European research on *Xylella fastidiosa*

Contributions of the H2020 EU-funded projects POnTE and XF-ACTORS

European conference on
Xylella fastidiosa
and project annual meetings

Palma de Mallorca (Spain)
13-16 November, 2017

POnTE
Pest Organisms Threatening Europe

European Commission

Horizon 2020
European Union funding
for Research & Innovation

XF ACTORS

This document has been prepared with the contribution of the Consortia XF-ACTORS and POnTE.

"*XF-ACTORS project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement N. 727987*"
www.xfactorsproject.eu

"*POnTE project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement N. 645636*"
www.ponteproject.eu

Content review and text editing: **IPSP-CNR, Institute for Sustainable Plant Protection, CNR, Unit of Bari (Italy)**
www.ipsp.cnr.it

Layout: **S-COM**
www.scom.eu

Cover: **NET7**
www.netseven.it

Contacts:

info@xfactorsproject.eu
communication@xfactorsproject.eu
info@ponteproject.eu

March, 2018

Contents

Introduction

An international scientific conference on *Xylella fastidiosa* research was held in Palma de Mallorca, Spain, from 13 to 15 November, 2017.

The conference was a joint venture organized by EFSA, the University of the Balearic Islands, the Euphresco network for phytosanitary research coordination and funding, the EU Horizon 2020 projects POnTE and XF-ACTORS, and the European Commission's Directorate-General for Research and Innovation (DG RTD).

The conference focused on outcomes of current European research programs on *X. fastidiosa* to disseminate the latest scientific advances and encourage dialogue, interactions and collaboration between research groups working on this emerging plant health threat.

Keynote lectures were presented by experts from America who articulated their knowledge and experience on the pathogen, vectors and disease management strategies, in support of on-going efforts to control the European outbreak of *X. fastidiosa*.

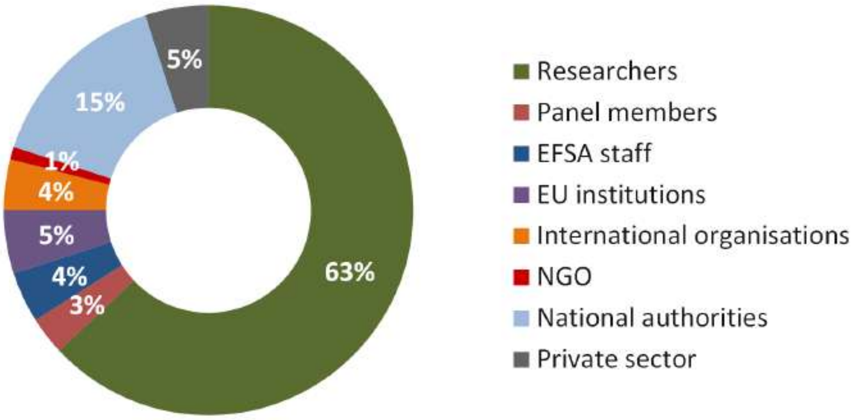
The 3-day conference was organized in 8 sessions covering all aspects related to the pathogen, its interactions with vectors and hosts, diagnosis and early detection, and pathology, from modelling disease dynamics and risk assessment to tools for management and analysis of socio-economic impact.

The H2020 research projects POnTE (635646) and XF-ACTORS (727987) contributed more than 70% of the scientific program reporting on activities and results obtained by these two EU research Consortia.

More than 260 participants from 20 EU and 14 non-EU countries (Europe, Middle East, North Africa, North, Central and South America, Australia and New Zealand) attended the conference. The participants came from different professionals fields, as stressed in the graph below.

The conference was followed, on November 15-16, by the joint annual meeting of the two H2020 projects, attended by project members, stakeholders supporting the projects and external observers.

During the 2-day internal meeting, brief reports were presented for each work package to describe ongoing experiments and share preliminary results not presented in the conference that addressed implementation of the work plan of both projects.



The key contributions of POnTE and XF-ACTORS projects presented at the conference and during the annual meetings covered the different aspects of the *Xylella*-induced diseases and are summarized as follows.

Insights into the genetics and genomics of *Xylella fastidiosa*

Genetic studies are critical to understand the epidemiology of *X. fastidiosa* diseases. These data help to identify drivers of pathogen spread through the environment and landscape; provides insight to host- or climate-adaptation and evolutionary history; identify genotypes and their route of dissemination; provide insights into the biology of different strains.

Research teams of both consortia worked to develop the largest worldwide dataset of *Xylella*-genomic sequences and functional genetic data to support epidemiology studies of the *Xylella* emerging diseases and biology of the EU strains.

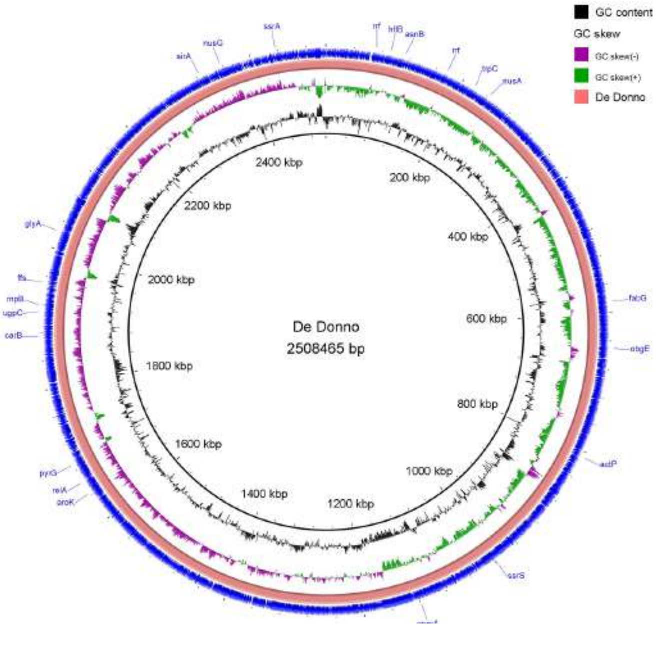
The Department of Environmental Science, Policy and Management of the University of California, Berkeley (USA) is coordinating a large next-generation sequencing (NGS) project, collecting and processing more than 200 isolates of different origin (California, Italy, Brazil, Costa Rica and Spain).

Bioinformatic pipelines have been specifically developed to rapidly analyze the huge dataset of raw sequences and retrieve useful genetic and epidemiological information.

Preliminary project results have advanced knowledge on the genetic diversity of *X. fastidiosa* worldwide and the technology/methodology to explore its diversity.

The following points summarize the major achievements presented at the conference:

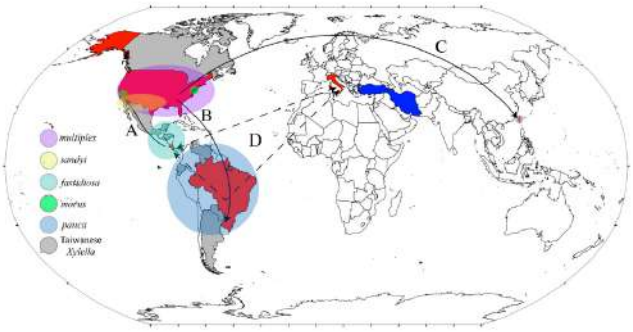
- Optimization and integration of different bioinformatic tools that resulted in the first complete genome of a European isolate of *X. fastidiosa*, the strain “De Donno”, recovered from southern Italy and associated with the severe disease epidemic on olive trees.



