# European Research on Emerging Plant Diseases

## Contributions of the H2020 projects **POnTE** and **XF-ACTORS**

## 2nd Joint Annual Meeting

BOOK OF ABSTRACTS

Valencia (Spain) | 23-26 October 2018

ivia
Instituto Valenciano
de Investigaciones Agrarias

GENERALITAT
VALENCIANA
Conselleria d'Educació,
Investigació, Cultura i Esport

POnTE
PestOrganisms
ThreateningEurope

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XF
ACTORS

# POnTE

PEST ORGANISMS THREATENING EUROPE

# XF-ACTORS

XYLELLA FASTIDIOSA ACTIVE CONTAINMENT
THROUGH A MULTIDISCIPLINARY-ORIENTED RESEARCH STRATEGY

# 2ND JOINT ANNUAL MEETING

POnTE
Pest Organisms
Threatening Europe

XF
ACTORS
Xylella Fastidiosa Active Containment Through a
multidisciplinary-Oriented Research Strategy

# BOOK OF ABSTRACTS

## VALENCIA, 23–26 OCTOBER 2018

**INSTITUTO VALENCIANO DE INVESTIGACIONES AGRARIAS (IVIA)**
**46113 MONCADA (VALENCIA), SPAIN**

## 2ND JOINT ANNUAL MEETING

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# THE PROJECTS IN BRIEF

# THE PONTE PROJECT

# (GA 635646)

# TOPICS

## PONTE FOCUSES 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 that 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.** *Ca*Lsol 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 *Ca*Lsol and its psyllid vector Bactericera cockerelli as quarantine pests, since non-solanaceous *Ca*Lsol haplotypes have now been found in Europe associated with diseased carrots and celery. The emergence of these *Ca*Lsol 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. are 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

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| P10 | LUKE, NATURAL RESOURCES INSTITUTE FINLAND, Finland |
| P11 | WU, WAGENINGEN UNIVERSITY, The Netherlands |
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| P13 | UCR, UNIVERSITY OF COSTA RICA, Costa Rica |
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# 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.

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| **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*

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TOPIC *CANDIDATUS* LIBERIBACTER SOLANACEARUM

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TOPIC EMERGING DISEASES OF FORESTS

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## 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.

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## 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.

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## 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 advice coming from other scientific communities and areas interested in the outcomes of the Project.

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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.

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# THE XF-ACTORS PROJECT

# (GA 727987)

# TOPICS

XF-ACTORS RESEARCH WORKPLAN COVERS DIFFERENT TOPICS RELATED TO *XYLELLA FASTIDIOSA*:

- BIOLOGY, GENETICS AND PATHOGENICITY
- ECOLOGY AND CONTROL OF VECTORS
- SURVEILLANCE PROGRAMS: TOOLS FOR EARLY DETECTION AND REMOTE SENSING APPROACHES
- EPIDEMIOLOGY AND PEST RISK ASSESSMENT
- INNOVATIVE AND SUSTAINABLE STRATEGIES FOR THE CONTROL OF XYLELLA-INDUCED DISEASES
- IMPROVING CAPACITY BUILDING AND PLANT HEALTH MANAGEMENT

**BIOLOGY, GENETICS AND PATHOGENICITY**. *Xylella fastidiosa* (*Xf*) is a xylem-limited, plant-pathogenic bacterium with a wide host range. Processes leading to plant colonization and the specific mechanisms leading to disease and the mechanisms of host plant specificity are still poorly understood.

Thus, there is high uncertainty with regard to the potential host range of *Xf* in the European flora as a wide range of European wild plant species have never been exposed to the bacterium and it is not known whether they would be hosts, and, if so, whether they would develop symptoms.
To this end, the importance of experimental work to determine the host range of pathogens remains paramount to demonstrate that individual genotypes are pathogenic to specific host plant species. Large dataset of genomic sequences will be cooperatively developed within this project, to gather critical elements to understand the evolution of the population in the contaminated areas, and to identify critical parameters involved in *Xf*-host interaction that could be used to design novel control methods.
Molecular characterization of EU *Xf* genotypes coupled with biological tests on host range and analysis of ecological parameters will be essential in the development of quarantine, containment, and control practices.

**ECOLOGY AND CONTROL OF VECTORS.** Vectors are required for the natural dissemination of *X. fastidiosa*. Therefore, a robust understanding of vector ecology is necessary for the development of management practices. Some aspects relevant to the emergence of Xf is that the bacterium lacks vector specificity, thus all xylem-feeder species are potential vectors and these are distributed worldwide.
Due to the relatively recent emergence of the Xf threats in Europe, there are no consolidated data on the distribution of various potential insect vectors are available in EU, with consequent uncertainties about the area where the bacterium can spread rapidly and cause serious disease outbreaks, the project work plan will investigate the biological processes involved in insect vectoring (feeding behavior, host preference, vibrational communication signals) and the use of innovative approaches (NGS analysis, microbiome and metagenomics sequencing) to produce massive information for the development of novel bio-control tools.

**SURVEILLANCE PROGRAMS: TOOLS FOR EARLY DETECTION AND REMOTE SENSING APPROACHES**. Surveillance and detection of *Xylella fastidiosa* through the area of potential establishment in the EU are keys for early identification of further outbreaks and a prerequisite to effective containment and control of the bacterium and its vector.

Both Xf susceptible host mapping and the early detections of *Xf* infections, have the potential to greatly improve pest spread models, which will specifically address the need to "establish more effective mechanisms and tools for risk assessment and prevention.

The development of methodologies for rapid and sensitive bacterial detection is one of the main project task, whose outcomes will support field surveys and inspections at the point of entry, and that coupled with the use of remote sensing technologies for discovering Xf-associated symptoms at the early stage aims to provide efficient and innovative tools for prevention.

**EPIDEMIOLOGY AND RISK ASSESSMENT OF XF DISEASES.** The epidemiology of *Xylella fastidiosa* diseases is dependent on a variety of ecological, biotic, and abiotic factors and infection dynamics are influenced by the extensive list of host plants species that can be infected, the plant-host specificity of different *Xf* genotypes, and the wide range of potential insect vectors, i.e the epidemiology of *Xylella*-diseases may change dramatically if vector species with different host plant preferences, feeding habits, and dispersal abilities are introduced.

It is also possible that ecological conditions limit the host range and/or virulence of pathogens, which may be 'released' in new environments where other vector species and host plants are present. A biologically-detailed process-based (mechanistic) spread models representing the potential expansion of an invasive species from a location of entry, based on its population dynamics and dispersal will be explored.

