTE PROJECT

PEST ORGANISMS THREATENING EUROPE

1ST ANNUAL MEETING

POnTE
PestOrganisms
ThreateningEurope

BOOK OF ABSTRACTS

MADRID, 12-13 DECEMBER 2016

AGRICULTURAL SCIENCES INSTITUTE
SPANISH NATIONAL RESEARCH COUNCIL
C/SERRANO 115 DPDO., 28006, MADRID, SPAIN
MEETING ROOM: "SALÓN DE ACTOS"

FOOD
H2020-SFS-2014-2 SUB CALL OF:
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Horizon 2020
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SUSTAINABLE
SECURITY
H2020-SFS-

SFS-03A-2014: NATIVE AND ALIEN PESTS IN AGRICULTURE AND FORESTRY
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1ST ANNUAL MEETING

BOOK OF ABSTRACTS

EDITED BY: M. MORELLI (CNR-IPSP)

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MEETING ROOM: "SALÓN DE ACTOS"

1ST ANNUAL MEETING
HORIZON 2020 PROJECT PONTE (PEST ORGANISMS THREATENING EUROPE)
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THE PROJECT IN BRIEF

TOPICS

PONTE WILL FOCUS ITS ACTIVITIES ON THE INVESTIGATION OF GENETICS, BIOLOGY, EPIDEMIOLOGY, VECTOR ECOLOGY AND ECONOMIC IMPACTS OF THREE MAIN PATHOSYSTEMS THAT THREATEN STRATEGIC CROPS AND NATURAL LANDSCAPES IN THE EU

XYLELLA FASTIDIOSA (XF) AND HEMIPTERAN VECTOR SPECIES. The harmful bacterium Xf is involved in a new and severe olive disease (Olive Quick Decline Syndrome – OQDS) firstly reported in 2013 in southern Italy (Apulia region, Salento peninsula). Preliminary investigations showed that symptomatic olive trees were affected by a biocomplex of pests and plant pathogens: the Gram-negative bacterium Xf, several pathogenic fungal species and Zeuzera pyrina (Leopard moth). Xf was previously isolated from olive in California, whose strain proved to be phylogenetically related to subsp. multiplex, and classified as "Genotype A". In contrast, Xf isolated from OQDS in Italy was identified as a novel and distinct genotype (denoted as "CoDiRO Xf strain"), which has a phylogenetic relationship with isolates of Xf subsp. pauca. In February 2014, a Xf strain with a genetic profile similar to the CoDiRO Xf was found and identified in oleander in Costa Rica. Although Xf is widely distributed and studied in the Americas due to diseases caused in grapevine, fruit trees, and landscape plants, the recent outbreak of Xf in olive trees in southern Italy, is the first confirmed presence of Xf in the EU. Xf is exclusively transmitted by xylem-fluid feeding insects. A preliminary survey of the hemipteran population in Xf foci area indicated that the primary xylem-feeding insect there was the spittlebug Philaenus spumarius. PCR assays of head capsules of P. spumarius collected from weeds in olive groves with OQDS in this area showed a high percent was positive for Xf and transmission tests proved P. spumarius main role in the Xf CoDiRO strain transmission.

'CANDIDATUS LIBERIBACTER SOLANACEARUM' AND PSYLLID VECTOR SPECIES. CaLsol is a recently described phloem-limited, Gram-negative, not culturable bacterium that has emerged as one of the most important pathogens affecting potato and other solanaceous crops (i.e. tomato and pepper) in the Americas and New Zealand. Recently EPPO has recommended member countries to regulate solanaceous haplotypes of CaLsol and its psyllid vector Bactericera cockerelli as quarantine pests, since non-solanaceous CaLsol haplotypes have now been found in Europe associated with diseased carrots and celery. The emergence of these CaLsol haplotypes in carrots and celery has raised serious concerns about the risk that they pose to potato and other solanaceous crops across the whole EU.

HYMENOSCYPHUS FRAXINEUS (ANAMORPH. CHALARA FRAXINEA) AND NEW AND EXOTIC PHYTOPHTHORA SPECIES. Hp is a pathogen introduced, for the first time in Poland in 2006, via plant trade, mainly affecting common ash (Fraxinus excelsior) and the narrow-leafed ash (F. angustifolia). The disease is usually fatal and has now been reported in most continental European countries as a very serious threat to ash populations. In addition, an increasing number of new emerging diseases affecting forest trees caused by several Phytophthora spp. is leading to significant economic losses and pose considerable risks to natural ecosystems. The knowledge of the genus Phytophthora is still limited and some hybrid species are still evolving, potentially increasing the risk of colonization of new forest hosts.

SPECIFIC OBJECTIVES

The specific objectives of POnTE are focused on the investigation of genetics, biology, epidemiology, vector ecology and economic impacts of four pathosystems that threaten strategic crops and natural landscapes in the EU in order to identify economically, technically feasible and environmental sustainable integrated management strategies for the containment of each pathosystem. For each target, the research activities will implement the state-of-the-art and provide a novel scientific background to sustain future management policies. The specific objectives will broadly cover all targeted pathosystems merging multidisciplinary research with the practical needs of the stakeholders and end-users.

LIST OF BENEFICIARIES

P1	CNR, ITALIAN NATIONAL RESEARCH COUNCIL, Italy
P2	UNIBA, UNIVERSITY OF BARI ALDO MORO, Italy
P3	INRA, FRENCH NATIONAL INSTITUTE FOR AGRICULTURAL RESEARCH, France
P4	ANSES, FRENCH AGENCY FOR FOOD, ENVIRONMENTAL AND OCCUPATIONAL HEALTH AND SAFETY, France
P5	IVIA, VALENCIAN INSTITUTE FOR AGRICULTURAL RESEARCH, Spain
P6	CSIC, SPANISH NATIONAL RESEARCH COUNCIL, Spain
P7	SG SASA, SCOTTISH GOVERNMENT–SCIENCE AND ADVICE FOR SCOTTISH AGRICULTURE, United Kingdom
P8	FORESTRY COMMISSION RESEARCH AGENCY, United Kingdom
P9	BFW, FEDERAL RESEARCH AND TRAINING CENTRE FOR FORESTS, NATURAL HAZARDS AND LANDSCAPE, Austria
P10	LUKE, NATURAL RESOURCES INSTITUTE FINLAND, Finland
P11	WU, WAGENINGEN UNIVERSITY, The Netherlands
P12	NIBIO, NORWEGIAN INSTITUTE OF BIOECONOMY RESEARCH, Norway
P13	UCR, UNIVERSITY OF COSTA RICA, Costa Rica
P14	ARO, AGRICULTURAL RESEARCH ORGANIZATION OF ISRAEL, THE VOLCANI, Israel
P15	UB, UNIVERSITY OF BELGRADE, Serbia
P16	CERTIS EUROPE B.V., The Netherlands
P17	AUREA IMAGING BVBA, Belgium
P18	VILMORIN S.A., France
P19	LOEWE BIOCHEMICA GMBH, Germany
P20	PHYTOPHTHORA RESEARCH AND CONSULTANCY, Germany
P21	ACLI RACALE–AGRICULTURAL COOPERATIVE SOCIETY, Italy
P22	AGRITEST SRL, Italy

P23 CITOLIVA FOUNDATION, INNOVATION AND TECHNOLOGY CENTER FOR OLIVE FARMING AND OLIVE OIL, Spain

P24 AGRICULTURAL VILLENA COOPERATIVE, Spain

P25 A L TOZER LTD, United Kingdom

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P1 DEPARTMENT OF AGRICULTURAL, FOREST AND FOOD SCIENCES (DISAFA) OF THE UNIVERSITY OF TORINO, ITALY

P2 CENTRO DI RICERCA, FORMAZIONE E SPERIMENTAZIONE IN AGRICOLTURA (CRSFA) "BASILE CARAMIA", ITALY

P10 DEPARTMENT OF AGRICULTURAL SCIENCES OF THE UNIVERSITY OF HELSINKI, FINLAND.

CONSORTIUM BODIES

THE COORDINATOR

His primary role is to represent the intermediary between the European Commission (EC) and the Consortium as well as to be the promoter and supervisor of the overall technical and scientific progress of POnTE.

Donato BOSCIA **(CNR-IPSP, Italy)**

THE SCIENTIFIC COORDINATION TEAM

The Scientific Coordination Team is a management body chaired by the Project Coordinator; it is composed by the sub-Coordinators in charge for the coordination of the research and dissemination activities related to the three pathosystems targeted by POnTE.

TOPIC XYLELLA FASTIDIOSA

Maria SAPONARI **(CNR-IPSP, Italy)**

TOPIC CANDIDATUS LIBERIBACTER SOLANACEARUM

María Milagros LÓPEZ **(IVIA-PPBC, Spain)**
Anne NISSINEN **(LUKE, Finland)**

TOPIC EMERGING DISEASES OF FORESTS

Ana PEREZ-SIERRA **(FORESTRY RES AG, United Kingdom)**

THE GENERAL ASSEMBLY

The General Assembly is the decision-making body of the Project. All Project partners are seated in the General Assembly, chaired by the Project Coordinator. The General Assembly meets annually, unless the interest of the Project may require intermediate meetings, to

consider the reports of the Project Coordinator, the Scientific Coordination Team, accounts for the past financial year, and to decide upon changes to the Implementation Plan.

THE MANAGEMENT BOARD

The Management Board is the decision-implementing body of the Project. Chaired by the Coordinator, the Management Board is composed of the following persons (WP leaders), each of them holding both scientific excellence and strong experience in large collaborative research and development projects.

Donato BOSCIA	**(CNR-IPSP, Italy) Coordinator, WP11 Leader**
Ana PEREZ-SIERRA	**(FORESTRY RES AG, UK) WP1 Leader**
Maria SAPONARI	**(CNR-IPSP, Italy) WP2 Leader**
Blanca Beatriz LANDA	**(CSIC, Spain) WP3 Leader**
María Milagros LÓPEZ	**(IVIA-PPBC, Spain) WP4 Leader**
Francesco PORCELLI	**(UNIBA-DiSSPA, Italy) WP5 Leader**
José BLASCO	**(IVIA-AC, Spain) WP6 Leader**
Pasquale SALDARELLI	**(CNR-IPSP, Italy), WP7 Leader**
Alfons Oude LANSINK	**(WU-BEC, The Netherlands) WP8 Leader**
Anne NISSINEN	**(LUKE, Finland) WP9 Leader**
Aleksa OBRADOVIĆ	**(UB-FA, Serbia) WP10 Leader**

THE COORDINATION TEAM

The Coordination Team, provided by CNR-IPSP, is made up by the Project Coordinator assisted by a sub-Coordinator and 2 personnel Units, one from administrative and one from research staff. The Coordination Team is in particular responsible for Project administration, consolidation of the annual Project reports, financial monitoring, management of the web resources and partner assistance.

Donato BOSCIA	**(CNR-IPSP, Italy) Coordinator**
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Luciana SAVINO	**(CNR-IPSP, Italy) Administrative Staff**

THE SCIENTIFIC ADVISORY GROUP

The Scientific Advisory Group consists of internationally acknowledged scientists and experts from outside the Project. The main role of the Scientific Advisory Group is to provide the Project with points of view and advices coming from other scientific communities and areas interested in the outcomes of the Project.