- Whole genome sequencing analysis based on single nucleotide polymorphism (SNP) was shown to be a valuable alternative to microsatellites analysis or multilocus sequence typing (MLST) for typing *X. fastidiosa* strains, with an in-depth genetic resolution to explore the genetic variability for epidemiological studies.

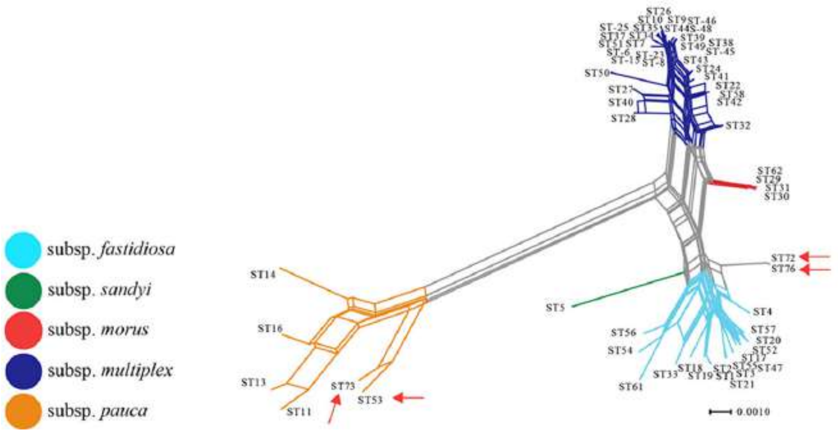
- Specific bioinformatics and statistics tools resulted in valuable comparative phylogenetic analyses to estimate the putative divergence date (approximately 35 - 50 years ago) between the predominant strains detected in France and those belonging to the same sequence type (ST) in the USA (i.e. ST6 and ST7 belonging to the subspecies multiplex).

- Using the abovementioned approaches, a common lineage was found between Costa Rican ST53 isolates and the strain “De Donno”, thus, supporting the hypothesis about the Central American origin of the Italian isolate. Indeed, lack of spatial structure (geographical differentiation) was detected in the population causing the epidemic in olives in southern Italy, suggesting that this is associated to a single and recent introduction.



- MLST approach was used to type strains detected in the outbreaks more recently emerged in Corsica and mainland France, and in the Balearic Islands and mainland Spain. Overall, results disclosed the presence in EU of isolates of the subspecies *pauca, fastidiosa,* and *multiplex* indicating that great genetic variability exists among the isolates detected in Europe and suggested that several independent introductions have occurred. More importantly, several strains detected in EU represent hitherto undescribed genotype – new sequence type – indicating that the current knowledge on the genetics of this pathogen is still far from being fully explored and that the pathogen is evolving.

- Typing of the EU isolates by MLST was rapidly achieved by using plant DNA (as the most common situation is the presence of infections caused by a single strain), i.e. without prior isolation and culturing, producing reliable results in most the samples analyzed, with few exceptions related to particular plants matrices rich in PCR-inhibitors or harbouring low bacterial populations.



Biology of *Xylella fastidiosa*: pathogenicity and host interactions

Biological and molecular investigations of host-pathogen interactions increase our understanding of the mechanisms and determinants involved in the host specificity/adaptation, pathogenicity, and responses to infections caused by genetically distinct strains of *Xylella fastidiosa*.

Considering that closely related genotypes can dramatically vary in their host range, knowledge on the specific biological and epidemiological features of the EU strains is a major task of the ongoing research programs that will lead to improve mitigation of *Xylella*-diseases and limit the spread of the pathogen.

As such, the recent emergence of several outbreaks on the EU territory poses challenges and underlines the need for studies to support pest risk assessment in relation to the different detected strains.

The list of susceptible hosts to the EU strains is continuously updated based on the official monitoring programs in the Member States, supported by the ongoing research activities mainly focussing on the identification of the subspecies (http://bit.ly/2F53Uky).

This information is also part of the implementation of the database on the host of *X. fastidiosa* worldwide curated by EFSA.

EUROPEAN COMMISSION
DIRECTORATE-GENERAL FOR
HEALTH AND FOOD SAFETY

Commission database of host plants found to be susceptible to Xylella fastidiosa in the Union territory - Update 10

efsa
European Food Safety Authority

Update of a database of host plants of Xylella fastidiosa

So far, olive is the major crop species affected by Xylella fastidiosa in Europe and the strain causing this severe epidemic in southern Italy appears to be the most virulent among EU strains.

As a consequence, major resources have been devoted to study this host-pathogen combination and to assess the susceptibility of a large number of olive cultivars and relevant EU crops and plant species to this strain.

Experiments of artificial inoculations have been successfully implemented in Italy, France, Belgium and the Netherlands.

Critical issues are:

- the requirement of large containment facilities;
- the long time needed to complete the experiments (pathogenicity studies are long-term experiments);
- the quarantine restrictions to manipulate different strains of the pathogen;
- the need to grow and maintain under confined conditions several plant species requiring different growing conditions.

Due to the fairly recent establishment of dedicated research on the biology of the EU strains (some of which never reported before), and the complex nature of this pathogen, no results are available yet from long-term studies.

However, experiments in southern Italy have produced preliminary important indications on the pathogenicity of the strain causing infection on olives and on susceptible host species:

- Infections upon artificial inoculations (using cultures of *X. fastidiosa* subsp. *pauca* ST53) on potted olive plants reproduced typical symptoms of desiccation and quick decline, confirming the pathogenic role of the bacterium in the emerging Olive quick decline syndrome (OQDS).

- Attempts to mechanically inoculate and establish infections in citrus and grapes failed, supporting field observations and findings that showed the immunity of these species to the Italian strain.

- Inoculations under controlled conditions indicated that latency of the infection in olive could be longer than 1 year.

- Experimental infectivity studies and surveys in olive orchards, found traits of resistance in the cultivar Leccino, although data on long-term productivity are not yet available.

Indeed, recent observations on olive trees under field conditions identified the olive cultivar FS-17®as an olive selection with possible resistance traits.

- The optimized procedure to inoculate and infect olives, has allowed initiation of a major program of olive cultivar screening for resistance to *X. fastidiosa*; so far, more than 70 selections have been either needle-inoculated under confined conditions or in field conditions exposed to high natural inoculum pressure or grafted on ancient infected trees.

- Transcriptome profiles unravelled differential gene expressions in infected cultivars displaying different phenotypes (symptomatic vs asymptomatic), that associated specific gene responses to the phenotype of the cultivar. Such findings are consistent with previous studies performed on *Vitis vinifera* or on *Citrus spp*. and prompted further investigations to understand the molecular mechanism and target genes involved in the olive resistance phenomena and accelerate progress for breeding programs and marker-assisted selection.

Besides this extensive research program in Italy, involving the collaboration of several project partners located in other Countries and support from local stakeholders, parallel experiments of artificial inoculations have initiated in Belgium, Brasil and France to study the biology of other EU strains and the susceptibility of different plants species/cultivars.