This approach complements the species distribution model, incorporating the temporal disease dynamics and mechanistic underpinning. The model will be an effective tool for regional risk assessment estimating the potential future spread and dispersal.

**INNOVATIVE AND SUSTAINABLE STRATEGIES FOR THE CONTROL OF *XYLELLA*-INDUCED DISEASES.** Successful *Xylella*-diseases management must use an integrated strategy that involves the principles of exclusion, eradication, and protection. Exclusion of the pathogen, avoidance of the pathogen, cultural practices, control of insect vectors and disease resistance are some of the control measures.

With regard to control measures, although there are some ongoing research lines, an effective control method of the pathogen applicable in the field is lacking. Control of Xf is therefore currently achieved by removing sources of inoculum, using healthy plant propagation material and controlling the vector(s).

The project ambition is then to include different actions (i) to control the pathogen and the vector developing novel tools (i.e. use of bacteriophage, antimicrobial peptides, endosymbiotic microrganisms, etc.); (ii) to enhance host defense mechanisms, (iii) to implement agricultural practices to reduce spread, and (iv) to produce pathogen-free propagating materials for the new plantations.

The overall ambition is to develop long-term sustainability of cropping systems that protect the natural biodiversity and landscape environments throughout the entire EU territory. Such control measures will be used to produce specific cultivation guidelines for pest and disease management and for low input/organic farming, taking into account the high numbers of host plants and vectors of this bacterium and the differences across production systems which may affect the effectiveness of the measures.

**IMPROVING CAPACITY BUILDING AND PLANT HEALTH MANAGEMENT.** An important ambition of the project is to contribute to the capacity building of the human resources involved at international and national level in the biosecurity plan.

New requirements for imports of non-EU plants have been introduced and movements of ‘specified plants’ (which includes the confirmed hosts of Xf in the EU and further afield) are only possible from areas in the EU where the pathogen is present if stringent conditions are met. In the general organization of the EU phytosanitary system, the national plant protection organizations of the member states are the main part of the official services for the implementation of such provisions.

This implies that the effectiveness of the quarantine program relies on (i) coordinated measures adopted by the Member States; (ii) harmonized practical guidelines for the implementation of the EU decisions; (iii) updated information on the susceptible hosts and on the phytosanitary status of the country of origin; (iv) suitable diagnostic tools for early detection; and (v) trained personnel, at different level, capable of organizing and performing efficient surveillance programs.

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| P2 | Centre International de Hautes Etudes Méditerranéennes (CIHEAM) |
| P3 | Università degli Studi di Bari Aldo Moro (UNIBA) |
| P4 | Institut National de la Recherche Agronomique (INRA) |
| P5 | Agencia Estatal Consejo Superior De Investigaciones Cientificas (CSIC) |
| P6 | Instituto Valenciano de Investigaciones Agrarias (IVIA) |
| P7 | Benaki Phytopathological Institute (BPI) |
| P8 | Julius Kuhn-Institut Bundesforschungsinstitut fur Kulturpflanzen (JKI) |
| P9 | Instituut voor Landbouw- en Visserijonderzoek (ILVO) |
| P10 | The Regents of the University of California (UC) |
| P11 | Centro de Citricultura (IAC) |
| P12 | National Taiwan University (NTU) |
| P13 | University of Costa Rica (UCR) |
| P14 | Natural Environment Research Council (NERC) |
| P15 | Instituto Politécnico de Bragança (IPB) |
| P16 | The University of Salford (USAL) |
| P17 | Joint Research Centre - European Commission (JRC) |
| P18 | Consiglio per la Ricerca in Agricoltura e l’Analisi dell’Economia Agraria (CRA) |
| P19 | Fondazione Centro Euro–Mediterraneo sui Cambiamenti Climatici (Fondazione CMCC) |
| P20 | Nederlandse Voedsel En Waren Autoriteit (NVWA) |
| P21 | Instituto Andaluz de Investigaciony Formacion Agraria Pesquera Alimentaria Y de la Producion Ecologica (IFAPA) |
| P22 | International Federation of Organic Agriculture Movements European Union Regional Group (IFOAM EU GROUP) |

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| P23 | EPPO |
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| P25 | ENBIOTECH Srl |
| P26 | AINIA |
| P27 | Sustainable Communication Aisbl (S-COM) |
| P28 | Stichting Nederlandse Algemene Kwaliteit Sdienst Tuinbouw (NAKTUINBOU) |
| P29 | CIVI-ITALIA |

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| P2 | CIHEAM – Bari and CIHEAM - Chania |
| P3 | CENTRO DI RICERCA, FORMAZIONE E SPERIMENTAZIONE IN AGRICOLTURA (CRSFA) "BASILE CARAMIA", ITALY |
| P27 | NET7 and ID Consulting |

# CONSORTIUM BODIES

### THE COORDINATOR

Her 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 XF-ACTORS.

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

### 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 in charge of monitoring the progress of the activities towards the overall objective of the project in order to deliver the results in due time and coherently with the budget allocation. It has the responsibility of the supervision of the research work flow, management and overseeing Project presentations and results dissemination, approve press release(s) and external communications related to the project. The Board members include all Workpackage Leaders as reported below.

| | |
|---|---|
| **Maria SAPONARI** | **(CNR-IPSP, Italy), Coordinator, WP1 Leader** |
| **Marie-Agnes JACQUES** | **(INRA, France), WP2 Leader** |
| **Pieter BECK** | **(JRC, Belgium), WP3 Leader** |
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## THE COORDINATION TEAM

The Coordination Team is responsible for Project administration, consolidation of the annual Project reports, financial monitoring, management of the financial resources and partner assistance.

| | |
|---|---|
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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.** | **Mariano CAMBRA** | **former IVIA, Spain** |
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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. Efforts have been made by the Beneficiaries for an active engagement of different stakeholders (i.e. growers, Plant Health Authorities, Nurserymen). The following members have already joined the Board, but in the course of the action, other participants will be offered the opportunity to join the Board, giving priorities to those stakeholders that are directly involved in the areas most affected by the socio-economical threat posed by Xylella fastidiosa.