Prof. Alexander PURCELL	**(University of Berkeley, CA, USA)**
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Dr. Giuseppe STANCANELLI	**(European Food and Safety Agency, EFSA)**
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Dr. Martin WARD	**(European Plant Protection Organization, EPPO)**
Prof. Niklaus J. GRUNWALD	**(University of Davis, CA, USA)**
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THE BOARD OF STAKEHOLDERS

The Board of Stakeholders is appointed from International and National Plant Protection and Quarantine services, from Policy makers at the EU level, growers, producers and nurserymen organizations and will ensure that the Consortium takes into account the interests of the stakeholders and end-users and operates for the benefit of the EU growers and of the Plant Protection Services. Besides the list below, several other members are expected to join the Board in the next months.

Dr. Mirkka SOUKAINEN	**(Finnish Food Safety Authority, EVIRA, Finland)**
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MEETING PROCEEDINGS

WORK PACKAGE 1

AETIOLOGY OF EMERGING DISEASES AFFECTING RELEVANT CROPS AND FORESTRY

XYLELLA FASTIDIOSA SUBSP. *FASTIDIOSA* DETECTED ON SWEET CHERRY TREES IN MALLORCA, SPAIN

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Following the identification in 2013 of *Xylella fastidiosa* in the southern of Italy, rapid emergency measures have been implemented in the EU for its prevention, detection and surveillance. In the framework of routine inspections performed in Mallorca, Spain, in October 2016, samples were taken from an open air nursery, and some sweet cherry plants were analyzed according to the protocol of the European and Mediterranean Plant Protection Organization (EPPO, 2016) and found positives for *X. fastidiosa* by two real time PCR tests. Besides, the bacteria was isolated in two media after 11 days of incubation. The MLST analysis of genes *leuA, petC, malF, cysG, holC, nuoL* and *glT* identified the isolate as *X. fastidiosa* subsp. *fastidiosa*.
ST-1. Subsequent sampling in the same nursery allowed the identification of two other cherry plants infected by the same bacterium. The cherry plants were grown in the nursery of Mallorca for the last four years and did not show typical leaf scorch symptoms. They were previously cultivated in another nursery in Tarragona (Spain) that imported them from The Netherlands. After confirmation of the analysis, the plants were destroyed right away as required by the EU legislation. The contingency plan of the Spanish Ministry of Agriculture (MAPAMA) was implemented and survey on potential host plants as well as sampling and testing within a radius of 100 m were performed around the infected plants and a buffer zone of 10 km, was designed. This is the second report of *X. fastidiosa* subsp. *fastidiosa* in an EU country after the detection of this subspecies in an oleander plant in Germany.

CHARACTERIZATION OF THE FUNGAL COMMUNITY OCCURRING IN THE SAPWOOD OF BOTH HEALTHY AND DISEASED OLIVE TREES SHOWING OLIVE QUICK DECLINE SYNDROME SYMPTOMS

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Several fungal species have been found associated with the Olive Quick Decline Syndrome (OQDS), among which some have long been recognized as primary pathogens of many trees and shrubs plants (i.e. *Phaeoacremonium* and *Pleurostomophora* spp.), causing wood discoloration, dieback and wilting. In order to characterize the fungal community occurring in the sapwood of both healthy and diseased olive trees showing OQDS symptoms, extensive field surveys were conducted in the main olive growing areas throughout the entire Apulia region, from the southernmost (Salento peninsula) to the Northernmost part (Gargano, province of Foggia). The presence of *X. fastidiosa* (*Xf*) in the samples was ascertained by using different PCR protocols. Fungal cultures were isolated from discolored sapwood of olive twigs and branches by using different agarized media. Results indicate that fungal species belonging to the *Phaeoacremonium*, *Pleurostomophora*, and *Pseudophaeomoniella* genera, occur both in healthy and in Xf-infected olive trees, as well as in the Northern and in the Southern Apulian olive growing areas, although with a higher frequency in the latter part. Moreover, morphometric characters and molecular analysis revealed that a high variability exists in the new genus *Pseudophaeomoniella*, thus suggesting that other species than *Ps. oleae* and *Ps. oleicola* could be identified. Specific pathogenicity tests were performed in a glasshouse on two-year-old olive plants (cv Cellina di Nardò), by inoculating some fungal strains, alone (*Ph. aleophilum* B1a, Ph. *rubrigenum* N20, Ps. *oleae* Fv84, *Ps. oleicola* M24, *Pseudophaeomoniella* sp. M51), or in combination with *Xf* CoDiRO strain. Preliminary results collected one year after the inoculation, seem to indicate that fungal strain(s) alone are able to colonize the olive xylem, although at variable extent, and to cause wood streaking, with limited number of twigs showing wilting in the lower part of the vegetation, close to the inoculum point on the main trunk. Conversely, the combined inoculations with *Xf* CoDiRO strain, induced typical and extensive OQDS wilting symptoms in the upper part of the plants. Further researches are in progress to better understand the pathogenic role of these fungi into the OQDS.

ASH DIEBACK MONITORING IN LOWER AUSTRIA

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Ash dieback causes damage and mortality of *Fraxinus excelsior* (common ash) and *Fraxinus angustifolia* (narrow-leaved ash) in Europe. In Austria, the first symptoms of the disease caused by the fungus *Hymenoscyphus fraxineus* (anamorph *Chalara fraxinea*) were recorded in 2005. To study the development and etiology, 14 permanent monitoring plots were installed in various parts of Lower Austria and continuously monitored almost every year since. Twenty mature ash trees per plot were assessed between July and August. The affected crown volume was visually estimated on a 5 % scale. In 2016 the mean crown dieback intensity of the remaining 248 trees was compared to their first assessment in 2008 showing an increase from 11% to 29%. Mean damage of the least affected plots also increased from 1% in 2008 to 11% in 2016.The most affected plot in 2008 remained the most severely damaged plot in 2016 with a mean value increase from 34 % to 71 %. Overall, mortality was low with 4 % and observed in five out of the 14 plots. Work is in progress to relate the long-term observation of ash dieback to climatic data.

CURRENT KNOWLEDGE ABOUT THE DISTRIBUTION OF *HYMENOSCYPHUS FRAXINEUS* IN SERBIA

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The problem of Ash decline has been for the first time observed in early 1990's in Poland. In the coming year disease has spread throughout Europe and produced serious damages in naturally established and planted ash stands. The disease has been identified in neighboring countries in 2005 in Romania, in Slovenia (2006), Croatia (western part 2009, eastern 2012) and Bosnia and Herzegovina (eastern part 2009 and central 2013, eastern 2014). In Serbia, the presence of *H. fraxineus* was monitored since 2011. Although decline of ash trees was present, symptoms were usually disguised with the presence of other disturbing abiotic (extreme drought 2012 and 2013, extreme wed 2014) and biotic (*Phytophthora* spp., *Stereonychus fraxini* – defoliation, etc.) factors. The first *H. fraxineus* resembling cultures were obtained in late autumn 2015. Ten cultures from three localities were identified as *H. fraxineus* after morphological and molecular analyses. Presence of *H. fraxineus* was confirmed for both *Fraxinus excelsior* and *F. angustifolia*, which were previously known as susceptible hosts. During 2016 extensive study of presence of the invasive alien ash dieback pathogen in wide range of forest ecosystems was performed. Forty-two localities were selected throughout Serbia and inspected for the presence of symptoms and tree decline. Variety of symptoms was observed on leaves, shots and in crowns of studied trees. Obtained knowledge about the symptoms, distribution, aetiology and biology of the ash dieback pathogen *H. fraxineus* will be presented.

ESTABLISHING A *PHYTOPHTHORA* BASELINE IN BRITISH SOILS

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Phytophthora trees diseases in the UK have increased in recent years and metabarcoding approaches suggest that many *Phytophthora* species are endemic in various soil environments. The aim of this project is to determine the range of *Phytophthora* species and the extent to which these might pose a risk to trees in the UK, especially those not previously thought to be present and therefore possibly cryptic or undetected. Traditional baiting methods will be used for isolation of *Phytophthora* from the soil, but simultaneously DNA will be extracted from the same soil samples, amplified with genus-specific primers and then massively parallel sequenced on an Illumina MiSeq platform. Both ITS1 and COX amplicons will be used for taxonomy assignments. Land use and planting history may influence the likelihood of *Phytophthora* occurrence, so survey sites include those categorised as 'disturbed' i.e. those frequently visited by the public, with recent and new plantings that can be linked to nurseries versus forest and woodland sites with little disturbance or interventional management ('undisturbed'). The study is planned to cover at least twelve sites (four in Scotland, six in England and two in Wales), each site comprising ten locations to be sampled during two different seasons. The locations within each site represent different environments, including soil types, land use, new plantings, and areas of recreation. An update of the results will be presented and the findings discussed.

DIVERSITY OF *PHYTOPHTHORA* SPECIES IN NATURAL FORESTS AND STREAMS AND IN RUBBER PLANTATIONS IN VIETNAM

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Many devastating tree declines are driven by non-native *Phytophthora* species which remain unnoticed in their native environment and after introduction to other continents became invasive, threatening a non-coevolved highly susceptible flora. There is an accumulating body of evidence that many important *Phytophthora* pathogens with global distribution are native to Southeast Asia suggesting this region might be one center of origin of the genus *Phytophthora*. Therefore, a 5 weeks *Phytophthora* survey was conducted in spring 2016 in 23 forest stands (tropical rainforests and montane forests) between 12 and 2903 m altitude in 4 National Parks, in 10 natural rivers and streams and in 14 rubber tree plantations across Vietnam. Using classical and molecular identification, the 793 oomycete isolates obtained could be assigned to 18 known species and informally designated taxa of *Phytophthora*, 21 new *Phytophthora* taxa, a diverse array of known and new taxa of *Phytopythium*, *Pythium* and *Elongisporangium* including 15 genotypes from the *Phytopythium vexans* complex, and a new sister genus of *Phytophthora* which is informally designated as *Nothophytophthora* gen. nov. The results from the Vietnamese survey contribute to clarify the origin of the two most destructive invasive *Phytophthora* pathogens of trees and natural ecosystems. Detailed morphological and physiological studies, sequencing of additional gene regions and multigene phylogenetic analyses are underway to characterise and officially describe the new *Phytophthora* species from Vietnam and the new genus *Nothophytophthora*. Soil infestation trials will be performed to test the potential threat posed by new *Phytophthora* species from Vietnam to European forests.

WORK PACKAGE 2

BIOLOGY AND PATHOGENESIS

A PARALLEL APPROACH IN UNDERSTANDING *XYLELLA FASTIDIOSA*: THE COSTA RICA CONTRIBUTION

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Xylella fastidiosa is endemic in Costa Rica. The bacterium was originally detected in the 1980´s in coffee plants causing a milder version of coffee leaf scorch named "crespera". Consequently, in the last decade, *X. fastidiosa* has been detected and isolated from more than 20 different economic important crops and ornamentals, and most importantly extending the geographic range of detection of the bacteria. However, although the bacterium has great potential for disease and it is widespread throughout the country, the symptoms related to infected plants are usually mild or asymptomatic. In 2015, the European Union closed the importation of ornamentals from Costa Rica, alleging that *X. fastidiosa* was introduced in this matter into Europe and that is related to the serious epidemic disease affecting Olives. It is known that *X. fastidiosa* strains isolated from Costa Rica represent a broader genetic variability than in other countries. The report of ST53 *X. fastidiosa* strains from Costa Rica is of particular interest. In this direction, our contribution is based on isolating *X. fastidiosa* from different hosts to allow a broader genomic characterization and comparison with the European strains. Complementary to genetic profiling, we are focused in phenotypic characterization of *X. fastidiosa* strains, through the standardization of bacteriological, biological and immunological based assays that could eventually be used in reference and research units for *X. fastidiosa* profiling.