Combinations of different conditions of temperature, water availability and fertilization have been set in Belgium to evaluate the effects on the host colonization and disease development in different plant species subjected to artificial infections with different *X. fastidiosa* strains.

Several strains (both coffee and citrus strains) belonging to subspecies *pauca* (the same subspecies to which belong the strain causing the epidemic disease in olive in southern Italy) have been used to inoculate different olive cultivars in Brazil, and evaluate the susceptibility and the severity of the infections in relation to the bacterial strain.

Relevant varieties of major crops (grape, olive and citrus) have been inoculated in France, comparing different strains.

Regarding ornamentals, infections and symptoms were successfully reproduced on *Polygala myrtifolia* 6-8 months post inoculations, using both the Italian strain (subspecies *pauca*) and a French strain (subspecies *multiplex*). Similarly, successful infections and symptoms developed on oleander plants inoculated with the Italian strain approx. 10 months post inoculation.

Lavandula stoechas has been selected as a model plant to evaluate effects of low temperature on plant colonization and bacterial multiplication, upon inoculation with the Italian strain.

Definitive results will be collected in the upcoming years and are expected to contribute to the knowledge on host susceptibility and the influence of different growth conditions for the development of the diseases.

Insect vectors of *Xylella fastidiosa*

A large diversity of sharpshooters and spittlebugs has been found in association with the spread of *X. fastidiosa* in the America. Because insect transmission of *X. fastidiosa* is known to be non-specific among xylem sap feeders, all xylem-fluid feeding insects are considered potential vectors in Europe, until proven otherwise.

The relevance and the role of vectors in the spread of the bacterium depends not only on the ability and efficiency to transmit the pathogen, but also on ecological and behavioral characteristics, host affiliation, seasonal movement between crop and wild species, interactions with other epidemiological components of the disease, such as source plants, pathogen dynamics in these hosts, *X. fastidiosa* genotype, and favorable environmental conditions.

In the framework of both projects faunistic surveys and studies are carried out on the presence, host affiliation and life history of xylem-feeders in different countries, covering southern and central Europe (Italy, Spain, Greece, Germany, Portugal, Serbia), as well as in olive groves in Brasil.

The objective is to acquire data on the potential risk posed by those species for the establishment and spread of *X. fastidiosa*. These studies are taking advantage from the methodology and procedures standardized within a project funded by EFSA RC/EFSA/ALPHA/2015/01 – CT1, allowing a common approach for sampling and studying population densities and dynamics to gather harmonized and comparable results.

Aphrophora alni

Aphrophora salicina

Philaenus spumarius

Neophilaenus lineolatus

Cercopis sanguinolenta

Cercopis vulnerata

Cicadella viridis

Graphocephala fennahi

Altogether, the ongoing research programs will contribute to maximizing the experimental work plan of the project on "Collection of data and information in the Balearic Islands on biology of vectors and potential vectors of *Xylella fastidiosa*" (GP/EFSA/ALPHA/2017/01), recently approved upon the findings of *X. fastidiosa* in the Balearic islands.

First results of the ongoing surveys indicate:

- Regional differences in the composition of the xylem-feeder fauna.
- *Philaenus spumarius* is the dominant species in Europe.
- Cicadellinae are rare in southern EU regions.
- High species richness in Brasil - 270 species, 97 sharpshooter species.

Surveys and characterization of xylem-feeders are combined with investigations focusing on the biology and ecology of insect species already identified as vector(s) in Europe, thus primarily targeting *P. spumarius*.

A major experimental drawback, for such investigations, is the difficulty of rearing *P. spumarius* for lab-studies, still requiring identification and optimization of environmental conditions that drive oviposition, egg diapause and hatching.

Overall research topics and preliminary results are:

• Acoustic communication and mating behaviour

- *P. spumarius* emits vibrational signals to accomplish mating
- Males are active from the first day of emergence as adults, while females start calling at least 60 days after emergence (from August on)
- The interactive calling of males and females enables males to localize females for mating, female call or its reply triggers emission of male courtship song

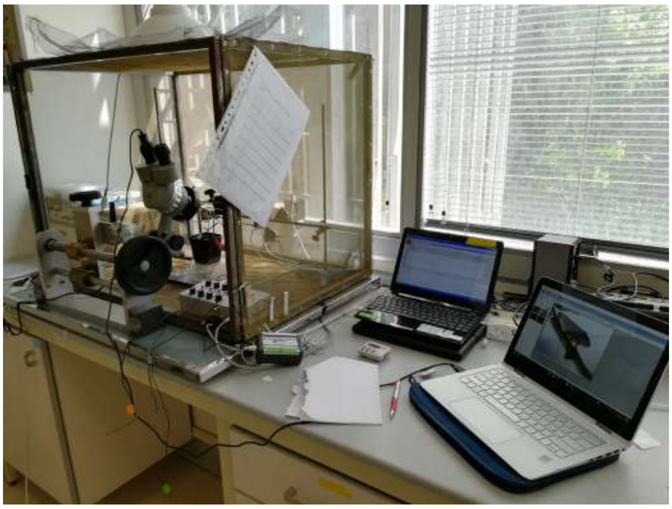
• Chemical ecology of *P. spumarius* and *Neophilaenus campestris*

- Olfactometer bioassay to assess the attractiveness of olive plants showed: (i) young *P. spumarius* females more responsive than young males; (ii) young spittlebugs more responsive than older spittlebugs; (iii) little preference for one cultivar among the two tested.
- Positive electrophysiological and behavioural antennal response from *N. campestris* to olive volatiles have been

demonstrated, and tests will now be performed on *P. spumarius*.

- **EPG-assisted observations of *P. spumarius* feeding behaviour**

- Five electrical waveforms were identified with *P. spumarius* feeding.
- Parameter description and correlation studies ongoing.
- EPG experiments by interrupting insect probing at specific signals have been performed with infected source plants, results are expected to be collected regarding optimum pathogen acquisition access period (AAP) during the next reporting period.



- **Assessment of the active movement of *P. spumarius***:

- Marked-release-recapture experiments repeated in two ecological different areas and for two consecutive seasons showed relatively good dispersal capacity (i.e. up to 120 m in 7-12 days), higher in the olive groves than in the meadow.

- **Assessment of the role of candidate vectors**:

- Adults of *Neophilaenus campestris, P. italosignus*, *Cicada orni* and *Cicadella viridis* have bene used in experimental transmission tests to assess their capability to acquire and transmit the bacterium, with consistent successful transmissions for *N. campestris* and *P. italosignus*, confirming they are competent vectors of the strain associated to the epidemic in southern Italy. In contrast, so far negative results have been obtained with *C. orni*, and no data are available yet for *C. viridis*.

- **Pilot study in Corsica to assess the presence of infected *P. spumarius***:

- Using a new nested PCR-MLST assay simultaneous identification of infected spittlebugs and typing of the strain(s) was accomplished.
- The higher sensitivity of this new approach compared to qPCR was demonstrated
- Infected spittlebugs were identified in areas where *X. fastidiosa* was not yet detected on host plants.

- **A collaborative work on microbiome and endosymbionts analysis** have been organized with results expected to be delivered in the next reporting period.