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# MEETING
# PROCEEDINGS

# SESSION 1

# EMERGING PATHOGENS OF FORESTRY

# *PHYTOPHTHORA*-FREE PLANT PRODUCTION AND UPDATE ON *HYMENOSCYPHUS FRAXINEUS*

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In order to minimize the risk of spreading *Phytophthora* species to forests and ornamental sites via nursery stock, BFW has been performing checks of plants of Common Alder (*Alnus glutinosa*) and European beech (*Fagus sylvatica*) from forest nurseries for contamination with *Phytophthora* spp. on a service-basis for the past 13 years. Following a scheme developed by BFW, samples (both plants and soil) are taken by BFW-technicians preferably most close to the time of selling. The samples are tested in BFW's lab by direct isolation from symptomatic plant tissues and by soil baiting. Identification of species is performed by morphological and molecular methods. The results (identified species of *Phytophthora*) are delivered to the nurseries and, in case of freedom of the samples from *Phytophthora*, a certificate is issued to state that the tested material was not contaminated. In order to push to a stable production of plants not infested by *Phytophthora*, the nurseries are informed on a range of requirements. To produce an international protocol for both a *Phytophthora*-testing-system and a guideline for nurseries to enable a stable guaranteed freedom of plantstock from *Phytophthora*, a questionnaire was produced in collaboration with nurseries in Austria to define the most essential challenges. The outcome is a list of prerequisites ranked by importance, reliability and practicability. Among the requirements, avoidance of introduction of *Phytophthora*'s into the nursery via plant import on the one hand and the need for irrigation of the plants with non infected water turned out the most essential challenges. As a preliminary result we can state that, regarding the spectrum of nurseries in Europe with respect to dimension, financial potential, but also availability of irrigation sources as well as infection-risks by specific environments, an international management guideline will have to comprise a certain flexibility to achieve *Phytophthora*-free plant production.

Ash dieback monitoring has been performed since 2008 on 14 sites distributed throughout Lower Austria. Common ash (*Fraxinus excelsior*) is present on 13 plots while one plot represents a pure stand of Narrow-leaved ash (*Fraxinus angustifolia*). Assessment of the disease performed on a yearly base results in a health status of the trees in a 5 % scale, reaching from 0 % (no dieback symptoms in the crown) to 100 % (crown dead). After ten years of continuous monitoring, data for 186 *F. excelsior* and 17 *F. angustifolia* trees in twelve different sites situated in Lower Austria are available. In the past few years, ash dieback has turned out to be a big problem for forest owners and the risk of trees losing their stability without showing symptoms of a disease in the crown raised the awareness and resulted in massive clear cuts and felling of single ash trees. Therefore, the total number of 280 trees in the year 2008 was reduced to 186 *F. excelsior* trees and 17 *F. angustifolia* trees in 2018. Within this time, mean crown dieback intensity of the remaining *F. excelsior* trees increased from 12% to 31%. Distribution in damage classes is as follows: 38 % (up to 10 %); 21 % (> 10 % - 25 %); 12 % (> 25 % - 50 %); 16 % (> 50 % - 90 %); 5 % (> 90 % < 100%) an 8 % (100 %) of dieback in the crown. However, crown dieback damage varies among the plots at the different sites. While in the healthiest plot all trees show less than 25 % of crown damage, with 79 % even less than 10 %, in the plot with the highest

disease intensity a majority of trees exhibits more than 50 % of crown damage and the mortality level increased to 40 % by the year 2018. Although *F. angustifolia* is as susceptible as *F. excelsior*, the single plot representing this species remains relatively healthy by 2018 and mean crown damage of the 17 remaining trees was 3 %. This high variation of mean crown damage among the tested plots is likely due to differences of infection pressure, forest site condition and tree size/age. Future work will contain analysis of this long-term observation of crown damage in relation to climatic data.

Ash dieback has recently been connected with a high security risk, and reports of ash trees falling down or being thrown have increased throughout Austria. Consequently, forest owners started to cut trees in order to minimize the danger. Therefore, we started to investigate the relationship between visually detectable symptoms caused by *Hymenoscyphus fraxineus* (including secondary pathogens) on tree stability. In a mixed *F. excelsior* stand in Lower Austria, affected by crown symptoms and basal lesions, a practical survey was performed in October 2017. Twenty trees were chosen and the occurrence of crown symptoms, basal stem lesions and secondary pathogens documented. Trees were then pulled over with a tractor mounted winch. By aid of a hanging scale, integrated into the pull rope, the tractive force applied to the trees was measured. The type of fraction (root or stem) was documented. Further, the size of the root system and the condition of the main roots were assessed. The samples collected for further analysis in the laboratory included root tissue, life stages of secondary pathogens as well as a stem disc of the basal part of each tree trunk. Preliminary results show that the relation between crown damage and the affected necrotic stem circumference is weak and both symptoms have to be considered separately in assessing tree health. The diameter of tree trunks had a significant impact on the maximum tractive force required to pull trees over. Results of the root assessment and the analysis of stem disks showed that there is a relation with rot in the stem and especially with root rot caused by *Armillaria* sp. Trees with visible demarcation lines in the stem discs were more affected by basal lesions and rot. Assessment of disease symptoms of ash trees affected by *H. fraxineus* should include a careful inspection of the tree base, especially on sites where *Armillaria* sp. are present. This first trial shows that inferring on the stability of ash trees makes it necessary to combine the occurrence of basal lesions caused by *H. fraxineus* and root rot caused by secondary pathogens.

# UPDATE OF *HYMENOSCYPHUS FRAXINEUS* ON DIFFERENT *FRAXINUS* SPP. AND NON-*FRAXINUS* SPECIES

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# LIFE CYCLE OF THE INVASIVE ASH DIEBACK PATHOGEN *HYMENOSCYPHUS FRAXINEUS* IN THE BALKAN REGION

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The alien invasive pathogen *Hymenoscyphus fraxineus* (T. Kowalski) Baral, Queloz, Hosoya has been imported from Asia in Poland at the beginning of 90's and started to cause ash dieback on native European *Fraxinus excelsior* L. and *F. angustifolia* Vahl. The pathogen has spread epidemically through the whole continent in the last 20 years and currently is not present only in countries belonging to the Mediterranean part of Europe.

In the affected regions damages ranges between 3 and 60% in older stands and up to 80% in younger stands, because of the mode of infection and dominance of shoot decline. A wide variety of symptoms has been observed on infected trees starting with the decline of annual shoots and development of the fungus into the wood of branches and causing a decline of more or less the whole crown and finally, the tree dies.