PATHOGENICITY OF *XYLELLA FASTIDIOSA* ON PLANTS OF INTEREST FOR FRANCE

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Xylella fastidiosa was detected for the first time outdoor in France in 2015. Since the detection of the first focus in Corsica several sequence types (STs) and subspecies have been identified on nearly 30 plant species. Other STs have been detected in Europe on intercepted coffee plants, in nurseries, and in natural settings. Determining the real threat that this diversity of strains represent for the main plant productions in France is the aim of the pathogenicity tests we launched. However, due to a strict adhesion to the 2008/61/CE directive, pathogenicity tests using this plant pathogen have to be monitored in type S3 confined growth chambers. In consequence only a limited number of individual can be tested, and due to technical constraints a certain degree of uncertainty surrounds these costly tests conducted during six to 18 months. We plan to test the pathogenicity of strains representing the subspecies *fastidiosa*, *sandyi*, *multiplex*, and *pauca* and especially the STs identified so far in Europe. The plant species and genotypes to be tested have been chosen to represent either an economic interest for mainland France or Corsica (grapevine, apple tree, pear tree, citrus, olive tree), or positive control (coffee, oleander and polygala) or indicator plant (tobacco and periwinkle). Monitoring the pathogenicity of *X. fastidiosa* strains is part of our involvement in both H2020 projects POnTE and XF-ACTORS.

HOST PLANTS COLONIZATION BY *XYLELLA FASTIDIOSA*

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Since 2012, Anses isolated several strains of *Xylella fastidiosa* from different intercepted coffee plants. Three of them are available at the CIRM-CFBP: one strain of *Xylella fastidiosa* subsp. *sandy/fastidiosa* (CFBP 8073) and two strains of *Xylella fastidiosa* subsp. *pauca* (CFBP8072 and CFPB8074). During pathogenicity test carried out with these strains in order to study colonization of host plant by *Xylella fastidiosa*, the presence of bacteria was observed in the different parts of the coffee plant, under and below the inoculation point and in roots. On *Citrus sinensis*, no migration of these strains has been observed but maintenance at the inoculation point ten months after contamination. The presence of the bacteria in the plants was assayed using the real-time PCR (Harper *et al*., 2010, Erratum 2013).

ISOLATION AND BIOLOGY TRAITS OF XYLELLA FASTIDIOSA ISOLATES RECOVERED IN APULIA

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The isolation in pure culture of the *Xylella fastidiosa* is one of the critical steps for establishing comprehensive research programs aiming at investigating its host range, genomics and population genetics. An isolation campaign was started in Apulia in spring 2016 to collect isolates representing several foci in the infected demarcated area of Apulia (southern Italy) and different hosts. Axenic cultures were successfully established either using hardwood cuttings squeezed on BCYE medium (olive, oleander, myrtle-leaf milkwort, cherry, almond and laurel) or by plating dilutions of the PBS-extracts from leaves on PWG plates (oleander, cherry, myrtle-leaf milkwort and lavender). The isolates (approx. 50) so far recovered are preserved at -80°C in 50% glycerol and available for genomic and genetic studies. Evaluation of the pathogenicity and host specificity of the selected reference olive strain "De Donno" was started at the end of 2014 within the EFSA Pilot project NP.EFSA.ALPHA.2014.07, and further implemented within the first year of the project POnTE, with the specific objective of consolidating the knowledge on the susceptibility of grapes and stone fruits upon mechanical inoculations or exposure to naturally infected vectors. Indeed, the pathogenicity on olives was confirmed by inoculating a new set of plants of the susceptible variety "Cellina di Nardò". Although the inoculation procedure (three inoculum points per plant) and the growing conditions (at 24-28°C) need to be further optimized, the procedure so far adopted proved to be efficient for the successful infection and the symptom development in the susceptible hosts, i.e. olive and myrtle-leaf milkwort. Within the 2016 surveys for the identification of susceptible hosts in the infected area, several novel and hitherto unknown susceptible species to the CoDiRO strain have been reported, including typical essences of the "Macchia Mediterranea" and common weeds.

'*CANDIDATUS* LIBERIBACTER SOLANACEARUM', DETECTED ON CARROT AND TOMATO IN TUNISIA

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In 2014, 2015 and 2016 symptoms associated in several countries to '*Candidatus* Liberibacter solanacearum' (CaLsol) were observed on carrot and tomato plants in Kairouan region, Tunisia. Yield losses and vegetative disorders were observed in affected fields. Samples were analysed by real-time PCR to investigate if such bacterium and/or to phytoplams and/or to *Spiroplasma citri* were detected. Carrot and tomato plants of local Tunisian cultivars, insect vectors and weeds were analyzed, with positive results for CaLsol in 14 out of 93 samples of carrots plants and 2 out of 87 samples of tomato plants. On the contrary 57 insect vectors were negative for CaLsol (49 *Bactericera trigonica* and 8 *B. nigricornis*) and also six samples from *Morella* sp. The obtained results showed the detection of CaLsol and the absence of phytoplasmas and *S. citri* in all samples. Furthermore, Tunisian carrot seeds of local production were also analyzed and were positive for CaLsol, with average cell viability around 55%, much higher than that of commercial European seeds (3-5%). Probably this big difference is related to the lack of treatments in Tunisian seeds. The haplotype of CaLsol present in positive carrot plants was analyzed and characterized as E and D, but interestingly, the haplotype D was also detected in the three lots of Tunisian carrot seeds. CaLsol haplotype of tomato plants is pending. This is the first report of the presence of CaLsol in Tunisia and the first in tomato outside America and New Zealand.

WORK PACKAGE 3

GENOTYPING AND GENETIC STRUCTURE OF THE PATHOGEN POPULATIONS

GENETIC ASSESSMENT OF XYLELLA FASTIDIOSA ISOLATES RECOVERED IN THE SALENTO PENINSULA

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Xylella fastidiosa is a plant pathogenic bacterium, recently introduced in Europe that is causing a decline in olive trees in the South of Italy. Preliminary genetic studies using Multi Locus Sequence Typing (MLST) analysis revealed that the bacterial genotype recovered from the infected olive trees belongs to the sequence type "ST53" within subspecies *pauca*. This genotype has also been reported to occur in Costa Rica, and more recently in France. Extensive MLST analyses coupled with next generation sequencing (NGS) approaches have been conducted to assess the population genomics of the isolates occurring in the epidemic area of the Salento peninsula. All the different tested susceptible hosts and all the olive trees sampled in the most recently reported outbreaks show to harbor the same sequence type ST53. NGS using complementary approaches based on Illumina and PacBio technologies was used to reconstruct the genome of the selected reference isolate "De Donno", being the first sequenced olive strain. Indeed, the draft genomes of two additional olive isolates were obtained both from the pure culture and total plant DNA. The sequence dataset of these novel reconstructed genomes was inferred with the recently sequenced draft genomes of the Costa Rican ST53 genotypes to assess their phylogenetic relatedness. Comparative analysis based on single nucleotide polymorphisms and the study of the pan-genome of these whole genome sequences along with those currently available for *X. fastidiosa*, confirmed that within the subsp. *pauca* the Italian and Costa Rican ST53 isolates formed a distinct phylotype in a clade divergent from the South American *pauca* isolates. The clustering and distinctiveness of the ST53 isolates support the hypothesis of their common origin, and the limited genetic diversity among these isolates suggests this is an emerging clade.

A SET OF VARIABLE NUMBER OF TANDEM REPEATS LOCI TO STUDY GENETIC DIVERSITY OF *X. FASTIDIOSA* POLULATIONS IN THE SALENTO PENINSULA

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Extensive sampling of diseased olive trees and other infected hosts in the epidemic area of Apulia demonstrated the widespread presence of a single Multilocus Sequence Type (ST53) which is indicative that a single pathogen introduction led to the current outbreak in Salento. Fast-evolving VNTR (Variable Number of Tandem Repeats) loci were selected to study the genetic diversity of field populations since they have been previously shown to provide adequate resolution for *X. fastidiosa* populations in other crops. About 40 VNTR loci, previously described for *X. fastidiosa,* were searched for their presence in the CoDiRO draft genome. Of those, 14 VNTR loci were selected and tested against DNA samples from 20 *X. fastidiosa* isolates obtained from different crops and locations in Apulia. Amplifications and sequencing was performed to validate their usefulness. Results indicated that 3 VNTRs show no repeats in CoDiRo-type isolates, 1 VNTR has two different repeats, 3 VNTRs are no informative among Italian and other *pauca* isolates, and only 5 VNTRs show polymorphisms within Italian strains. Additionally, 26 new VNTRs were selected on the CoDiRO draft genome and new primer pairs designed. Those 26 VTNR loci are being validated by PCR amplification and fragment sequencing of different *X. fastidiosa* subspecies and the same collection of *X. fastidiosa* from the outbreak referred above. This set of VTNR loci can be used to study the genetic diversity of *X. fastidiosa* field populations in the Salento Peninsula, as well as the consequences of vector transmission of *X. fastidiosa* on the structure of these populations.

DIVERSITY OF *XYLELLA FASTIDIOSA* IN FRANCE

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While listed as a quarantine pest for Europe *Xylella fastidiosa* emerged in France and this is evidenced through the survey of coffee plant trading from Latin America as well as the survey of natural settings. Since the discovery of the first contaminated foci of *Polygala myrtifolia* shrubs showing leaf scorch symptoms in France during 2015 summer, numerous plants have been screened, ending in the identification of more than 300 foci. Here, we report on the diversity of *X. fastidiosa* infections over the year 2015 in France in natural settings and in imported coffee plants. Multilocus sequence typings revealed that several subspecies and sequence types are associated to the emergence of *X. fastidiosa* in France. Evidences of recombination and mixed infections in a same plant have been found. The genomes of some strains of *X. fastidiosa* subsp. *multiplex* that were isolated in France were sequenced and compared to their American relatives. Pathogenicity of these strains is also tested on *P. myrtifolia* and other potential hosts. Altogether, these analyses suggest that *X. fastidiosa* has been introduced several times in France via various plant material from different origins. This work was done as part of INRA's duties in WP2, task 2a and WP3, task 3a of POnTE project.

GENETIC VARIABILITY OF '*CANDIDATUS* LIBERIBACTER SOLANACEARUM' ASSOCIATED WITH APIACEOUS CROPS IN FRANCE

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'Candidatus Liberibacter solanacearum' (Lso) is emerging as an important pathogen of several solanaceous and apiaceous crops worldwide. There are currently five recognized Lso haplotypes (designated A, B, C, D and E). The objective of the present work was to identify the Lso haplotypes in France and to determine the genetic relationships among Lso haplotypes. For this purpose, 192 apiaceous samples were collected from 2012 to 2016 in different geographical regions and were tested for Lso infection by real-time PCR assay. In addition to carrot and celery, Lso was detected for the first time in four other apiaceous crops: chervil, fennel, parsley and parsnip, suggesting that Lso has a wider host range within the Apiaceae family than expected. Sequencing of the 16S rRNA and 50S rplJ-rplL genes revealed the occurrence of haplotypes D and E in France. Then, we assessed the phylogenetic relationships between strains isolated in France and a worldwide collection of Lso isolates by using the rplJ-rplL gene sequences. The rplJ-rplL based phylogenetic tree delineated five clusters corresponding to the five Lso haplotypes, with LsoD and LsoE being phylogenetically closely related. To further clarify the genetic structure of Lso, we are now undertaking a multilocus phylogeny scheme based on housekeeping genes by referring to the complete genome sequences of Lso. The selected genes will be sequenced from a large sample of Lso isolates representing the known diversity of the bacterial species. Altogether, the data presented here provide new insights into the genetic structure and evolutionary history of Lso.