- **Potential predator species** (*Zelus renardii, Synema globosum, Araniella cucurbitina)* have been tested with encouraging results at the laboratory level.

Knowledge and research progress on the abovementioned activities are aimed to develop sustainable strategies for the control of vector populations. Once validated, information from these activities should lead to novel tools for vector control.

These include but are not limited to: (i) use of vibrations to support sustainable control strategies (i.e. mating disruption or lure & kill techniques); (ii) chemical signals and plant volatiles to enhance trap catches; (iii) use of endosymbiotic bacteria as a potential biological control.

In addition, field trials for testing different approaches mainly targeting the control of *P. spumarius* have been started: (i) use of non-host plants as cover crops in olive groves; (ii) use of trap border trees; (iii) testing different formulations for applications under integrated management or organic farming regime.

Regarding insecticides to control *P. spumarius*, pyrethroids and neonicotinoids were, by far, the best among the different tested formulations in terms of efficacy and persistence.

The other products and formulations resulted in low persistence and very low mortality rates especially for the organic products tested.

These aspects coupled with the need to define the number and time of the applications, the development of integrated pest management approaches, strongly suggested that much more research on new formulations, and new testing are necessary to pursue a more sustainable and effective vector control.

Disease control in the host plants

The search for traits of resistance in olive germplasm, is considered one of the most promising areas for research that will result in a medium-term strategy to control the epidemic spread of *X. fastidiosa* in southern Italy and support, in the long-term, breeding activities.

In parallel, different research approaches are also explored towards the development of molecular interventions to restrain bacterial multiplication and movement in the host plants to contain the impact of the infections.

Experiments testing different formulations/molecules and control agents, i.e. bacteriophages, endophytes, competing microorganisms and antagonists have been initiated although these activities are mainly at the *in vitro* stage of testing, or as in the case of bacteriophages or endophytes/antagonists, at the attempt to isolate, select and characterize the putative beneficial microorganisms.

The lack of suitable model plants/hosts that, when infected, quickly develop disease-symptoms, is a major limitation to evaluate the efficacy of any of the control strategies under investigation, that perhaps require long-term efforts and multiple year experiments.

- **Testing formulations and antimicrobial peptides**

Based on some promising research conducted in Brazil over the past 5 years on citrus variated chlorosis caused by *X. fastidiosa* subspecies *pauca* in citrus, a 2-year pilot program of treatments with N-acetyl cysteine (NAC) was initiated in OQDS-affected olive groves, in comparison with different elicitors of plant resistance factors (fosetyl aluminium, acibenzolar-S-methyl, COS-OGA, σ–β Hairpin proteins, *S. cerevisiae*).

While elicitors were applied to trees by spraying, NAC was tested by endotherapy, fertirrigation or complexed with organic substances and supplied to the soil.

None of the tested elicitors produced any measurable positive effect on diseased trees nor reduced the progress of the new infections in the plantations, whereas, a slight attenuation of the severity of the symptoms was observed with NAC in endoterapy or in combination with soil application. These encouraging results prompted further integrated management experiments which are currently in progress in the framework of the H2020 project POnTE.

More recently, experiments have been started to test antimicrobial peptides (linear lipopeptides and cyclic decapeptides) as an inhibitor of the bacterial growth and/or multiplication.

Preliminary results from *in vitro* assays showed that the linear peptides are powerfully effective against *X. fastidiosa* cultures belonging to different subspecies, but are showing lower efficacy against the olive-infecting “De Donno” strain. Studies are planned to verify the toxicity to plants of these peptides.

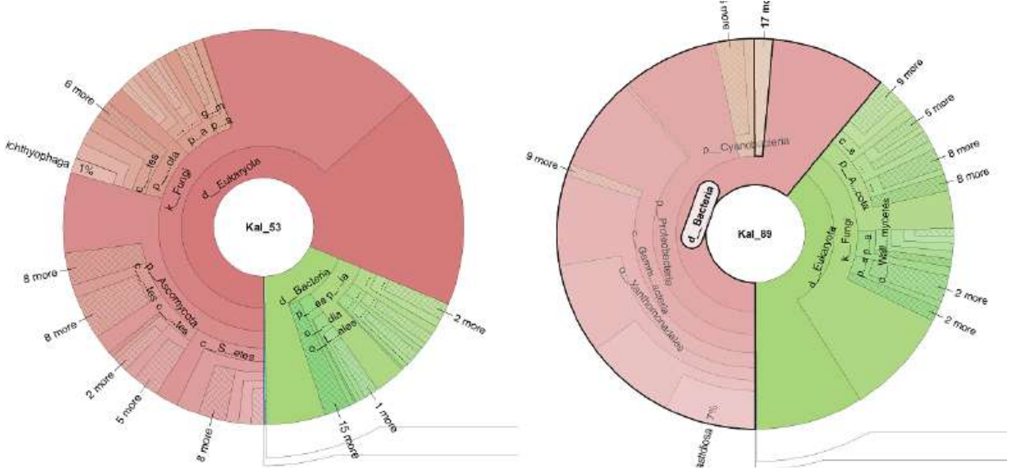
- **Exploring the microbiome of olive trees**

An integrated approach has been undertaken to explore the endophytic bacterial community in olive trees: whole-metagenome (WMGS) shotgun analysis, 16s rDNA gene sequencing, traditional isolation of cultivable bacteria from the xylem tissues of healthy and infected olive trees, as well in olive trees grown under different cultivation management were performed.

Several isolates of *Pseudomonas, Bacillus, Lysinibacillus, Pantoea, Microbacterium, Stenotrophomonas*, and *Methylobacterium* spp. have been isolated and cultured, with a general trend indicating higher population size in the healthy trees compared to infected trees.

Previous studies in olives analyzed root/soil microbial communities but not bacterial communities in the xylem tissues.

Data gathered from preliminary analyses of the WMGS datasets provided important insight on microbial communities associated in xylem tissues of olives and suggested a strong perturbation of the microbiome occurs in infected



Krona pie charts of the metagenomes of a healthy (left panel) and infected (right panel) olive trees.

susceptible cultivars where the bacterium dominates the whole endophytic niche.

Analyses are ongoing to determine correlations among molecular (WMGS and 16S rDNA) data and results from culture-dependent isolations, which will enable the description of the xylem microbiome of infected plants and select candidate endophytes to be screened *in vitro* and *in vivo*.

In the meanwhile, experiments have started using *Paraburkholderia phytofirmans PsJN*, based on studies in California indicating a possible competing role with *X. fastidiosa* in Pierce's disease infected grapevines.

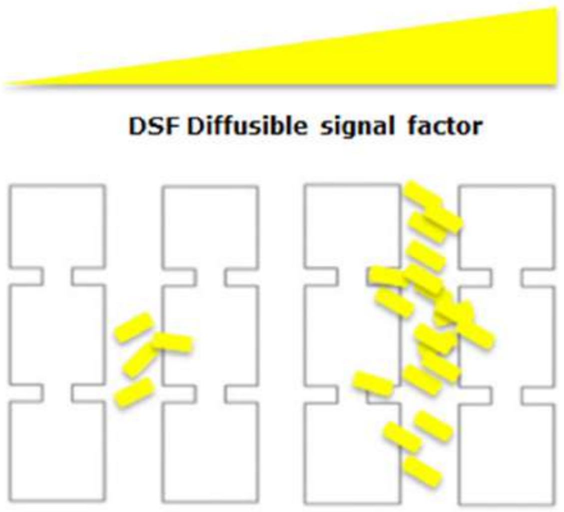
Data on *P. phytofirmans* colonization of olive trees are not yet available. However, endophytic colonization of vascular tissues was successfully achieved in olive potted plants needle-inoculated with the bacterium.