In the Balkan region, the pathogen *H. fraxineus* was at first reported in Croatia close to the Slovenian border. Very shortly after this report, the disease was isolated from the declining trees in western Bosnia and Herzegovina (BiH) (2009) and in 2013 pathogen was reported from the nursery in the central part of BiH. The disease was progressing through the massif of Dinaric Alps towards Serbia and Montenegro, where it was reported 2015 and 2017, respectively.

Study of the life cycle and evaluation of the impact of *H. fraxineus* in Serbia was performed during four year period. The damages in *F. excelsior* and *F. angustifolia* stands range from 4-38%, according to the adopted defoliation evaluation methodology. Lifecycle was slightly different than in western and northern Europe, suggesting that pathogen is adapting to the local environmental conditions.

# HOT-WATER TREATMENT OF EUROPEAN ASH (*FRAXINUS EXCELSIOR*) FRUITS: OLD-FASHIONED BUT POTENTIALLY EFFECTIVE AGAINST *HYMENOSCYPHUS FRAXINEUS*

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The content of this work is currently only available to the registered members of POnTE and XF-ACTORS research consortia.

# AN UPDATE ON THE GENETIC POPULATION STRUCTURE OF THE INVASIVE ASH DIEBACK PATHOGEN *HYMENOSCYPHUS FRAXINEUS*

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Understanding the genetic diversity and structure of invasive pathogens in their source of origin or in areas where they have been recently introduced is crucial to reconstruct the course of invasion(s) and to establish effective control measures. *Hymenoscyphus fraxineus* (*Hf*) jumped from Asian ash species (*Fraxinus* spp.) where it is a leaf pathogen with little impact to European ash (*F. excelsior*) and narrow-leafed ash (*F. angustifolia*) in Europe on which is causing severe disease symptoms and often mortality. Previous studies showed that the European *Hf* population was originated by two divergent haploid individuals. Subsequent introductions from this source would greatly increase the adaptive potential of this pathogen and represents a potential threat to European ash. Therefore, a need to analyse the *Hf* genetic population structure in new areas was identified as the main objective of this study.

In this study 11 microsatellite markers (MM) were used to characterize the genetic structure and diversity of 111 new isolates obtained from nine locations (five of them with no previous MM information in Serbia and the UK) and compare them with a published dataset of 1377 isolates from a total of 11 European countries, Russia and Japan. After combining the two data sets, 270 alleles were observed from 1488 isolates resulting in 778 Multilocus genotypes (MLGs) which were collapsed, based on Bruvo's genetic distance matrix, into 591contracted MLGs that were used to draw minimum spanning networks (MSN). MSN, STRUCTURE and PCA analyses validated previous findings that indicated that European and Japanese populations are partially intermingling although consistently associated to separate clusters. Results did not reveal any evident population structure of European strains, including the *Hf* isolates sampled from new locations, pointing to a single introduction in Europe by few individuals and a recent genetic bottleneck.

# IDENTIFICATION, DIVERSITY AND POPULATION STRUCTURE OF *PHYTOPHTHORA* INFECTING FORESTRY IN EUROPE

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An increasing number of reports of new emerging diseases affecting forest trees caused by several *Phytophthora* spp. indicate that this pathogen pose considerable risks to natural ecosystems. The knowledge of the genus *Phytophthora* is still limited as shown by the high number of new species that are being identified in recent years. In addition, the existing high risk of emergence of new species through hybridization indicates that the knowledge of the taxonomy and biology of the genus is incomplete. The objective of this work was the identification and characterization of new species of *Phytophthora* populations damaging broadleaf and conifer trees based on the use of next generation sequencing (NGS) of PCR amplicons of ITS rRNA. However, since some *Phytophthora* species are closely related and cannot be discriminated based on their ITS sequence we also explored the use of the cytochrome c oxidase subunit I (COI) as a barcode gene. PCR products were amplified from soil samples collected from 104 locations in England, Scotland and Wales from 'disturbed' sites (sites frequently visited by the public, with recent and new plantings, link to nurseries; 83 locations sampled in 9 sites) and from more 'natural' forest and woodland sites (with little disturbance or management; 51 locations sampled in 5 sites). *Phytophthora* was detected in all disturbed and undisturbed sites and in more than 80% of all samples when primers targeting the COI region were used and in a slight lower number when primers targeting the ITS were used. NGS analyses of the ITS region revealed the presence of other oomycetes (7,9% of sequences) in the soil samples apart from *Phytophthora* spp., while those using the COI region revealed lower specificity as compared to ITS since amplified several sequences from diatoms, algi, fungi, an unidentified insect, and several oomycetes different from *Phytophthora*. Results from NGS analysis of ITS sequences identified 36 *Phytophthora* species/phylotypes, whereas those using the COI region identified 28 species/phylotypes, and the combination of both allowed the detection of up to 39 *Phytophthora* spp.. The taxonomy assignation of ZOTUs was obtained after comparing the NGS data with different curated *Phytophthora* databases. We found that the taxonomic assignation of some ZOTUs to a specific *Phytophthora* species varied with the database used, which makes it necessary to have a common curate database to avoid misidentifications, and also to isolate in parallel some of those problematic specimens to be able to confirm their species identity.

# CLIMATIC SUITABILITY FOR THE ESTABLISHMENT OF *HYMENOSCYPHUS FRAXINEUS* IN EUROPE

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# UNDERSTANDING *HYMENOSCYPHUS FRAXINEUS* INFECTION ON ASH FRUITS

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# SESSION 2

# *CANDIDATUS* LIBERIBACTER SOLANACEARUM AND PSYLLID VECTORS

# SECOND YEAR OF MONITORING OF '*CANDIDATUS* LIBERIBACTER SOLANACEARUM' IN CARROT FIELD TRIALS

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Since the first detection in 2008 of '*Candidatus* Liberibacter solanacearum' (*Ca*Lsol) in Spain (in Villena, Alicante), this pathogen has caused year after year variable economic losses in carrot production for the fresh market. In this area, the transmission of the bacterium has been reported mainly by the insect vectors *Bactericera trigonica* and sometimes by *Trioza apicalis*. As part of the objective of providing practical solutions for the management and containment of the diseases associated with this pathogen, challenges with different treatments based on one horticultural mineral oil and three biopesticides were performed by partner 16 (Certis Europe), throughout the second half of 2017, to test their efficacy against vectors and *Ca*Lsol damages.