GENETIC DIVERSITY OF '*CANDIDATUS* LIBERIBACTER SOLANACEARUM' DETECTED IN CARROTS, PARSNIP AND PSYLLIDS IN SOUTHWESTERN FRANCE

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Carrot proliferation associated with phloem-limited bacteria transmitted by carrot psyllids was recorded in France in the 1970s (Giannotti et al., 1974). '*Candidatus* Liberibacter solanacearum' (CaLsol) of different ribosomal haplotypes have recently been associated with carrot disorders in several European countries. In order to reassess disease impact and characterize the CaLsol strains associated, three carrot production areas of Southwestern France were surveyed for CaLsol in 2016. CaLsol could be detected by Taqman real-time PCR (Teresani et al., 2014) in carrots exhibiting stem proliferation and stunting as it was previously reported in Czech Republic (Franova et al., 2000), as well as parsnip and parsley. The disease had a low to moderate incidence in 2 organic carrot fields but was absent in 3 other fields surveyed. Two ribosomal haplotypes could be characterized: a first haplotype D2 differing to the haplotype D by two SNPs in the 16S rDNA and in the 16S-23S spacer sequence and a second corresponding to the haplotype E. Genotyping using the five most variable gene markers described by Glynn and collaborators (2012) showed that haplotypes D2 and E differed for all five markers *dnaG*, *metG*, *recA*, *mutS* and *adk*. *mutS* being the most discriminant with 8 SNPs between haplotype D2 and E. Both haplotypes were detected in carrot and parsnip as well as in *Triozidae* psyllids The preliminary identification of infected psyllids as *Bactericera trigonica* requires further confirmation.

GENOMIC SEQUENCE OF '*CANDIDATUS* LIBERIBACTER SOLANACEARUM' HAPLOTYPE C

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Candidatus Liberibacter solanacearum haplotype C is a pathogen of carrot that occurs in several countries in northern Europe and is transmitted by the carrot psyllid (*Trioza apicalis*). Two whole genome assemblies, FIN111 and FIN114, of *Ca.* L. solanacearum haplotype C were derived from two carrot psyllid individuals harbouring the bacteria. After sequencing by Illumina HiSeq2000 and PacBio, the Liberibacter-related reads were extracted from the metagenomic data and used for *de novo* assembly, and then the contigs of assembly FIN114 were joined using long-range PCR, primer walking and Sanger sequencing methods. The size of this genome assembly is 1.24 Mbp, and it includes two prophage regions in addition to the bacterial chromosome. The average GC content of the genome is 35.2%, and the number of predicted protein coding genes is 1067. Genomic comparisons between the haplotype C and the previously sequenced haplotypes A and B revealed differences in the genome organization, resulting from large inversions and other recombination events. Comparison of the predicted protein-coding genes of the three haplotypes indicated that the core genome of *Ca.* L. solanacearum consists of 885 orthologs, with the pan-genome consisting of 1327 orthologs. Both the pair-wise comparisons by ANI and supermatrix-based maximum likelihood phylogeny tree suggested that the core genome of haplotype C is more closely related to haplotype A than to haplotype B.

DETECTION OF *PHYTOPHTHORA* ON ENVIRONMENTAL SAMPLES BY A NEW NEXT GENERATION SEQUENCING (NGS) APPROACH

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We have tested different primer sets to be used by NGS approach using Illumina platform to assess *Phytophthora* populations in environmental samples. PCR amplifications of ITS1 and ITS2 (two primer sets), *cox*1 gene (1 primer set), *cox*2 gene (2 primer sets) and *cox*1-2 spacer (1 primer set) have been tested with DNA extracted from soil and bark of infected trees using direct or nested PCR approaches. Results indicated good amplification for direct PCR with four ITS primer sets and one *cox*2 set as well as using a second-round PCR approach with one ITS and one *cox*2 primer sets. The genes were amplified and barcode-multiplexed from the different DNA samples and then subjected to Illumina MiSeq sequencing. Amplification with *cox*2 or *cox*1-2 spacer failed, but good amplification was obtained for *cox*1 region either using direct or second-round PCR approach. Initial bioinformatics analyses indicated that although direct PCR targeting ITS regions is more convenient for methodological purposes it can oversight the presence of *Phytophthora* spp. on the samples. On the contrary direct amplification of the *cox*1 region using primers cox1levup/cox1levlo and partial bidirectional amplification of the amplicons or nested-PCR amplification of *cox*1 using cox1levup/cox1levlo in the 1st round and HVshort/cox1levlo in the 2nd round and complete bidirectional sequencing allowed detection of several *Phytophthora* spp. and other oomycetes in soil and wood samples. Comparison of results obtained with this new protocol targeting the cox1 region with that from Scibetta et al. (2012) using a nested approach targeting the ITS region is under progress.

WORK PACKAGE 4

IMPLEMENTATION AND VALIDATION OF DIAGNOSTIC KITS FOR EARLY AND RAPID DETECTION OF TARGET PATHOGENS IN HOST PLANTS AND VECTORS

RECENT DEVELOPMENTS FOR *XYLELLA FASTIDIOSA* DIAGNOSIS: EVALUATION, VALIDATION AND IMPLEMENTATION OF ROUTINE TESTING METHODS

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The implementation and validation of diagnostic tests included serological and molecular approaches with the aim of pursuing sensitive and reliable identification of the bacterium in a wide range of plant species, including symptomatic and asymptomatic hosts. Serological assays focused on ELISA and DTBIA procedures.
The newly developed polyclonal antisera, raised against the CoDiRO strain, was processed as ELISA and DTBIA reagent, thus evaluated for testing a wide range of plant matrices. Indeed, an *X. fastidiosa* MopB recombinant protein was developed for being used as standardized reference positive control in the diagnostic kits, avoiding the manipulation and the use of bacterial suspension preparations.
In addition, experiments have been made to determine the best procedure for preparing and preserving non-infectious samples (with inactivated bacterium) for diagnostic purposes, including the preparation of the samples to be used for interlaboratory validation and performance test studies. Specifically, freeze-drying and dehydration protocols were used to prepare experimental samples that can be safely manipulated by different diagnostic laboratories without specific quarantine requirements.
For molecular assays, a commercial kit (DNeasy mericon food kit, Qiagen) was successfully adapted for the automatized purification of high-quality DNA from a wide range of host plants. The qPCR assay was implemented by including the simultaneous detection of a plant DNA internal control (multiplex qPCR). A sensitive bacterial detection was also obtained through the development of a real-time LAMP assay based on crude plant sap. All the currently available methods were then tested and compared to a panel of asymptomatic infected plants (olive, oleander, cherry). All approaches were able to identify at a different level the presence of the bacterium, with qPCR assays being the most sensitive tests detecting up to 10^2 CFU/ml.

DEVELOPMENT OF NOVEL AND HIGH-THROUGHPUT DIAGNOSTIC PROCEDURES TO DETECT *XYLELLA FASTIDIOSA* (XF) IN PLANTA

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The development of novel diagnostic procedures to detect *Xylella fastidiosa* is attempted by two different methodical approaches: a serological rapid test (immunostrip or LF assay) and a molecular method, based on a sandwich type DNA-hybridization assay (NALF). Both assays will allow fast, easy and direct detection of the bacteria in the field without the need of a laboratory.

Specific Oligos have been designed and optimized for the development of the NALF, meeting the special requirements of the test system. Conjugation protocols for labelling of Oligos to gold nanoparticles have been evaluated and the reaction between the immobilized capture oligos and the Oligo-gold conjugate using a *Xylella fastidiosa* specific ss-DNA as positive control has been demonstrated, with a detection limit of $1.2 \cdot 10^{10}$ molecules. So far the method failed to detect genomic DNA of *Xf* or a *Xf* PCR amplicon and further optimization experiments for Oligo conjugation and PCR conditions are carried out.

For immunostrip (LF) development on a serological base, a new antiserum against *Xylella fastidiosa* was produced and IgGs from different bleedings have been evaluated in DAS-ELISA. While detecting six tested *Xf* subspecies so far, the new antiserum exhibits an increased specificity towards *Xf* CoDiRO, especially in terms of detection limit. A lateral flow prototype has been produced and specificity, cross reaction and healthy background reactions have been tested, with results being in agreement with ELISA data. *Xylella fastidiosa* from infected olive branches could easily been detected in both LF and ELISA and a demonstration video has been prepared.

EVALUATION AND VALIDATION OF DIAGNOSTIC PROCEDURES TO DETECT XF IN PLANTA AND VECTORS

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Xylem sap-feeding insects belonging to Hemiptera order and Auchenorrhynca sub-order are known to be the main way for X. fastidiosa spreading. The laboratory of entomology of ANSES identified forty-seven species belonging the Aphrophoridae, Cercopidae, Cicadellidae and Cicadidae families as potential vector that are present in mainland France and twelve in Corsica island. The Aphrophoridae Philaenus spumarius, known as vector in Apulia (Saponari et al., 2014, Cornara et al., 2016), is commonly distributed in mainland France and Corsica. The objective of the present work was to evaluate and validate a method for detection of Xylella fastidiosa directly on the vector Philaenus spumarius. A method based on Real-Time PCR (Harper et al., 2010) after QuickPick™ Plant DNA kit (Bio-Nobile) DNA extraction has been validated on plant host for X.f detection. In order to assure high throughput, the performance criteria of this method has been evaluated for the use of DNA extractions, KingFisher™ (Thermo Fisher Scientific) robots allowing 15 or 96 samples serial extractions. The analytical sensitivity of this method ranges from 102 bact./mL to 105 bact./mL depending of host matrices. A duplex Real-Time PCR (Harper et al., 2010, Ioos et al., 2009) with internal insect control was tested with Philaenus spumarius contaminated artificially with X.f. The samples were prepared by grinding individual head of one insect in 200µL of sterile water after removing of eyes. This method allowed detecting 103 b/head of Philaenus spumarius. More than three hundred P. spumarius specimens collected from October to December 2015 on eight Corsican outbreaks were tested individually. The observed contamination rate varied, according to the outbreak points, from 4% to 25% with an average of 8.8%. The rate of contamination was of 6% on a batch of 50 P. spumarius provided by CNR (Bari) for which the expected value was of 10%.

RAPID SCREENING TESTS FOR DIFFERENTIATING *XYLELLA FASTIDIOSA* ISOLATES

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The bacterial pathogen *Xylella fastidiosa* (*Xf*) is characterized by a wide plant host range and insect vectors, and on the basis of phylogenetic studies it was subdivided into different subspecies. Results from strain typing, phylogenetic analyses, and other data comparisons have shown that phylogenetic clusters exhibit host-based genetic relationships. Until now, different molecular tests can be used for the differentiation of *Xf* isolates, among which MLST/MLSA represents the most common method to determine classification and phylogenetic placement of novel isolates. *Xf* outbreaks in EU motivated the search for accurate and faster approaches for detection and identification of the bacterium in different plant matrices. Because MLST/MLSA requires several PCR reactions and sequencing analyses, we have developed two independent approaches for rapid taxonomic assignment of uncharacterized isolates: (1) single-nucleotide primer extension (SNuPE) method for the multiplex amplification of six *Xylella* DNA sequences (targeting all subspecies and three genotypes within *Xf* subsp. *pauca* including the type-isolate infecting olive in Italy); (2) high-resolution melting (HRM) analysis of the amplicon recovered from the gene encoding the conserved HL protein. Both assays proved to clearly differentiate *Xf* isolates currently known to occur in the Italian and France outbreaks. Indeed, validation on a larger panel of isolates covering the different subspecies consistently allowed to rapidly differentiate the isolates in different clusters. In conclusion, these approaches could represent a useful tool for pre-screening and selection of infected samples to be further analyzed by MLST or whole genome sequencing. Future project activities will be focalized on the implementation of both protocols on a larger number of isolates and new variants of Xf as they will appear.