Hence, a coordinated program of co-inoculations on olive plants has been planned for the next vegetative season based on these preliminary positive results.

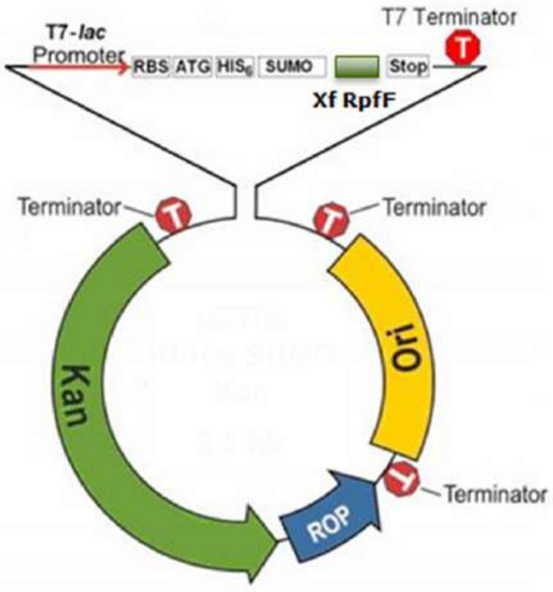
- **Pathogen confusion strategies**

This research task refers to the exploitation of a "pathogen confusion" strategy, which relies on modulating the accumulation of the "diffusible signalling factor (DSF)", a fatty acid synthesized by the RpfF gene of *X. fastidiosa* involved in quorum sensing.

Initial studies are dealing with the characterization of the DSF produced by the specific strain associated to the epidemic in southern Italy, by Gas Chromatography-Mass Spectrometry analysis.



Indeed, a parallel biotechnological approach aims at the production of the DSF *via* a recombinant plant viral-based vector engineered to contain and transiently express *in planta* copies of the Xf-RpfF gene.



The expected outcome is to use this approach to mitigate virulence of the bacterium in susceptible hosts such as olive plants.

- **Production of *Xylella*-free plant propagation materials by implementing a certification program**

This project task is implemented through cooperation between researchers and stakeholders. The main "actors" contributing to this topic are the organizations and associations representing nurserymen and/or in charge of plant health controls and inspections, under the umbrella and supervision of EPPO.

Discussions on the topic have started within a small working group, which drafted a preliminary proposal for a "**Voluntary System Preventing Pests (VSPP)**". The objective is to propose a common EU certification system and certification standards, by taking stock from the current emerging scenario on *X. fastidiosa* in Europe.

An international committee will be formally appointed to prepare the general requirements of this proposed certification process. The network of experts involved in this task is not limited to the project members but external experts (from different countries and working on different crops/species) have been involved to ensure high convergence and consensus on the outcome of this task.

Diagnostic and tools for early detection

One of the main research tasks within both H2020 projects is to promote, under the coordination of EPPO, the use of common and harmonized diagnostic procedures, ensuring the availability of common diagnostic standards for the laboratories in charge of the official testing in the different Countries.

Several diagnostic assays are available from the literature and many research groups have worked toward the implementation of the plant extraction procedures. This is, in fact, a critical issue, due to the vast number of putative host plants to be screened.

Large inter-laboratory validations have been conducted to assess the proficiency of the different diagnostic tests and the performance among different laboratories.

These validations resulted in a panel of recommended assays to be used according to the target area (i.e. in demarcated *vs* non-demarcated area), which are part of the **EPPO standards** (bit.ly/2FuLL34), as well as part of the **official database of validated tests of the Commission** (bit.ly/2FANuUJ).

Beside the collaborative efforts for the harmonization and validation of diagnostic procedures, continuous research efforts are committed on two key aspects:

(i) sampling methods, with particular reference to the asymptomatic plant materials, the detection of the bacterium during the latent stage of the infections and the reliability of the different tests when using pooled samples; (ii) testing and validation of rapid procedures for the highly sensitive detection in the host plants and in the vectors.

To further support the efficacy of the monitoring programs, experiments are ongoing to search for candidate "spy plants", starting with *Polygala myrtifolia*, one of the common host for the different subspecies found in different EU outbreaks.

Potted plants of *P. myrtifolia* have been positioned (left for at least 1 month during summer) in different selected sites, both in the demarcated "infected" and non-demarcated area of Apulia region (southern Italy).

oepp eppo

EUROPEAN COMMISSION
DIRECTORATE-GENERAL FOR HEALTH AND FOOD SAFETY

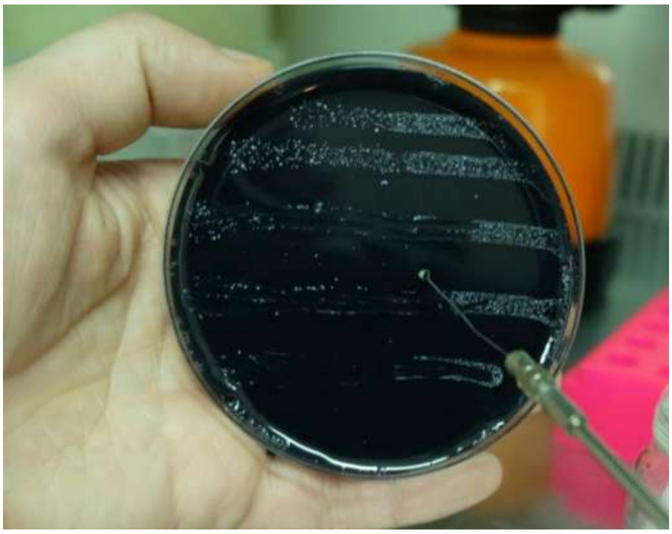
So far encouraging results have been obtained, with positive detection occurred in the "spy myrtle-leaf milkwort plants" positioned in the infected and containment areas, as soon as 1-month after their exposure in the environment, even if the plants were still symptomless.

Indeed, detection occurred in one spy myrtle-leaf milkwort plant positioned as far as 500 m from a known outbreak (infected olive grove). Thus, the first season of experimentation (summer 2017) confirmed that infections of the spy plants occur in infected sites; a promising evidence that can be useful to better focus sampling and testing approaches for disease risk assessment.

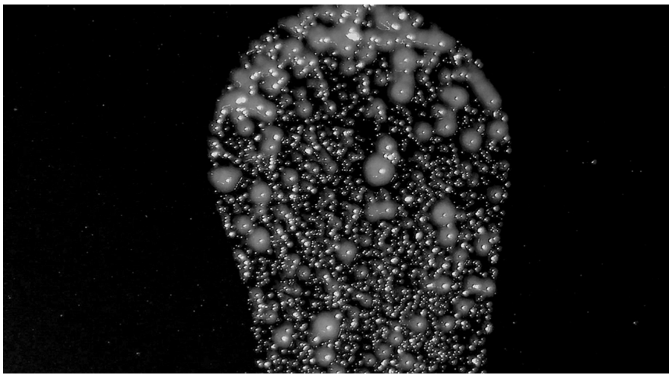
Within the general framework of diagnosis, early detection of *Xylella*-infections (*i.e.* prior the manifestation of clear and evident symptoms) is one of the major and ambitious tasks to accomplish.