A carrot seed lot of `Soprano F1´ cultivar, tested negative for *Ca*Lsol, was sown in two open air plots, close to each other. Each plot was divided into 24 squares of ~~12~~ 36 $m^2$ each. Four treatments against insect vectors were tested: maltodextrine, natural pyrethrins, paraffin oil and the entomopathogenic fungus *Beauveria bassiana*, using acetamiprid as a compound of known activity against insect vectors. Each treatment was applied several times in four squares per field randomly distributed, and four received no treatment. At harvest, the plants were inspected for CaLsol symptomatology and ten samples of carrot leaves per square were analysed by real-time PCR for *Ca*Lsol detection in both fields. These samples were also analysed for the universal detection of `*Candidatus* Phytoplasma´ (*Ca*Phy) and for *Spiroplasma citri*.

The samples that had been treated with any of the assayed compounds showed an average level of *Ca*Lsol infection similar to untreated plants (81-91% positive samples), but only 5-10% of the samples showed symptomatology compatible with that described for *Ca*Lsol or *Ca*Phy. Interestingly, all *Ca*Lsol-positive samples analyzed were also positive for *Ca*Phy, while *Spiroplasma citri* was not detected in any of them. All these data suggest that the field and environmental conditions assayed were not appropriate for expression of the bacterial symptomatology and/or the treatments were playing a role in stimulating plant defences or other mechanisms.

# ROBOTIC SOLUTION TO DETECT ASYMPTOMATIC PLANTS INFECTED BY *CANDIDATUS* LIBERIBACTER SOLANACEARUM IN HORTICULTURAL CROPS USING MULTISPECTRAL COMPUTER VISION

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A field robot has been designed and built to automate the capture of images and the mapping of a horticultural field using different computer vision equipments. The robot moves by the impulse of two electric motors coupled to the front wheels and is operated by remote control. A telescopic system allows adapting the width of the robot to the needs of the field in a range between 100 and 200 cm because the geometry of the crop allows the wheels to circulate only by a few lines separated by a certain distance, which can vary from one crop to another. On the other hand, this system allows to reduce its size to be transported in a van. The robot equips three DSLR (Digital Single Lens Reflex) cameras (EOS 600D, Canon Inc, Japan), two of them modified to capture images in near infrared (NIR) from 700 to 1000 nm, and blue NDVI (normalized difference vegetation index), a thermal camera (A320, FLIR Systems, USA) and a multispectral camera (CMS-V, Silios Technologies, France) capable of capturing eight monochromatic images in 558, 589, 623, 656, 699, 732, 769 and 801 nm.

The cameras have been placed facing the ground (the plants) at a distance of approximately one meter from the plants. To avoid the influence of sunlight, the scene is protected from outside light by a canvas. In addition, four halogen spotlights illuminated the scene to provide artificial light. A GPS has been used to geolocate the images and to be able to reference each plant in the images with its physical location in the field. The cameras and the GPS are connected to an industrial computer. An application running on the industrial computer captures the signal from an inductive sensor coupled on a robot wheel and triggers the cameras to synchronise the image acquisition with the robot's advance.

Surveys were carried out in two experimental carrot plots located in Villena (Spain) with the aim of detecting asymptomatic plants infected with '*Candidatus* Liberibacter solanacearum', a vascular bacterium that causes vegetative disorders in *Apiaceae* and *Solanaceae* plants. The plot 1 was inspected monthly at different vegetative status while the plot 2 was inspected only once before harvesting, with the robot carrying the proximal sensing equipment. Several

maps of the field have been created using spectral indices at a resolution of 0.5 mm/pixel using the images captured by the DSLR cameras, and 2.5 mm/pixel using the images captured by the other cameras.

During the last survey of the field, 100 plants were marked to be later identified in the images. These plants were collected separately and taken to the laboratory to undergo a spectral analysis with a hyperspectral camera and a molecular analysis using specific real-time PCR, to determine the presence or absence of infection in the leaves and compare them to the maps created by the robot.

# COMPARISON, CHARACTERIZATION AND VALIDATION OF DIFFERENT DNA EXTRACTION PROCEDURES FOR THE DETECTION OF '*CANDIDATUS* LIBERIBACTER SOLANACEARUM' ON PLANT HOSTS AND INSECT VECTORS

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'*Candidatus* Liberibacter solanacearum' (Lso) is a phloem-limited, Gram-negative, unculturable bacterium that is spread by psyllid insect vectors. Lso is associated with diseases in solanaceous crops in New Zealand and Americas and with diseases in apiaceous crops in Europe and North Africa. This study reports the comparison, characterization and validation of different DNA extraction procedures for the detection of Lso on host plants and insect vectors. As regards the host plant samples, four DNA extraction methods were assessed: CTAB and three different commercial kits for the DNA extraction on nine different types of plant material. The CTAB method, the NucleoMag® Plant kit and the NucleoSpin® Food kit (Macherey-Nagel) provided the best universality as they were able to detect Lso on all the tested matrixes and gave the best analytical sensitivity with most of the matrixes. As for the insect vectors, three DNA extraction methods were assessed for DNA extraction. All the tested DNA extraction methods allowed the detection of Lso in the vector and the identification of the psyllid species.

# EFFECT OF TEMPERATURE, *TRIOZA APICALIS* FEEDING AND '*CANDIDATUS* LIBERIBACTER SOLANACEARUM' INFECTION ON THE CARROT GROWTH

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# ASSESSING THE POTENTIAL ECONOMIC IMPACT OF *CANDIDATUS*

# LIBERIBACTER SOLANACEARUM IN EUROPEAN CARROTS

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# HOW MANY HAPLOTYPES? DETECTION OF '*CANDIDATUS* LIBERIBACTER SOLANACERUM' IN WEED SPECIES