OCCURRENCE OF *CANDIDATUS LIBERIBACTER SOLACEARUM* IN IMPORTANT CARROT GROWING AREAS IN WESTERN EUROPE AND MEDITERRANEAN BASIN

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It has been recently discovered that symptoms of purplish discoloration of leaves on carrot crops, commonly associated with a complex of viruses or environmental disorders, can also be caused by emerging pathogens in Europe such as *Candidatus Liberibacter Solacearum*, *Spiroplasma* sp. and *Phytoplasmas*. Thanks to its large experimental network in Europe and the Mediterranean basin, Vilmorin has initiated a survey in 2013 in order to estimate the occurrence of *Candidatus Liberibacter Solanacearum* (CalSol) in the main carrots growing regions. Samples from 23 carrots trials located in 6 different countries were analyzed between 2013 and 2016. Each sample exhibited at least one of the following symptoms: dwarfed shoots, dense hairy growth of secondary roots, yellowish and/or purplish discoloration of the leaves, proliferation of shoots. CalSol was detected in samples from 11 trials in 4 countries: France, Spain, Morocco and Portugal. For 4 samples, we also detected the presence of at least one of the following pathogens: *Phytoplasma*, *Spiroplama* and CMDV. No CalSol was detected in samples from UK and Holland. The DNA from 9 samples will be transferred to ANSES and INRA for further molecular diversity analysis. In parallel, a DNA bank from 80 carrots seedlots was prepared. It will be provided to other participating partners for validation of protocols to detect CalSol on carrot seeds. This includes the CalSoL detection methods developed in Vilmorin, submitted and recognized internationally by the ISHI-veg. The development of such a protocol will avoid the dissemination of CalSol through contaminated seeds.

THE DETECTION AND DIAGNOSTICS OF PSYLLID VECTORS OF '*CANDIDATUS* LIBERIBACTER SOLANACEARUM'

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SG SASA (P7) specializes in the identification (ID) of psyllids. The high number of psyllid species and the lack of taxonomically useful characteristics between some species present a challenge for the identification of vectors. To achieve reliable species ID, we use a combination of classical and molecular taxonomy and maintain voucher specimens after DNA sequencing. We have already collaborated with POnTE partners and external partners, providing them with species ID services (Task 5e). Psyllids are also obtained from suction traps in the UK. POnTE partners in Finland are providing catches from summer 2016 for ID at SASA, with two further suction traps in Spain and Belgium being organized for collection during summer 2017.

DNA sequences obtained from identified specimens are used to design diagnostic real-time PCR assays for target species. We have designed and validated an assay for *Bactericera cockerelli*, the main vector in the US, using the ITS2 (internal transcribed spacer 2) region of the genome. Due to a high similarity in ITS2 sequences between remaining vector species, alternative genome regions are being explored to design assays for the remaining known and suspected vectors (*Trioza apicalis, B. trigonica, B. nigricornis,* and *B. tremblayi*) (Task 4c). We have plans to organize a ring-test of the *B. cockerelli* assay and will be taking part in future interlaboratory/ring-tests for CaLsol (Task 4e).

Details of a Psyllid ID workshop that will be held 23-24 March 2017 at SASA, will be provided.

WORK PACKAGE 5

VECTOR-PATHOGEN IDENTIFICATION AND DISEASE EPIDEMIOLOGY

ASSOCIATION OF *CA*. LIBERIBACTER SOLANACEARUM WITH THE CARROT PSYLLID *BACTERICERA TRIGONICA* AND WITH CARROT YELLOWS IN ISRAEL

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Yellows disease of carrot is one of the main limiting factors of growing carrots in Israel. This is due mostly to reduced yields in infected fields and poor quality of symptomatic carrots. In the past, disease symptoms were shown to associate with the presence of the Gram-positive phytopathogen phytoplasma, which was considered as the main causal agent of carrot yellows in Israel. In recent years, many reports, mostly from Europe, describe the association of a different, Gram-negative bacterium, Ca. Liberibacter solanacearum (CLso), with carrot yellows. To date, five genetic haplotypes of CLso have been reported, haplotypes A and B infecting solanaceous crops and haplotypes C-E infecting Apiaceae crops, mostly carrot. A survey in two commercial carrot fields was conducted in Israel to test the possible association of CLso with yellows symptoms, and the possible presence of a CLso vector. The survey included yellow sticky traps collected and replaced every two weeks, symptom assessments and correlation with CLso detection. In both fields, large numbers of psyllids were captured throughout the growing season. Captured psyllids were identified as Bactericera trigonica, a known vector of CLso around the Mediterranean, and were positive for CLso. Carrots showing typical yellows symptoms appeared towards harvest and tightly correlated with the presence of CLso. Genetic analysis revealed that the common CLso haplotype in Israel was haplotype D. Our study reveals that carrot yellows in Israel is tightly associated with CLso presence, suggesting that CLso is the dominant agent causing this disease in Israel at present.

VECTOR PREFERENCES AS A DRIVER OF *CANDIDATUS* LIBERIBACTER SOLANACEARUM EPIDEMIOLOGY

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Candidatus Liberibacter solanacearum (Lso) is a phloem-limited bacterium transmitted by different psyllid species to *Solanaceae* and *Apiaceae* plants. The transmission of Lso mediated by European psyllids is not well understood because the feeding habits of these insects on different host are unknown. We examined the settling, oviposition and feeding behaviour of *B. trigonica* and *B. tremblayi* and determined the likely implications for the vertical transmission of Lso. *Bactericera trigonica* was unable to colonize potato plants and consequently failed to efficiently transmit Lso to potato (percentage of transmission ≤ 3). Additionally, *B. trigonica* showed a clear preference to ingest from the phloem, settle and oviposit on carrot and celery. As a consequence, Lso was efficiently transmitted to both carrot (80%) and celery (70%). *Bactericera tremblayi* preferred leek over carrot and potato, with potato the less preferred host. *Bactericera tremblayi* ingested from the phloem of infected carrots and consequently acquired Lso (acquisition =11%); However, *B. tremblayi* failed to transmit Lso from carrot to carrot (0 infected plants/30 plants tested). Therefore, based on our results, the risk of Lso transmission from *Apiaceae* to potato mediated by *B. trigonica* is very low, and *B. tremblayi* is not a likely vector of Lso.

POTENTIAL VECTORS OF *XYLELLA FASTIDIOSA* IN THE IBERIAN PENINSULA OLIVE GROVES: DISTRIBUTION AND RISKS

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The recent introduction of *Xylella fastidiosa* in Europe, causing serious epidemics and yield losses in olive groves in Southern Italy together with its generalist nature entail a great risk to European agriculture. The bacterium is transmitted by xylem feeders belonging to the Order Hemiptera, Suborder Cicadomorpha. Understanding the biology and ecology of the potential vectors of *X. fastidiosa* is essential for risk assessment of pathogen spread and to develop preventive control strategies. Our ongoing work is focused on the population dynamics of the main potential vectors of *X. fastidiosa* in the Iberian Peninsula. We selected 14 olive groves (organic, conservation tillage and conventional) distributed along the Iberian Peninsula and sampled the olive canopy and the associated ground vegetation. After using different sampling methods, our results suggest that aerial sweeping and branch beating inside plastic bags were the most adequate procedures to estimate xylem-feeder populations. Conversely, yellow sticky traps were very inefficient to capture xylem-feeders associated to olive groves. Our results indicate that *Philaenus spumarius*, the main vector in Italy, is also present in some sampling sites but at low population densities and limited to humid regions where cover crops or natural vegetation are present most time of the year. Furthermore, we have found other potential vectors such as *Neophilaenus campestris*, quite abundant on grasses below trees, but almost none in olive canopies. *Cercopis intermedia* and *Lepyronia coleoptrata* were also present in low densities. Our preliminary findings suggest that xylem-feeders are at low density and not well adapted to most of the olive-producing regions in Spain.

TRANSMISSION STUDIES ON *PHILAENUS SPUMARIUS* AND CANDIDATE VECTORS OF *XYLELLA FASTIDIOSA* IN APULIA (SOUTHERN ITALY)

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Recent surveys, aimed at describing Auchenorrhyncha occurring in olive groves in the Apulian region (southern Italy), identified the presence of at least 15 Taxa, among which the most abundant was *Philaenus spumarius* (L.), the ascertained vector of *Xylella fastidiosa* strain CoDiRO. In this first year of the project, several transmission experiments were set in order to (i) evaluate the influence of olive cultivar on the efficiency of acquisition and transmission of *X. fastidiosa* by *P. spumarius*; (ii) assess the role of other Auchenorrhyncha species as vectors of the bacterium. For the first objective, three independent transmission tests were carried out under field conditions using infected trees of two highly susceptible cultivars: "Cellina di Nardò" and "Ogliarola", and trees of the tolerant cultivar "Leccino". Adult spittlebugs collected in the pathogen-free area, were caged on the branches of the selected infected trees for 48-72 hours of acquisition and then transferred (in groups of five) onto healthy young olive trees for 48h. All the insects that survived the transmission tests were collected and tested in qPCR for the presence of *X. fastidiosa*. The results show a significant difference in the acquisition, with only 6-8% of insects retaining the bacterium after feeding on the trees of the tolerant variety vs 38-40% of positive specimens after feeding on the susceptible olive trees. Although the assessment of the efficiency of transmission is ongoing, this result is expected to be related to the acquisition rates.

For the second objective, transmission tests have been conducted using adult specimens of *Anoplotettix* spp. and *Thamnotettix zelleri* (Cicadellidae), *Agalmatium* spp. (Issidae), *Cercopis sanguinolenta (*Cercopidae) *Neophilaenus campestris* and *P. italosignus* (Aphrophoridae), and *Cicada orni* (Cicadidae).

Preliminary results on their infectivity rate (under natural and/or experimental conditions) showed that the majority of the insects tested negative for *X. fastidiosa*, with few insects yielding results close to the detection threshold. Conclusive assessment regarding their capability and role in the spread of the bacterial infections will be obtained upon sampling and testing the receptor plants in the following 6-12 months.