Taking advantage from the studies and experience gained for other pathogens causing severe disease in olives, i.e. different pathotypes of *Verticillium dahliae*, a 2-year remote sensing campaign was carried out covering approx. 200.000 olive trees in the infected area of Apulia (southern Italy).

Hyperspectral and thermal images were collected at the sub-meter resolution and, concomitantly, a selected number of olive groves were inspected through a series of periodic field inspections, for scoring the presence and the severity of the symptoms to validate remote sensing dataset.

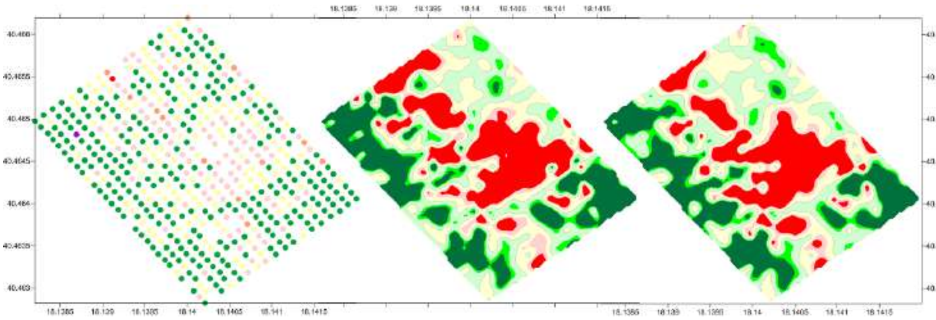


High-resolution imaging spectroscopy and thermography identified changes in plant functional traits, revealing the disease at the pre-visual stage when the symptoms were not yet evident, a critical requirement for effective surveillance programs and for timely implement an effective eradication program.



Disease dynamics, modeling and pest risk assessment

Initial insights into the spatio-temporal spread of *X. fastidiosa* infections have been gathered from study areas in southern Italy.



Disease incidence and severity

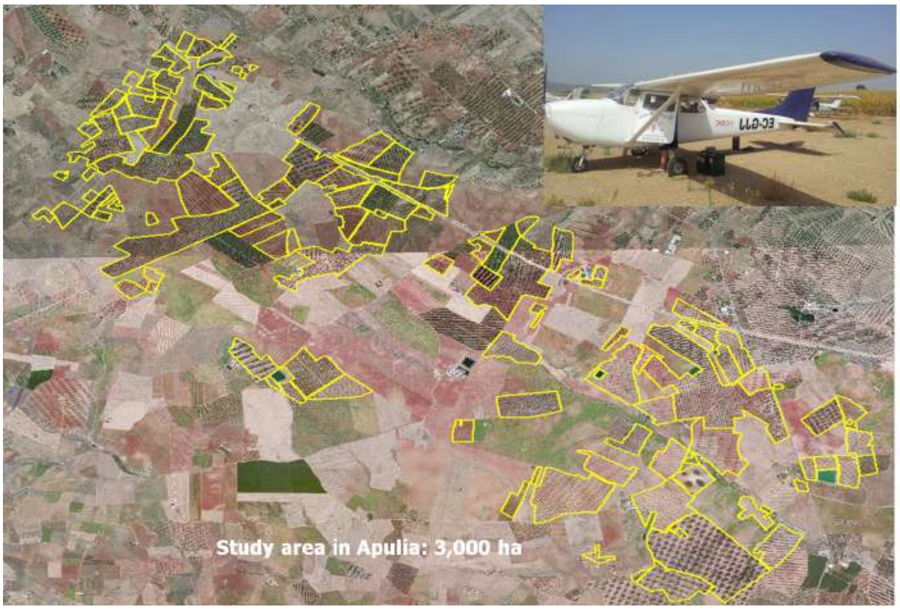
Understanding spatio-temporal dynamics of the infections and the associated disease in olive groves is crucial for addressing an appropriate management strategy. Although preliminary, important information was obtained after a multiple-year survey in properly selected olive plots with low infection rate and disease severity.

The spatial pattern retrieved using the Spatial Analysis by Distance Indices (SADIE) indicated the existence of aggregated spatial patterns (i.e. newly infected trees are linked to the old infections), confirming that secondary spread occurs from the initially infected trees to the neighboring non-infected tree.

This is in agreement with the finding that, so far, no wild species/alternative/riparian plants have been found extensively infected in the olive orchards and suggests that infections occur and spread mainly from olives to olives.

This spatial pattern was less pronounced in olive groves with resistant cultivars. It has been shown that bacterial concentrations in the resistant olive trees significantly lower, with direct negative consequences on the vector-transmission efficiency (i.e. significantly lower). These findings are consistent with the spatial pattern distribution detected in the case of the resistant cultivar.

To establish more effective mechanisms and tools for risk assessment and prevention, as well as preferred strategies for eradication or containment, simulation models were developed that



represents an important task to which several EU research groups are contributing to.

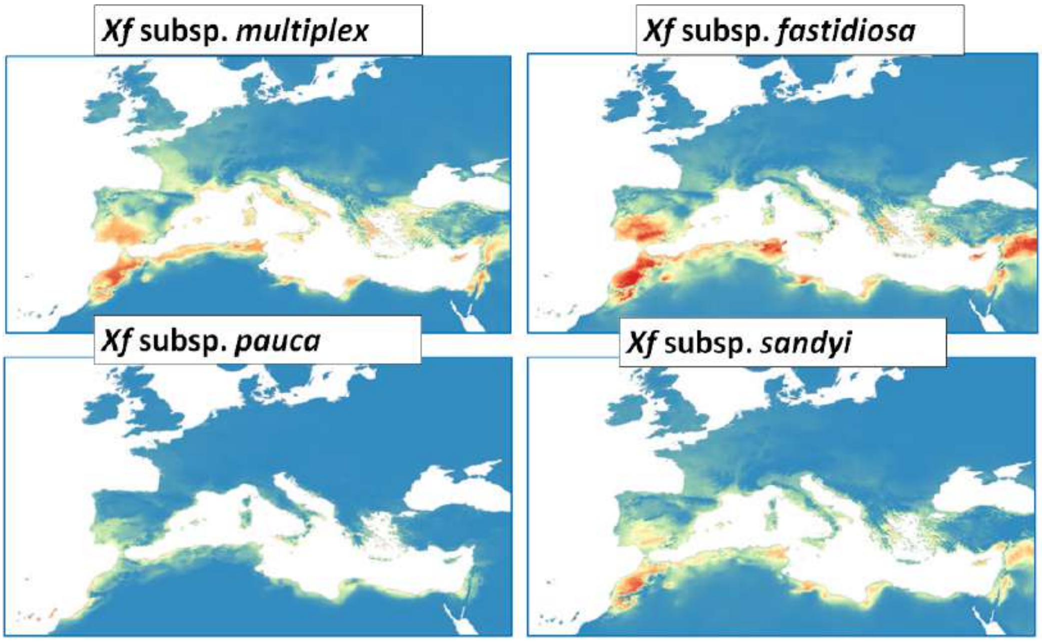
Different complementary methodologies are in place to evaluate risk assessment models at regional scale, using relevant parameters, e.g. epidemiology of *X. fastidiosa* diseases, host(s), vectors, governance, and environmental conditions. As a consequence, performance and robustness of these simulation models are greatly affected by the availability of biological and epidemiological data.