# AND NOVEL PSYLLID HOSTS ASSOCIATED WITH CARROT FIELDS IN SCOTLAND AND SUCTION TRAP SAMPLES

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# TREATMENT PROGRAMS DESIGNED IN SPAIN TO REDUCE THE INCIDENCE OF

# BACTERICERA SP. ON CARROT FIELDS: RESULTS PREVIEW

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Three integrated pest management programs were designed in two trials conducted on carrot crops placed in Villena (Alicante, Spain). Different biopesticides have been included according to previous results showing different effects on eggs, larvae and adults of *Bactericera trigonica* considered the main vector of *Candidatus* Liberibacter solanacearum (*Ca*Lsol) affecting carrots. A complete randomized block design was used with 4 replications (70 $m^2$ per replicate); carrot variety Soprano was used and seeds were previously analysed with negative results to the presence of *Ca*Lsol. Trial protocols included untreated control, straight applications of paraffin oil (54.6% EW at 1% v/v) and three different programs applying alternatively treatments with maltodextrin (59.8% SL at 25L/ha), *Beauveria bassiana* (10.7% OD at 1L/ha), natural pyrethrins (4% EC at 0.75L/ha), acetamiprid (20% SP at 50g/hl) and paraffin oil. First applications were scheduled in alignment with the first captures detected in yellow sticky traps placed in both experimental areas. Treatments and evaluations will cover the crop cycle. Number of larvae and eggs in 100 leaves per replicate are being determined weekly in order to quantify the efficacy of each treatment. At the end of the trials, total production will be evaluated taking 20 plants per each replicate. Trials and evaluations are still being done, with the first treatments applied in August 2018 and the finalization of the crop expected for November 2018. Although the incidence in the untreated check is still low in both trials (maximum detected 1.53 and 2.1 larvae per leaf and 1.53 and 0.35 eggs per leaf, respectively), first results shown a slightly decrease of insect incidence after applying the designed programs when compared with straight applications of paraffin oil. Program development including the available active ingredients to control the levels of the vector population and working in a sustainable frame, will be the key target helping to fight against the disease dissemination in the field.

# SESSION 3

# *XYLELLA FASTIDIOSA*: DIAGNOSIS, GENETICS, HOST RANGE AND CONTROL

# THE MICROBIOME OF OLIVE TREE XYLEM: CAN THEY BE THE KEY TO CONTROL *XYLELLA FASTIDIOSA*?

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Microbial endophytes are well known to protect host plants against pathogens, thus representing a promising strategy for the control of *Xylella fastidiosa* in olive trees. Although their high potential for being used as biocontrol agents, there are pending questions regarding to which extent endophytes enhance plant defences. For example, how microbial fluctuations in the endosphere correlate to plant health? Also, what are the traits that enable endophytes to protect the host plant from pathogens? A better understanding of these questions will assist in the design of new strategies for the control of *X. fastidiosa*. In this study, the xylem microbiome of two olive cultivars with different susceptibilities to *X. fastidiosa* (*i.e.* cvs. FS17 is resistant while cv. Kalamata is susceptible) and with different amounts of *X. fastidiosa* in their xylem vessels, were analysed and compared. Twigs of three cultivars growing in the same orchard in the outbreak area in Italy were collected, and used to assess *X. fastidiosa* infection status by qPCR and xylem-associated microbiome. After DNA extraction from xylem tissue, both bacterial and fungal communities were assessed through sequencing (Illumina MiSeq) of the 16S rRNA V4 amplicons (pair primers 515f/806rB) and ITS1-spanning amplicons (pair primers ITS1F-ITS2), respectively. A total of 86 bacterial and 310 fungal OTUs inhabiting the xylem sap of the three cultivars were identified. There are bacterial and fungal families that are preferential colonizers and the OTUs distribution within these families differs among the olive tree cultivars. Xylem core microbial community of the cv. Kalamata was dominated by Enterobacteriaceae (within bacteria) and Cucurbitariaceae / Herpotrichiellaceae (within fungi), while the xylem of cv. FS17 was colonized mostly by Sphingomonadaceae (within bacteria) and Teratosphaeriaceae (within fungi). A distinct core communities of bacteria and fungi associated with each infection status of the olive trees (highly or poorly infected by *X. fastidiosa*) was also identified. The most parsimonious assumption is that the core microbiome comprises microorganism with relevance to olive tree health. The potential role of these microorganisms in conferring olive tree protection against *X. fastidiosa* should be studied in the future.

# REMOTELY-OPERATED FIELD ROBOT TO DETECT OLIVE TREES INFECTED WITH *XYLELLA FASTIDIOSA* USING PROXIMAL SENSING

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A simple and flexible robotic solution has been developed that equips proximal sensing technology to inspect olive fields in search of early infection by *Xylella fastidiosa*. The robot is operated remotely. Two batteries allow continuous use for six hours, which allows the inspection of a field of approximately 4 ha. Due to the height of the trees, a system of elevation of the cameras has been designed so that they can be raised up to 200 cm. An encoder connected to the axis of one of the motors measures the distance travelled by the vehicle allowing the computer to trigger the cameras at programmable intervals. The system has been tested in a field of olive trees potentially infected by *X. fastidiosa* located in an area of Italy declared under containment in the province of Lecce (Apulia Region). A series of tests have been carried out in an olive testing orchard which showed mild symptoms of *Xf* infection. The sensing equipment consisted of a digital SLR camera, another digital SLR camera modified to capture BNDVI images and a multispectral camera capable of acquiring eight wavelengths in the region of 550 to 850 nm. The system was programmed to capture an image for each meter of the robot's advance. In addition, a 2D LiDAR scanner was used to obtain three-dimensional (3D) structural characteristics of the trees. Preliminary tests were carried out in which the robot captured images and data with all the sensors in different weather conditions (from intense sun to intense rain). The vehicle advanced in each row acquiring the images of the trees on one side and returning in the same row acquiring the images of the trees on the other side, thus obtaining information of the whole tree. These tests served to adjust all the developed software, electronics, and sensors, as well as to improve some key aspects of the robot in terms of battery life and ease of operation.

The robot worked properly during the tests in a field of relatively tall olive trees, being capable of continuously inspecting the whole field without interruptions while capturing valid data. The batteries lasted for more than six hours. During each survey, the robot captured around 35000 images (one every meter) with all cameras. The LiDAR information allowed the 3D reconstruction of the trees in an ulterior off-line process with a total execution time of 850 s for the entire field.

# EVALUATION OF A RAPID DIAGNOSTIC DETECTION ASSAY OF *XYLELLA FASTIDIOSA* BY ISOTHERMAL AMPLIFY RP®

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# UPDATE ON *XYLELLA FASTIDIOSA* SITUATION IN COSTA RICA: STRAIN DIVERSITY, GEOGRAPHIC LOCATION AND HOSTS

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# A FIELD STUDY ON LEAF IONOME AND CALCIUM-RELATED GENE EXPRESSION PROFILES IN *XYLELLA FASTIDIOSA*-INFECTED OLIVE CULTIVARS

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Field observations carried out in Salento (Italy) in the area infected by *Xylella fastidiosa* (*Xf*) evidenced that olive trees of the cultivar 'Leccino' show milder symptoms, if compared to those observed in the susceptible cv. 'Ogliarola salentina'. A transcriptome profiling of the two cultivars infected with the *Xf* De Donno strain revealed different expression profiles of genes controlling calcium homeostasis. In particular, a calcium-dependent protein kinase (CDPK1) is highly overexpressed in 'Ogliarola salentina' infected leaves. Calcium is an important secondary messenger, whose downstream transduction pathways trigger different physiological processes, including stress response and plant defense. Moreover, previous studies have shown that Ca accumulation in leaves is associated with symptomatic tobacco, blueberry, grapes and pecan plants infected with *Xf*.