STAKEHOLDER-ORIENTED *PHILAENUS SPUMARIUS* SAMPLING PROCEDURES

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The sampling of *Philaenus spumarius* (L.) (Ps), as developed in the EFSA Procurement [*Collection of data and information on biology and control of vectors of Xylella fastidiosa* (RC/EFSA/ALPHA/2015/01)] is based on an intensive sampling design aiming at providing detailed information on the phenology and the local population dynamics of the pest. Monitoring for control purposes requires the survey of wide areas to the purpose of forecasting the biological events that elicit the control actions. In accordance with the objectives of POnTE WP5 we have been elaborating sampling procedures and tools to gather data useful for the design of control methods and candidate vector surveys. *Philaenus spumarius* lifecycle is peculiar as it is hemimetabolous with juveniles living in a froth and free-living adults. This biological peculiarity strongly suggests to use two different sampling protocols, one tailored for juveniles in spittles and another for the adults on herbaceous and woody hosts. The sampling unit should include a full herbaceous cover (for the spittle identification and counting) where spittle are counted in subsampling units framed by a transect. We collect Ps juvenile(s) by cutting the plant part with the spittle and dipping it in a single saline solution flask together with unit data. Nymphs in saline solution can be easily sieved out later, and transferred in ethanol with the accompanying dataset. To collect Ps adults from herbaceous or woody hosts, we use a normalized (38 cm Ø) sweeping net. We modified the net by replacing the conventional fabric sac with a net-like screen (1,5 x1,5 mm) one provided with a detachable single-sampling plastic collecting bag. After few swept (sampling subunit) on herbs or tree branches, we promptly add data into the plastic bag before detached and seal it. Bags are stored in fridges for days to kill the collected insect, then samples are stored in ethanol. Field trials with absolute beginner volunteers showed that, following 30 minutes of training, most of the volunteers are able to sample for juveniles and adults. Subsampling for juveniles takes about 10' by a two-person team that can also sample for adults ten subunits in the same time lapse. Finally, skilled operators shall identify the specimens.

WORK PACKAGE 6

FIELD AND AUTOMATED SURVEILLANCE SYSTEMS FOR VECTOR AND DISEASE MONITORING

SPATIAL AND TEMPORAL DYNAMICS OF CALSOL IN FINLAND - PRELIMINARY RESULTS

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To study the dynamics of CaLsol spreading in carrot, an experiment was carried out on a commercial farm in the area of the highest occurrence of CaLsol in Finland. Carrot psyllid flight activity was monitored at the experimental plot by yellow sticky traps. According to the experimental design, three carrots were sampled at 25 points within the field plot once a month starting from late June. The plants were weighed, and the total number of leaves and the number of damaged and discoloured leaves were recorded, and samples were cut for CaLsol test. DNA was extracted by DNeasy Plant Mini kit protocol (Qiagen), and presence of CaLsol was determined by probe qPCR. As expected, the plant samples collected at the first time point were not infected with CaLsol, whereas many samples collected at the later time points were infected. Variogram was used to measure to what extent the variation in the psyllid feeding damage is dependent on the distance between the samples. Clear spatial correlation up to distances of 40-50 m as well as clear temporal correlation in the psyllid damage (r=0.46) was found in the beginning of the season. However, these correlations gradually declined towards the end of the season. 16% of the total variation was explained by the temporal variation and 19% by the spatial variation. The results will be shown as maps and as variogram curves for each sampling time, to illustrate the development of the psyllid feeding damage, and the spreading of CaLsol.

FIELD AND LABORATORY MONITORING OF CALSOL USING OPTICAL DEVICES MOUNTED ON AERIAL AND TERRESTRIAL VEHICLES

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A total of six flights over the Villena test field in four different dates between July and August 2016 were carried out by Aurea Imaging using an unmanned aerial vehicle (UAV) with an on board multispectral camera capable of acquiring images in five bands in the visible (red, green, and blue), red-edge, and near infrared (NIR). Maps of the field showing several indexes of the vegetation were obtained such us normalized difference vegetation indices (NDVI, green NDVI, and Red Edge Index (NDRE)), anthocyanin reflectance index (ARI) or carotenoid reflectance index (CRI). No signs of *Candidatus Liberibacter solanacearum* (CaLsol) were found in the crop nor in the spectral information acquired during the monitoring period. Therefore, other two flights were done in October 2016 at different ground sample resolutions in a field of carrot in La Roda (Albacete) where some plants (approx. 75%) had clear symptoms of CaLsol infection. In combination to flights, several monitoring tests have been carried out at ground level by IVIA. Static VIS/NIR hyperspectral and thermal images were acquired on selected plots of the Villena test field. Moreover, an electric vehicle was built and used to transport colour, NIR and NDVI high-resolution cameras along the whole field acquiring images of the carrots. Additional static tests using the colour, NIR and NDVI cameras were also carried out on November, monitoring the carrot field in La Roda. Finally, several tests have been done at laboratory conditions analysing potentially healthy and infected plants (carrot and celery) using hyperspectral, colour, NIR, NDVI and UV-induced fluorescence imaging (IVIA), and NIR spectroscopy (Aurea Imaging). The plants (7 healthy, 4 infected by CaLsol, 4 infect by phytoplasmas, and 46 infected by both pathogens) without visible clear symptoms in the field except if the stalks are carefully observed, were analysed by the department of bacteriology of IVIA using PCR to precisely determine the presence/absence of CaLsol and/or phytoplasmas. Data are still being analysed but first results indicate that using colour and NIR images the detection of infected plants seems not be possible. As a remarkable result, it was observed the presence of UV-induced fluorescence in one plant of celery with curled stems that was positive both for CaLsol and phytoplasmas, although this finding has to be reproduced in the future to ensure that it was not due to external factors.

SPATIAL AND TEMPORAL DYNAMICS OF OLIVE QUICK DECLINE SYNDROME IN PUGLIA, SOUTHERN ITALY

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The spatial dynamics of Olive Quick Decline Syndrome (OQDS) caused by *Xylella fastidiosa* subsp. *pauca* are being determined in 20 olive plots in a selected olive growing area within the infected zone of the Lecce province in Puglia by the IAS-CSIC team in collaboration with CNR-UNIBA by assessing disease incidence and severity (on a 0-5 rating scale) by the end of June, 2016. Of them, eight plots with a wide range of initial disease incidence and severity values were selected for further analyses in early October 2016 to assess the spatial and temporal dynamics of the OQDS in Puglia region. Data analyses are in progress and include the use of the Spatial Analysis by Distance Indices (SADIE) to quantify the spatial pattern of OQDS incidence and severity. The spatial association between the two time periods was analyzed using the SADIE association Index that measures the local association between clustering indices of both time periods. The spatial pattern of symptomatic olive trees was estimated as regular (22.2% of plots), non-aggregated (33.3% of plots) or aggregated (44.4% of plots). Both, disease incidence and severity increased in the second assessment in all plots by an average of 28.7±4.2% and 0.53±0.27, respectively. The spatial pattern was characterized by the occurrence of several clusters of infected trees. Increasing clustering over the two time periods was indicated by stronger values of clustering index over time and by the increase in the size of patch clusters. A significant spatial association was found in the clustering of diseased trees over time.

DETECTION OF OLIVE QUICK DECLINE SYNDROME (OQDS) BY HIGH RESOLUTION THERMAL AND HYPERSPECTRAL IMAGERY

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High resolution thermal and hyperspectral imagery have proven their potential for early detection and identification of plant diseases. A collaboration was established between JRC and the POnTE consortium to develop a robust and accurate method for the automatic classification of *Xylella fastidiosa* (*Xf*) infections and severity at large scale. To this end, JRC andIAS-CSICperformed an airborne and ground survey campaign in collaboration with CNR-IPSP and UNIBA in a selected olive growing area within the infected zone in the north part of the Lecce province on the Adriatic coast in Puglia. Remote sensing sensors (narrow-band thermal, multispectral and micro-hyperspectral) were placed on a manned airborne platform, which flew over an area of 1,200 hectares. Concurrently with the airborne campaign, field visual inspections and measurements at leaf and tree-crown levels were conducted in 20 olive plots, with approximately 4,000 trees scored for OQDS incidence and severity. Narrow-band reflectance indices related to physiological condition were calculated from pure-crown spectra extracted from the imagery, such as i) tree crown structure, caused by the sensitivity of the near-infrared bands to the foliar scattering of the canopy; ii) epoxidation state of the xanthophyll cycle caused the absorption of three carotenoid pigments that absorb in the 530-570 nm region; iii) chlorophyll a+b, caused by the absorption at spectral bands located in the green, red, and red edge region; iv) blue/green/red ratio indices; v) chlorophyll fluorescence emission by photosystems I-II, and vi) spectral disease index. Data analyses are in progress and include several multivariate approaches as Linear Discriminant Analysis combined with machine learning analyses as Support Vector Machine.

WORK PACKAGE 7

SIGNALLING PATHWAYS, MOLECULES AND GENES CONTRIBUTING TO PEST RESISTANCE IN FIELD

METAGENOME STUDIES ON THE MICROBIOME OF *XYLELLA FASTIDIOSA*-INFECTED OLIVES

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The CoDiRO strain of *Xylella fastidiosa* (*Xf*), which is devastating olive trees in the Apulia Region (southern Italy), represents the first documented establishment of this plant pathogenic bacterium in Europe. Diseases induced by *Xf* have no chemical cure and its pathogenic mechanism(s) are not completely understood. Nevertheless, profiles and relationships of microbial communities and their effects on the expression of symptoms of *Xf*-infected plants are poorly studied. The microbiome of *Xf*-infected olives was investigated by a shotgun metagenomic DNA sequencing approach that avoids the limitations of amplicon sequencing. 28,333,924 and 29,096,610 reads from *Xf*-infected and healthy plants were analyzed by MetaPhlAn, a metagenomic abundance estimation tool which maps reads to a set of selected marker sequences. A complex community of small symbiotic bacteria of insects, i.e. *Candidatus* Zinderia insecticola and *Candidatus* Carsonella ruddii represented the 31% and 22% of the total population was found in libraries from xylem tissues. In infected plants *Xf* reaches the 12% of the total microbial community. Studies are ongoing to characterize the microbial communities in the xylem sap of tolerant and susceptible olive cultivars, to develop a control strategy based on the manipulation of these resident communities and to identify endosymbiont(s) which may be used to reduce the severity of symptoms. Moreover, the evaluation of an endosymbiont bacterium for its potential to colonize *Xf*-infected olive tissues is underway.

CHARACTERIZATION OF THE DIFFUSIBLE SIGNALING FACTOR OF *XYLELLA FASTIDIOSA* CODIRO STRAIN

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Recent studies demonstrated that the virulence of *Xylella fastidiosa* relies on a fine balance between more motile bacterial forms, able to move and proliferate within xylem vessels, and sticky cells forming a biofilm, which are responsible for vessels blockage and insect acquisition. This different behavior is regulated in a cell density-dependent manner by diffusible signaling factors (DSFs), produced by the *rpfF* gene that initiates a transduction cascade resulting in up- or down-regulation of several genes. Mainly DSFs identified in *Xf* subspecies *fastidiosa* and having biological activity, consist of 2-cis unsaturated fatty acids and particularly a 2-tetradecenoic (XfDSF1) and a 2-hexadecanoic acid (*Xf*DSF2). The characterization of these molecules has a direct application in a "pathogen confusion" strategy for reducing the impact of *Xf*-infections since the approach consists in tricking the pathogen, by altering DSF level *in planta* and lowering its virulence.

DSFs produced by the CoDiRO strain of *Xf* were extracted from bacterial culture media with equal volumes of ethyl acetate (EtOAc). The EtOAc fractions, after drying over Na_2SO_4, were analyzed by Gas Chromatography -Mass Spectrometry analysis. Preliminary results showed that a family of unsaturated fatty acids with a chain length of 12-18 carbon atoms is produced. Their further full characterization and principally localization of the unsaturated bond is in progress by ^{1}H nuclear magnetic resonance (NMR) and ^{13}C NMR spectra.