To this end, efforts were made to coordinate and ensure continuous and effective exchange of information and networking between the researchers developing new knowledge on the pathogen, the vector, host prevalence and the host-pathogen interactions, and the "modellers".

Assimilation of these biological/ epidemiological data into the simulation models will result in more accurate predictions of potential establishment and spread, providing key elements for more targeted interventions (surveillance strategies, prioritization of areas/hosts for surveys and sampling, eradication, criteria for the demarcation of the areas, etc.).

The availability of monitoring datasets from the National Phytosanitary Authorities is an additional important element for the development and the calibration of the models.

Thus, based on the monitoring datasets and on the available knowledge on the host(s) prevalence, different models have been developed and tested to estimate the introduction (i.e. in Corsica), to reveal the presence of "hidden component" (i.e. niches serving as source of inoculum), to understand the history of invasion and to draw forward projections of *Xylella* dynamics under different scenarios.



Regional climatic suitability and potential geographic distribution in relation to the different subspecies

The overall results are summarized as follows:

- Spatio-temporal models tested using the Apulian monitoring dataset confirmed the high disease spread under favourable environmental conditions.

- A preliminary descriptive analysis indicated that environmental variables were quite homogenous in the study area of Apulia, thus, they are not expected to be so much influent in the models.

- A strong influence of the spatial component exists in *X. fastidiosa* distribution.

- The analysis conducted on the data collected in Corsica, opened a scientific debate about the introduction date, with evidence in support of a putative introduction dated back in 1985.

- *Cistus spp.* (Cistaceae) (one of the main infected host species in Corsica) most likely contributes to the hidden component.

- Modelling identified the critical need for early detection, if removal of plants is to be effective.

- Spread modelling highlighted that buffer and containment zone widths are important to halt the expansion of spread due to long-range dispersal. When constrained by budget, wider containment and buffer zones are likely to reduce risk, as opposed to more concentrated surveillance.

- The predicted sensitivity to long-distance dispersal on spread rates, surveillance and control indicates that further research is required to quantify this ecological process.

Meeting with risk assessors and risk managers will be organized in order to critically discuss the methodology and the expected outcomes by end users, as well as to better select and validate entry parameters and the analysis to be run.

Because a systematic approach to risk management and prevention requires the involvement of stakeholders, two independent analyses were performed by collecting the feedback of ad hoc prepared questionnaires. Psychological and sociodemographic factors associated with olive growers' perceptions and intentions to participate in the control of *X. fastidiosa* in southern Italy were studied.

Preliminary results indicated that farmers in the infected area are very much concerned about the economic losses caused by the disease, and that 97% of the olive growers intended to report a suspected infection to the competent authorities. Likewise, 84% of farmers showed a high intention to apply best practices to slow down the disease spread. Only 26% indicated a willingness to take part in phytosanitary treatments against the disease's vectors.

The intention among growers to participate in the removal of infected trees upon compensation equal to the olive tree market price was limited to 38%, lower (30%) in the scenario of no compensation.

Feedback from stakeholders

The conference and the projects annual meeting were animated by dedicated sessions of discussion with stakeholders (farmers, nurserymen, plant health inspectors and risk managers).

A general consensus was expressed about:

- the **relevant knowledge and scientific advances made in few years by the European research teams** (knowledge on the EU susceptible hosts and on the genetic/biology of *Xylella*-strains, EU vector(s) and preliminary information on their host preference and biological cycle, development of promising novel surveillance approaches like use of remote sensing, identification of resistant olive cultivars, etc.).

- the **high level of international cooperation** in the ongoing research programs, a good basis to effectively reach important goals.

- **impacts** of *Xylella*-outbreaks are not limited to **agricultural productions and landscape**, but significant **economic losses** affect the **nursery** industry (strict rules for the production and commercialization impact the nurseries located in the demarcated areas).

From the stakeholder perspective, the major needs for the research were identified as:

- early detection system/easy **quick field tests**.

- **guidelines for surveillance** of low-level presence of the pathogen/sampling asymptomatic plants.

- **treatments (curative)** for infected plants (i.e. formulations, thermotherapy extended to other species than grapes).

- need to define the **requirements of the facilities for the production of *Xylella*-free plant** propagation materials (size/type of the screen; means for vector control before trading consignments).

- **extend knowledge** of the **vector** behaviour under different EU climate conditions and on the **vector dispersal capability**.

- develop **environmentally acceptable treatments to control the vectors** applicable on different crops.

- test/screen as many as possible plant species for the **identification of non-susceptible hosts/resistant cultivars** for replanting in the affected areas.

- increase or initiate **breeding programs for resistance** to the pathogen.

Overall concluding remarks

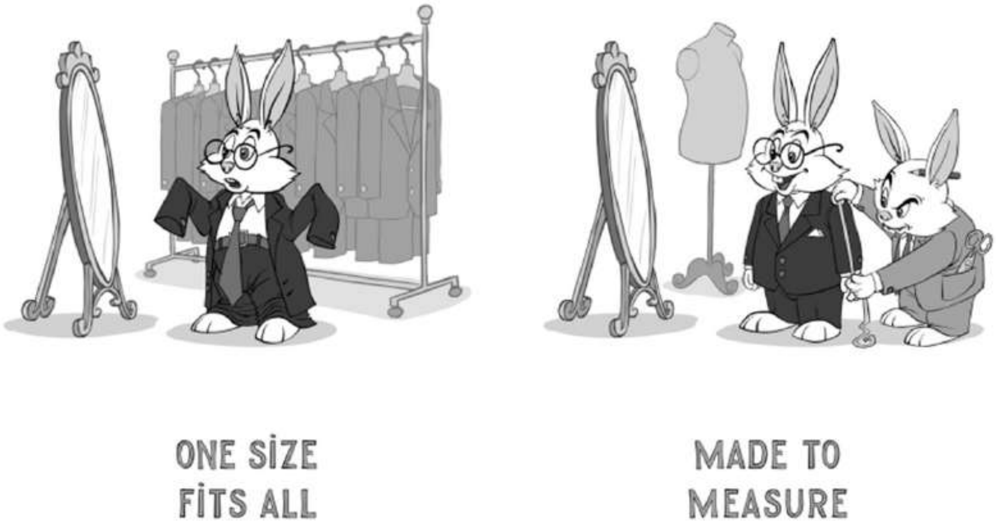
- Significant progress and relevant cooperation on *Xylella* research in Europe have been achieved in the last few years but developing sustainable management strategies requires long-term EU research programs.

- Scientists are conscious of the importance of their research toward the prevention, management and control of the *Xylella*-diseases, and, as such, they are sharing and discussing their results even if unpublished yet.