We pursued a field study that determined the ionome and CDPK1 expression levels of infected leaves of the cvs. 'Leccino' and 'Ogliarola salentina', to evaluate the correlation between Ca, Ca-mediated responses and disease severity in *Xf* olive infection.

Comparison between the two cultivars revealed changes in ionome and CDPK1 gene expression in symptomatic vs asymptomatic tissues. 'Leccino' symptomatic leaves had significant higher Ca concentration as compared to asymptomatic leaves, while differences for the susceptible cv. 'Ogliarola salentina' were non-significant. CDPK1 expression in 'Ogliarola salentina' was significantly increased relatively to 'Leccino' trees grown in the same field and the increase was higher in symptomatic leaves.

These data confirmed that, in olive, as previously observed in other species, Ca plays a role in disease progression. Moreover, *Xf* infection in olive induces a re-modeling of the ionome, which correlates with the degree of disease severity and the susceptibility of the cultivar.

# GENOMIC ASSESSMENT OF *XYLELLA FASTIDIOSA* ISOLATES RECOVERED IN THE BALEARIC ISLANDS AND MAINLAND SPAIN

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The quarantine bacterium *Xylella fastidiosa* has recently emerged as a serious threat to the European and Mediterranean agriculture and landscape, with several outbreaks detected in Italy, France, and Spain. The first finding of the bacterium in Spain occurred in 2016, when cherry (*Prunus avium*) and *Polygala myrtifolia* plants were reported to be infected by strains of *X. fastidiosa* subsp. *fastidiosa* ST1 and of *X. fastidiosa* subsp. *multiplex* ST7 and 81. Since then, numerous outbreaks of the bacterium have been reported in the major Balearic Islands (Mallorca, Menorca, and Ibiza), where more than 15 host species have been found to be infected by different subspecies and STs of *X. fastidiosa,* including the subspecies *pauca* ST80 in Ibiza. Later on in 2017, *X. fastidiosa* subsp. multiplex ST6 was detected for the first time in the mainland Spain, on almond trees in Alicante province (Valencian Community).

Draft genome sequences of two *X. fastidiosa* subsp. *multiplex* ST6 strains ESVL and IVIA5901 from almond in Alicante, and a *X. fastidiosa* subsp. *fastidiosa* ST1 strain IVIA5235, from a cherry tree in Mallorca, were obtained by high throughput WGS using a HiSeq4000 Illumina platform. Pairwise comparisons of the chromosomal genomes of the two *X. fastidiosa* subsp. *multiplex* strains showed an average nucleotide identity higher than 99%. Interestingly, the two strains differ for the presence of the plasmids pXF64-Hb_ESVL and pUCLA-ESVL detected only in the ESVL strain. In the case of *X. fastidiosa* subsp. *fastidiosa* strain a plasmid, named pXFAS_5235 showing high sequence similarity with the conjugative plasmid pXFAS01 which was reported in *X. fastidiosa* subsp. *fastidiosa* strain M23 from almond, was also identified. To confirm the presence/absence of the different plasmids PCR tests were carried out on different cultured strains, and on several infected plant samples collected in the almond orchards in Alicante and in a collection of several host from Balearic Islands. PCR results confirmed the WGS data, and disclosed the occurrence of strains of *X. fastidiosa* subsp. *multiplex* ST6 infecting almond trees harboring the two plasmids, only plasmid pUCLA-ESVL or none. Additionally, the presence of plasmid pXFAS_5235 was demonstrated in all host infected by *X. fastidiosa* subsp. *fastidiosa* ST1 in Mallorca.

The availability of these draft genomes will contribute to extend the European genomic sequence dataset, a first step toward setting new research to elucidate the pathway of introduction and spread of the numerous strains of this subspecies so far detected in Europe.

# IMPLEMENTATION OF SAMPLING PROCEDURES FOR TESTING COMPOSITE SAMPLES FOR *XYLELLA FASTIDIOSA*

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Inspections and controls for *Xylella fastidiosa* are mandatory on consignment and in place of productions for the most susceptible host plants listed in the EU Decision 2017/2352, as well as on the long list of "specified plants" when propagated in nurseries located in the infected, containment and buffer zones. The main constrains for testing samples collected from lots of plants are the large number of units to be sampled and the large amount of materials (n. of leaves or shoots/cuttings) to be processed at laboratory level.

We carried out experiments by simulating composite samples containing different proportions of *Xylella*-infected plant tissues, to verify the diagnostic sensitivity of serological and molecular tests. Petioles recovered from infected leaves of *Polygala myrtifolia*, *Nerium oleander* and *Olea europaea,* and scraped xylem tissue from infected cuttings of *Prunus avium* were pooled at different ratio with healthy materials and processed by ELISA, LAMP and qPCR. Indeed, a protocol based on the extraction of large amount of tissues (40gr of plant material, 100-200 leaves) was also tested.

The results provided preliminary important indications both for sampling and laboratory testing: (i) minimum n. of leaves to be collected from the single unit of the lot; (ii) the maximum n. of units that can be pooled and processed using either the standard extraction procedures or the protocol adapted for large tissue amount. The minimum number of the leaves to be collected from the individual plant of the sample unit, ranged from 2 to 6 according to the host species, allowing to pool up to 10 plants or more in relation to the extraction protocol used.

Composite sampling is increasingly becoming an acceptable practice when a large number of samples have to be selected to satisfy sample size requirements, keeping the number of diagnostic tests affordable.