TRANSCRIPTOME PROFILING OF TWO OLIVE CULTIVARS IN RESPONSE TO *XYLELLA FASTIDIOSA* INFECTION

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Olive Quick Decline Syndrome (OQDS) is a destructive disease of olive (*Olea europaea*) described in Apulia (Italy), to which the CoDiRO strain of *Xylella fastidiosa* subsp. *pauca* (*Xfp*) is associated. Differently from the severely affected cv. Ogliarola salentina, *Xfp*-infected plants of the cv. Leccino develop reduced symptoms, which are compatible with olive production. Comparison of the transcriptomes of the two olive cultivars, infected or not by *Xfp*, was performed to ascertain whether a tolerant condition of cv. Leccino exists, which could be exploited for lessening the economic impact of the disease on the local olive industry. 659 and 447 genes were differentially regulated in *Xfp* infected cvs Leccino and Ogliarola salentina, respectively, whereas expression of 512 genes was altered between the two infected cultivars. The study indicated that plants of both cultivars perceive the presence of *Xfp*, mainly involving cell wall-associated proteins. The tolerant response of cv. Leccino, which is missing in cv. Ogliarola salentina, consists on the up-regulation of genes encoding receptor-like kinases and receptor-like proteins. Likely, the active response of the cv. Leccino is responsible for a lower pathogen concentration, suggesting that it may harbor genetic constituents and/or regulatory elements which counteract *Xfp* infection. These findings suggest that cv. Leccino is endowed with an intrinsic tolerance to *Xfp*, which makes it eligible for further studies.

DOES THE MICROBIAL ENDOPHYTIC COMMUNITY AFFECT THE SEVERITY OF OLIVE QUICK DECLINE SYNDROME? PRELIMINARY REPORT

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Endophytes are usually defined as microorganisms that exist and colonize tissues without causing any visible symptoms to the host plant, and can be isolated from surface-sterilized plant tissue or extracted from within the plant. Although the inadequate elimination of nucleic acids after disinfection of plant surfaces, makes the traditional definition of endophyte less suitable for non-cultured species, the introduction of molecular detection techniques in endophyte research greatly contributed to expand the knowledge about the hidden "endosphere" world.

Endophytes promote plant healthiness by the production and secretion of plant growth regulators, the antagonistic activity against phytopathogens, the induction of resistance mechanisms, and the supply or mobilization of nutritional elements. One of the factors that may influence the behavior towards the quick decline syndrome is the nature of the endophytic microbial community occurring in *Olea europea* plants. However, knowledge about the endophytic community of fungi and bacteria inhabiting olive tree xylem is scarce to date, and very little is known about the factors affecting the microbial distribution into the plants. Therefore, objectives of the research activities were i) to characterize the fungal and bacterial endophytic population occurring into the xylem of olive trees by an isolation-dependent approach, ii) to verify the ability of selected bacterial endophytes in colonizing the olive xylem and reduce the disease severity.

Preliminary results indicate that under field conditions, the population level of cultivable endophytic fungi and bacteria is highly variable, being mainly affected by the host genotype, host age, and wilting severity. Among the endophytic strains tested, *B. subtilis* MBBS1 artificially introduced into the xylem, was able to colonize the trees, but it induced slight disease severity reduction as compared to the untreated control.

FIELD EVALUATION OF DIFFERENTIAL CULTIVAR RESPONSE TO *XYLELLA FASTIDIOSA* INFECTION IN OLIVE

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While different sources of natural resistance to *Xylella fastidiosa* (*Xf*) have been described in grapevines and citrus, lack of consolidated information exists on the wide panel of cultivars characterizing the vast olive germplasm. Preliminary observations and analysis on few cultivars, support the evidence that differential responses to *Xf* infections exist. To extend these studies to a large panel of cultivars a first experimental plot was realized under natural infection conditions in April 2015, as part of a pilot study commissioned by the European Food Safety Agency (EFSA). This plot, initially including 10 olive cultivars, was further extended, in April 2016, with additional 19 different varieties. The plot is located in the Apulia Region (Italy) in the demarcated infected area, surrounded by *Xf*-heavily affected olive groves. Twenty-four trees of each of the 29 cultivars were planted in randomized blocks and exposed to *Xf* infection by the natural vector populations occurring in the area. Indeed, to increase the probability of vector transmission, trees from the first set of 10 cultivars were caged with 15-20 specimens of *Philaenus spumarius,* collected from the neighboring *Xf*-infected olive groves. Visual inspections and diagnostic assays are being periodically performed. Laboratory tests confirmed the infectivity of vector populations occurring in the Apulian contaminated area and the *Xf* susceptibility of the olive cultivars tested. 50% of the trees tested positive to *Xf*, with an infection incidence ranging from 25% (cv. 'Leccino') to 78% (cv. 'Koroneiki'). Symptoms of shoot dieback started to appear 1-year after planting, limitedly on few replicates of cv. 'Cellina di Nardò'. In-depth investigations to monitor the bacterial titer and expression of target genes in different infected cultivars, are also underway.

STUDIES ON SUSCEPTIBILITY OF OLIVE CULTIVARS TO *XYLELLA FASTIDIOSA*

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Olive germplasm in Italy is characterized by a large number of autochthonous cultivars. The recent epidemic of Xylella fastidiosa on olive trees in Salento prompted a survey for the evaluation of the susceptibility of different varieties to bacterial infection. Preliminary observations indicated that some olive cultivars respond to Xf infection with different severity of symptoms. Particularly, cv 'Leccino' seems to be tolerant to Olive Quick Decline Syndrome (OQDS) to which the local strain of X. fastidiosa, CoDiRO, is associated. An olive orchard was selected in the outbreak area having 25 years-old trees planted with a regular 5x5 meters planting distance. The 291 olive plants of the orchard belong to cultivars Carolea, Picholine, Gioconda, Nocellara and Leccino.

Observations started in July 2015 and were continued with a 6-month cadence. Each plant was visually evaluated for the expression of symptoms, to which a progressive degree of severity from 0 to 5, where 0 was the absence of symptoms and 5 the death of the plant, was assigned.

Detection of X. fastidiosa was assessed by DAS-ELISA on each plant at the beginning of the observations and after one year. Results of these assays showed that after one year the rate of infection of trees of the cv. Leccino increased from 2,8% to 36%, while those of the other cvs changed from 24.7% to 84.5%. In addition, field observations confirmed the tolerant response of the cultivar Leccino to X. fastidiosa infections since plants of this cultivar showed less severe OQDS symptoms as compared to the other cultivars in the orchard.

WORK PACKAGE 8

PLANT DISEASE RISK ASSESSMENT AND SUPPORT FOR PLANT HEALTH

NETWORK MODELS FOR ASSESSING THE POTENTIAL SPREAD OF PLANT DISEASES ON A CONTINENTAL SCALE

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Different modelling approaches are currently available to assess the potential spread of plant diseases at continental scale. Pathway analysis and epidemiological networks were identified as the most promising modelling approaches for the pathogens targeted in the POnTE project, CaLsol, Xf, Hf and Phy. Key partners among the project were identified to define the locations potentially relevant to the introduction process (nodes), as well as movements and carriers which may disseminate these pathogens through Europe. A timeline of activities was defined for each pathogen, including expert elicitation, draft network scheme, data collection, network model development and model comparison. The pathogen tackled first was CaLsol, in particular haplotypes D/E affecting carrot, celery and parsnip. In the expert elicitation session, seeds were identified as the main carrier of the pathogen at continental scale. Data of seed trade and crop area planted in Europe are being currently searched in EUROSTAT, considering a resolution of NUTS2 and NUTS3 level. Data sources on the prevalence of CaLsol in seed lots from different countries and effectiveness of seed treatments for pathogen suppression were identified. In the expert elicitation session it was also identified that temperatures above 27ºC were limiting for the epidemic development of haplotypes D/E of CaLsol. This temperature threshold might reduce the magnitude of the exposure component in the network model, and so the establishment potential of the pathogen. Therefore, high-resolution maps including maximum monthly temperature for Europe were assembled based on the WorldClim database. Similar approaches will be followed for the other three pathogens, Xf, Hf and Phy, targeted in the POnTE project.

ASSESSING THE GLOBAL POTENTIAL DISTRIBUTION OF *XYLELLA FASTIDIOSA* USING CLIMEX AND MAXENT NICHE MODELS

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Species distribution models (SDMs) determine the relationships between sampled locations for a species and associated environmental variables that are used to estimate the ecological requirements of the species. SDMs provide realistic scenarios to explain the influence of bioclimatic variables on the epidemiology of plant pathogens, particularly in the context of emerging plant diseases. We used semi-mechanistic and correlative niche models to quantify and map the global patterns of the potential geographic distribution of *Xylella fastidiosa*. Climex was calibrated using species-specific physiological tolerance thresholds available in the literature, whereas for the correlative model MaxEnt we used species occurrences and climatic spatial data. Long-term climate data were obtained from the WorldClim website. The global distribution of *X. fastidiosa* was obtained from EFSA (EFSA Journal 2016, 14(2): 4378). Overall, projected potential distribution from both models conformed well to the current known distribution of *X. fastidiosa*. The application of MaxEnt models to the most prevalent *X. fastidiosa* subspecies will be discussed.

SOCIAL IMPACT APPRAISAL OF EMERGING PESTS IN EUROPE

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According to the International Association for Impact Assessment, "Social impact assessment includes the processes of analyzing, monitoring and managing the intended and unintended social consequences, both positive and negative, of interventions (policies, programs, plans) and any social change processes invoked by those interventions. Its primary purpose is to bring about a more sustainable and equitable biophysical and human environment." In current PRAs, the social impact appraisal (SIA) is composed of only a few qualitative questions. By POnTE we aim to design a transparent and quantitative SIA process to assess the social consequences of plant pests and mitigation options among the EU in a balanced way. This requires the definition of significant social indicators which will be derived from studies on applications in related fields and by expert surveys. A new method developed by USDA, SoIVES, will be used to quantify and map the social values on the indicators spatially, which makes it feasible to assess the social impacts of Xylella fastidiosa (Xf) and Candidatus Liberibacter solanacearum (CaLSol) on EU level. Actual quantification of the social impact indicators will depend on the insights obtained from the spread and establishment analyses as performed by WP8 partners and the mitigation analyses as performed by WP9 partners.

AN INTEGRATED FRAMEWORK TO ASSESS THE IMPACTS OF EXOTIC PLANT PESTS IN EUROPE

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Various integrated impact assessment (IIA) frameworks are used by governments and intragovernmental agencies to aid in planning and decision making for the management of natural disasters, such as floods or earthquakes, and planned interventions, such as mining operations or housing development. IIA considers economic, environmental and social impacts. IIA takes a hierarchical approach in which the impacts are decomposed in a stepwise manner down to a point at which they are concrete enough to make an assessment using data, expert judgement or models. The completed partial assessment results are combined using decision rules to achieve overarching assessments. IIA has similar objectives as pest risk analyses made by plant health authorities; however, the principles and approaches of IIA have not been widely applied in plant health. An inherent difficulty in the integrated approach is the assessment of non-monetary societal impacts. The classical approach to pest risk analysis follows the steps of entry, establishment, spread and impact, representing the mechanistic pathway towards harm. This approach may not necessarily provide the most effective way to assess the different kinds of impacts that need consideration, such as impacts on plant health, ecosystem functioning, and communities that depend for their livelihoods on agricultural plant production or ecosystem services. The question is what PRA can learn from the practice of IIA. As part of the POnTE Project, we will explore options to use IIA to support plant health authorities developing measures for prevention and control that deliver maximum total benefit to society.