- Control of *X. fastidiosa* is complex and needs to be tailored for each case (region) by understanding the specific pathosystem involved (pathogen, vectors, host, environment).

POnTE | XF-ACTORS
ANNUAL MEETING
15-16 NOVEMBER 2017
PALMA DE MALLORCA, SPAIN

One size does not fit all!



Author: "No Bugs" Hare
http://bit.ly/2HxzdVO

XF-ACTORS Consortium

1. **Consiglio Nazionale delle Ricerche (CNR)** - Istituto per la Protezione Sostenibile delle Piante

2. **Centre International de Hautes Etudes Méditerranéennes (C.I.H.E.A.M.)**

3. **Università degli Studi Aldo Moro (UNIBA)** - Dipartimento di Scienze del Suolo, della Pianta e degli Alimenti

4. **Institut National de la Recherche Agronomique (INRA) -** Research Institut for Horticulture and Seeds and Fruit Biology and Pathology

5. **Agencia Estatal Consejo Superior De Investigaciones Cientificas (CSIC) -** Institute For Sustainable Agriculture and Institute Of Agricultural Sciences

6. **Instituto Valenciano de Investigaciones Agrarias (IVIA) -** Department of Plant Protection and Agro-Engineering

7. **Benaki Phytopathological Institute (BPI) -** Department of Phytopathology and Department of Entomology and Agricultural Zoology

8. **Julius Kuhn-Institut Bundesforschungsinstitut fur Kulturpflanzen (JKI)** - Institut für Pflanzenschutz in Obst- und Weinbau

9. **Instituut voor Landbouw- en Visserijonderzoek (ILVO)**

10. **The Regents of the University of California (UC) –** Department of Environmental Science, Policy, and Management

11. **Centro de Citricultura (IAC) -** Centro de Investigación en Biología Celular y Molecular

12. **National Taiwan University (NTU) -** Department of Entomology

13. **University of Costa Rica (UCR) -** Centro de Investigación en Biología Celular y Molecular

14. **Natural Environment Research Council (NERC) -** Centre for Ecology & Hydrology

15 Instituto Politécnico de Bragança (IPB)

16 The University of Salford (USAL) - School of Environment and Life Sciences

17 Joint Research Centre - European Commission (JRC) - Institute for Environment and Sustainability - Forest Resources

18 Consiglio per la Ricerca in Agricoltura e l'Analisi dell'Economia agraria (CREA) - Centro di ricerca per la Patologia Vegetale

19 Centro Euro–Mediterraneo sui Cambiamenti Climatici Scarl (CMCC) – Department of Impacts on Agriculture, Forests and Ecosystem Services

20 Nederlandse Voedsel En Waren Autoriteit (NVWA)

21 Instituto Andaluz de Investigaciony Formacion Agraria Pesquera Alimentaria Y de la Producion Ecologica (IFAPA)

www.xfactorsproject.eu/partners

22 International Federation of Organic Agriculture Movements European Union Regional Group (IFOAM EU GROUP)

23 European and Mediterranean Plant Protection Organization (EPPO)

24 RUSSEL IPM LTD

25 ENBIOTECH Srl

26 AINIA - Bioassays

27 Sustainable Communication Aisbl (S-COM)

28 Stichting Nederlandse Algemene Kwaliteit Sdienst Tuinbouw (NAKTUINBOUW)

29 CIVI-ITALIA

Third linked parties

- CIHEAM - Bari
- CIHEAM - Chania
- CRSFA Basile Caramia
- NET7
- ID Consulting

POnTE Consortium

1. **Consiglio Nazionale delle Ricerche (CNR)** - Istituto per la Protezione Sostenibile delle Piante

2. **Università degli Studi Aldo Moro (UNIBA)** - Dipartimento di Scienze del Suolo, della Pianta e degli Alimenti

3. **Institut National de la Recherche Agronomique (INRA) -** Research Institut for Horticulture and Seeds and Fruit Biology and Pathology

4. **Agence Nationale de Securite Sanitaire de l'Alimentation, de l'Environnement et du Travail (ANSES) -** Laboratoire de la Santé des Végétaux

5. **Instituto Valenciano de Investigaciones Agrarias (IVIA) -** Department of Plant Protection and Agro-Engineering

6. **Agencia Estatal Consejo Superior De Investigaciones Cientificas (CSIC) -** Institute For Sustainable Agriculture and Institute Of Agricultural Sciences

7. **Scottish Government (SG) -** Science and Advice for Scottish Agriculture Edinburgh

8. **Forestry Commission Research Agency (FR) -** Centre for Ecosystems, Society & Biosecurity

9. **Bundesforschungs-und Ausbildungszentrum für Wald, Naturgefahren und Landschaft (BFW) -** Forest Protection

10. **Luonnonvarakeskus (LUKE) -** Plant Production Research

11. **Wageningen University (WU) -** Department of Social Sciences

12. **Norsk Institutt for Biookonomi (NIBIO)** - Plant Health and Plant Protection

13. **University of Costa Rica (UCR) -** Centro de Investigación en Biología Celular y Molecular

14. **The Agricultural Research Organisation of Israel (ARO) - The Volcani Centre -** Department of Plant Pathology and Weed Sciences

15 University of Belgrade (UB) - Faculty of Agriculture and Forestry

16 Certis Europe B.V. - Development and Registration Certis Europe B.V.

17 Aurea Imaging BV

18 Vilmorin

19 LOEWE Biochemica GmbH

20 Phytophthora Research and Consultancy (PRC)

21 Acli Racale Soc. Agr. Coop.

22 Agritest SRL

23 Fundacion Citoliva, Centro De Innovacion Y Tecnologia Del Olivar Y Del Aceite - Fundation CITOLIVA

24 Agricola Villena, Coop. V.

25 A L Tozer Ltd

Third linked parties

- **University of Turin**
- **University of Helsinky**
- **CRSFA Basile Caramia**

www.ponteproject.eu/partners

XF-ACTORS - Supporting Organisations

AESAVE - Asociación Española De Sanidad Vegetal

AIPH - The International Association of Horticultural Producers

ANVE - Associazione Nazionale Vivaisti Esportatori

APROL

ASAJA Córdoba

CIA - La Confederazione Italiana Agricoltori

Citoliva

CONFAGRICOLTURA, Confederazione Generale dell'Agricoltura Italiana

Copa-Cogeca

ENA – European Nurserystock Association

IOC - International Olive Council

InnovaPuglia

MIPAAF - Ministero delle politiche agricole, alimentari e forestali

Ministry of Environment and Energy, Greece

Ministry of Rural Development and Food, Greece

POnTE - Supporting Organisations

DGAL - Ministry of Agriculture (France)

Coldiretti

ANVE - National Association of Nurserymen Exporters

MAPAMA - Plant and Forestry Health (Spain)

ASAJA Córdoba

APROL Lecce

DOP EVO Consortium "Terra D'Otranto"

EVIRA - Finnish Food Safety Authority

Plant Protection Service - Ministry of Agriculture (Costa Rica)

Junta de Andalucia

ENA - European Nurserystock Association

Inter-professional organization of Spanish olive oils

FEPEX

NVWA - Dutch Food Safety Authority

Plant Health Service Apulia (Italy)