WORK PACKAGE 9

INTEGRATED DISEASE MANAGEMENT AND MITIGATION STRATEGIES

ASSESSMENT OF THE EFFICIENCY OF KAOLIN PARTICLE FILM AND SAPONIN EXTRACT TO REDUCE CALSOL SPREAD ON CARROT

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The content of this work is currently only available to the registered members of POnTE research consortium.

IMAGINE THERE'S PHILAENUS SPUMARIUS CONTROL

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Insect vectors of plant microorganism are primary targets of insect-borne pathogens control. Evidence about the vector role of *Philaenus spumarius* (L.) (Hemiptera Aphrophoridae) (*Ps*) in transmitting *Xylella fastidiosa* Wells, Raju *et al.* subsp. *pauca* OQDS strain (*Xf*), makes the control of this species of major importance. Data about vector life cycle, behaviour, population dynamics and bionomics are needed to design IPM control strategies (focused on the prevention of plant pathogen transmission) for both Integrated or Organic farming. *Ps* has an univoltine life cycle, it overwinters as an egg, nymphs develop in spring and female adults undergo a summer ovaric diapause. In OQDS and adjacent areas, the egg hatching occurs during the end of February and March. Juveniles live on herbs and bush twigs in "spittle" made purposely frothing mucus and faeces. *Philaenus spumarius* adults are active from May to December. Newly-hatched nymphs are gregarious but shift to solitary later in their development. Adults are food search-driven in their mass movements and shift from host to host following sap availability. The population is at maximum as eggs, and it decreases during pre-imaginal life-stages for several, not yet perfectly known mortality factors. Adults are still abundant in fall when they start to lay eggs. Population drops down almost to zero in middle November, but rare individuals are still active in middle December. In OQDS habitats, a mass movement from drying herbs to olive trees occurs around flowering time, and results in *Xf* acquisition from infected plants and subsequent new infections. We figure a *Ps* integrated control based on two control actions, namely a) mechanical control of nymphs and b) chemical control of adults. Careful timing both control actions is of paramount importance. We consider nymph control the key control action while adult control either may be a supplementary action in case of high vector population or in case of ineffective control of juveniles. In organic farming, only mechanical control of immatures can be applied. Unfortunately, we have no fully effective insecticides, or further control means options, by the time. Despite pyrethroids or Sweet Orange Essential Oil appear somewhat effective against the adult spittlebugs, their negatively impact on the olive biocenosis because they are broad spectrum. Finally, we are developing our index of impact for vector control actions based on Regione Puglia land use stats and willing to share and discuss the impact of control options with POnTE stakeholders.

FIELD EVALUATION OF DIFFERENT INSECTICIDE FORMULATIONS AGAINST *PHILAENUS SPUMARIUS* L. (*HEMIPTERA APHROPHORIDAE*) ON OLIVE TREES

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Upon the discovery of *Xylella fastidiosa* outbreaks on olive trees in Apulia (southern Italy), search for candidate vector(s) revealed that the hemipteran *Philaenus spumarius* is so far the only vector species known to be responsible for the bacterial spread in the infected area. Preliminary studies in Apulia have shown that adults of *P. spumarius* are competent vectors able to acquire and transmit the bacterium from/to olive and other known susceptible hosts, during the entire season when the adult populations occur in the olive groves. Thus, for the implementation of the containment strategies there is a need to reduce the infectious insect vector populations in the outbreak area. Since none of the current commercially available insecticides is registered for the specific control of *P. spumarius* on olives, four field trials to address the effectiveness of 13 different formulates were set up in the contaminated olive groves. Specifically, a constant number of *P. spumarius* specimens was confined, before treatment and at different period (days) post-treatment, on the branches selected on the olive trees sprayed with the different formulations. Neonicotinoids (acetamiprid and imidacloprid), pyrethroids (deltamethrin and lambda-cyhalothrin), showed rapid effects and high mortality rates; even if slower in action, similar results were obtained with etofenprox. Conversely, dimethoate (tested by using two different commercial formulations) proved to be less efficient. Whereas, buprofenzin, pymetrozine and spirotetramat proved to be not effective. With regard to the persistence, neonicotinoids, pyrethroids and etofenprox showed good persistence up to 7 days after the application. Among the organic compounds tested (kaolin, extract of citrus oil and natural pyretrine), the strongest insect knockdown effect was obtained with the formulation based on citrus oil extracts, followed by the natural pyretrine. However, no persistence was recorded for any of these compounds. Altogether, these results provide preliminary evidence on the efficacy of different formulations for their potential use for the biological and integrated control of *P. spumarius* toward the implementation of containment strategies for *X. fastidiosa*.

CONTROL OF VECTOR POPULATIONS FROM *BACTERICERA SP.* RESPONSIBLE FOR TRANSMISSION OF *CANDIDATUS* LIBERIBACTER SOLANACEARUM USING DIFFERENT BIOPESTICIDES UNDER CONTROLLED CONDITIONS

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In the search to design environmental friendly strategies for the control of vector populations responsible for the transmission of *Candidatus Liberibacter solanacearum* (CaLsol), different biopesticides were selected to develop integrated pest management programs. *Trioza apicalis*, *Bactericera trigonica*, *B. tremblayi* and *B. nigricornis* are described as main vectors. In this work, different products with a range of active substances derived from natural sources like maltodextrin (59,8% SL at 25L/ha) applied with and without silica adjuvant, *Beauveria bassiana* (10.6% SC at 1L/ha), natural pyrethrins (4% EC at 0.75L/ha) and paraffin oil (54.6% EW at 1% v/v) were tested using a Potter spray tower (2.0 ± 0.2 mg/cm^2 and water volumes equivalent to 200L/ha) to determine the efficacy against the hatching of *Bactericera* sp. eggs on carrot leaves with reference product fenoxycarb (25% WG 0.06% a.i./v) affecting the embryogenesis and vs untreated check. First results showed the improvement of efficacy for maltodextrin with silica adjuvant (77% vs 61%), followed by natural pyrethrins with (74%) efficacy. The same protocols to control larvae are ongoing. At field level, 2 trials have been conducted on carrot crops placed in Villena (Alicante, Spain) where presence of *B. trigonica* and *B. tremblayi* were previously reported. All products referred above were applied following 7 application dates and results are being processed. Next steps include new field trials during next crop season for carrots and potato to define the best positioning according to results previously achieved under controlled and/or field conditions to be used in programs targeted against CaLsol vectors.

MONITORING OF '*CANDIDATUS* LIBERIBACTER SOLANACEARUM' IN EXPERIMENTAL CARROT PLOTS

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'*Candidatus* Liberibacter solanacearum' (CaLsol) was detected for the first time in 2008 in Villena (Spain), and it has caused severe economic losses in carrot production for the fresh market. The transmission of the bacterium by the insect vectors *Trioza apicalis* and *Bactericera* spp. was reported in Finland and Spain. Moreover, it has been demonstrated that CaLsol is a seed-borne pathogen in carrot.

Two carrot seed lots of `Soprano F1´ cv., were analysed by real-time PCR to confirm the presence/absence of CaLsol and one was positive with a viability of 3%, and the other negative. Then, in WP9 context, three experimental carrots plots were sown in Villena: plot 1, an open air plot with CaLsol positive seeds, plot 2, a plot cover with an insect- proof tunnel (vector free) with the same positive seeds, and plot 3, an open air plot with CaLsol negative seeds. Our survey consisted in collecting 200-400 plant samples every 30 days from each plot. Up to now, positive results for CaLsol detection have been obtained in plot 1 (21 positive samples out of 600 samples) after 120 days post germination (dpg) (3.5%); in plot 2, (10 positive out of 1600 samples) after 150 dpg (0.63%); and in plot 3 (10 positive out of 500 samples) after 150 dpg (2%). Although the assay is still in course, the results in the plot 2 confirm that Calsol is a carrot seed-borne pathogen in natural conditions, and the results in plots 1 and 3 suggest the relevance of bacteriliferous vectors in the area.

ADAPTING SCREENING METHOD FOR CALSOL AT A L TOZER

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P25 (A L TOZER) compared extracting DNA with the Microzone DNAmite Plant kit (Microzone, UK) and the CTAB method from EPPO protocol. The DNA extraction efficiency of CaLsol with the Microzone kit is not as good as the reported CTAB method. However, pelleting the crude extract (14,000g for 10 min) before extraction proves to improve the extraction efficiency in the kit method. PMA treated and non-treated samples were tested with real-time PCR with a positive control from P5 (IVIA) and positive and negative controls purchased from Plant Print, Spain. PMA treatment appears to be effective to distinguish living and dead bacterium cell. Seven Celery crops, fourteen Parsnip crops and seven Carrot crops grown in different regions in Europe and harvested in different years from 2008 to 2015 were tested. Insect-proof growing conditions could help to keep seeds clean of CaLsol. Carrot seeds could be a better host than Parsnip seeds. Our test results were also compared with IVIA results on the same seed lots. The results suggest that sampling could heavily affect the test results.

WORK PACKAGE 10

DISSEMINATION AND TECHNICAL AND SCIENTIFIC RESULTS AND DEMONSTRATIVE FIELDS

THE H2020 PONTE PROJECT WEB SITE: A MULTITASKING PLATFORM FOR COMMUNICATION AND DISSEMINATION ON EMERGING PEST DISEASES

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The International Research Consortium POnTE (Pest Organisms Threatening Europe) is being funded by the European Commission under the Horizon 2020 programme to investigate four pathogens (i.e. *Xylella fastidiosa*, *Candidatus* Liberibacter solanacearum, *Hymenoscyphus fraxineus* and *Phythophthora* spp.) representing a major threat to strategic crops and natural landscapes in the EU, and identify integrated management strategies for their containment. The wide range of studies conducted within the Project tasks on key emergent pests and the rising request for accessing up-to-date references over the Internet, suggested the need to provide a larger variety of real-time information about the project and its targets for a much wider variety of end-users. A WordPress-based web portal (www.ponteproject.eu) has been created by the Coordination Team to support collaborative platform functions, enhance the project's visibility and provide in a flexible manner a rapid dissemination of valuable information, fostering raise of general knowledge and public awareness on relevant themes in plant pathology. Answering to the modern challenges, accounting for an effective web-based pest information system, the resource is intended as an open-access platform to share scientific achievements, upload promotional material and fact sheets, communicate conferences and training courses, report press review and legislative regulations. A social media presence on Twitter and Facebook channels was set up from the early stages in order to enable a two-way communication with a web-active audience and work towards a continuous engagement of the major plant pathology networking platforms and institutional accounts. To keep Project partners and interested parties always informed of the web site updates and encourage frequent visits, a newsletter is being released on a weekly basis.

DISSEMINATION EFFORTS FOR A WIDE SCOPE OF PONTE PROJECT RESULTS

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Dissemination tasks are increasingly important in order to ensure that the results of a project reach the stakeholders and society in general. Scientific dissemination in specific journals or symposiums are part of this job but equally necessary is making the transfer of knowledge to a less expert public affected by the results of the research. And this is especially important in a project as PONTE, whose findings affect to a large degree to farmers, companies, public administration, etc. For that reason, the focus on stakeholders is indispensable, as well as the organization of specific events for them adapted to their level of knowledge of the topic and the use of effective communication. Several events of this type will be held during PONTE project, starting with a workshop in Madrid scheduled on 14th December 2016 and targeted mainly to farmers and companies, forestry companies and managers, associations and bodies representing olive, vegetable and / or forestry, public authorities with responsibility for agriculture, forestry and / or plant health and policy makers and stakeholders, in general.

INDEX OF CONTRIBUTIONS

WP1

WP2

WP3

WP4

WP9

WP10