# OBTENTION OF SPECIFIC MONOCLONAL ANTIBODIES AND ANTISERA AGAINST *XYLELLA FASTIDIOSA* AND THEIR USE FOR DETECTION AND DIAGNOSIS

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Monoclonal antibodies (MAb) specific to *Xylella fastidiosa* were obtained by fusion of a nonsecreting myeloma cell line with spleen cells from immunized BALB/c mice by intraperitoneal injections of 0.1 ml of $10^8$ cfu/ml of *X. fastidiosa* subsp. *fastidiosa* (LMG17159 strain) (somatic antigens O) emulsified in Freund's incomplete adjuvant. Specific antibody-secreting hybridoma selected by indirect-ELISA was three times cloned under conditions of limiting dilution and established hybrids were grown in HT medium. Ten MAb lines producing the highest bacterial titre were selected, isotype determined and their specificity tested. Three MAbs (MAb2G1/PPD, MAb1C6/PPD and MAb9F7/PPD) were selected for their wide reaction spectrum against *X. fastidiosa* strains and good specificity. Furthermore six polyclonal antisera against *X. fastidiosa* were raised in CalifornianxNeozelander rabbits with O antigens from Conn Creek, LMG15099 and LMG17159 strains. LMG17159-O antiserum was selected for the higher titre and because it recognized all the *X. fastidiosa* strains challenged. Polyclonal immunoglobulins as trapping/coating antibodies and specific MAb2G1/PPD as intermediate-detecting antibodies (DASI-ELISA method) reached a sensitivity of $10^5$ cfu/ml of *Xylella fastidiosa* in almond extracts and of $10^5$-$10^6$ in olive extracts. A DAS-ELISA prototype was then developed, prior to commercial distribution, using MAb2G1/PPD conjugated with alkaline phosphatase. The sensitivity reached was $10^5$ cfu/ml and showed excellent specificity. One hundred twelve samples of different almond tree plots from the Demarcated Zone for *X. fastidiosa* in Alicante (Spain) were analysed comparatively by the developed DAS-ELISA, the LOEWE kit and the protocols of real-time PCR by Harper et al. (2010) and Francis et al. (2006). The agreement between the techniques was almost perfect according to the estimated Cohen's kappa index, even in symptomless almond trees. The production of specific MAbs to *X. fastidiosa* will supply a continuous source of homogenous and well characterized antibodies to increase the accuracy of diagnosis and detection methods. A direct tissue-print or DTBIA kit is being also validated in order to supply an available user-friendly system to test in a low cost, fast, discreet, sensitive, an accurate manner this harmful bacterium in samples from nurseries, gardens and wide surveys, such as is available for other plant pathogens.

# EFFICACY OF SYNTHETIC FUNCTIONAL PEPTIDES AGAINST SUBSPECIES OF *XYLELLA FASTIDIOSA*. *IN VITRO* AND *IN PLANTA* STUDIES.

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# CHARACTERIZATION OF *METHYLOBACTERIUM* SPP. POPULATION, ENDOPHYTES OF OLIVE SAPWOOD

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Endophytic bacteria are of biotechnological and agronomic interest as they promote plant healthiness. Investigations on the bacterial endophytic population occurring into the xylem of healthy and *Xylella fastidiosa* subsp. *pauca (Xfp)* infected olive trees showed that under field conditions, the population level of cultivable endophytic bacteria is highly variable, being mainly affected by the host genotype, host age, and wilting severity. Among the different groups, the cultivable microbial community of olive trees, either infected or not by *Xfp*, are *Pseudomonas*, *Bacillus*, *Lysinibacillus*, *Pantoea*, *Microbacterium*, *Stenotrophomonas*, and *Methylobacterium* spp. Bacteria of the genus *Methylobacterium* are facultative methylotrophs, and different species are endophytes in a variety of plants. As endophyte they promote plant growth and root formation by producing phytohormones and stimulating germination, enhance plant systemic resistance, supply or mobilize nutritional elements (siderophores production), and some species have also been reported as antagonist of bacterial and fungal phytopathogens. Several studies focused on *Methylobacterium* spp. community, which occupy the same ecological niche of *Xfp* in xylematic vessels of citrus plants. Buffered Charcoal Yeast Extract Agar (BCYEA) and Methanol Mineral Salts Agar (MMSA) behaved better than other microbiological media in estimating the population of spp. in the olive sapwood, and data indicated a higher population density in *Xfp*-infected olive sapwood than in the healthy ones, although without any statistical significance. Species of *Methylobacterium* have been reported as potential biocontrol agents, but it depends on which species. *M. extorquens* has a synergistic action, favoring the growth of *Xfp* and increasing the symptoms severity on citrus; conversely, the presence of *M. mesophilicum* reduce the growth of *Xfp* and consequently the symptoms severity. Colonies are pink to light red, circular and smooth with non-pigmented after growth on MMSA, for 6 days at 30°C. The analysis of 16S rRNA gene sequence and phylogenetic data by comparison with ten reference strains, showed that isolates GR18, GR19, GR22 and GR23, obtained from *Xfp*-infected olive tissues, cluster both with *M. fujisawaense* and with *M. radiotolerans*. Therefore, these isolates shows few differences to clearly discriminate the species. *M. radiotolerans* was also isolated from citrus plants infected by *Xfp* in Brazil, but no information about the effects on the pathogen are available. Further research are in progress to better characterize the different *Methylobacterium* strains, using molecular and biochemical approaches, and evaluating *in planta* their activity on olive quick decline syndrome.

# GENOMIC CHARACTERIZATION OF PSEUDOPHAEMONIELLA SPECIES OCCURRING IN THE SAPWOOD OF HEALTHY AND DISEASED OLIVE TREES AFFECTED BY QUICK DECLINE SYNDROME

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# EPPO DIAGNOSTIC PROTOCOL ON *XYLELLA FASTIDIOSA*, CONTRIBUTION OF XF-ACTORS & PONTE

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The first EPPO diagnostic protocol on *Xylella fastidiosa* was adopted in 2004. The first revision was adopted in September 2016 and took into account the experience gained by different laboratories after the *Xylella fastidiosa* outbreak in Europe. A second revision was adopted in April 2018. The diagnostic protocol was published on the EPPO Bulletin and is available on https://gd.eppo.int/download/standard/148/pm7-024-3-en.pdf. This recent version takes into account the outcomes of different projects and in particular an interlaboratory comparison performed in the framework of POnTE, XF-ACTORS and a Euphresco project. The EPPO Secretariat is in close contact with the researchers involved in the different projects and the next revision is already planned and a first Exert Working Group was organized in September 2018. The revisions envisaged for the next version of the protocol will be presented highlighting how outcomes of the different research projects can assist with a timely update of the diagnostic protocol.

# FURTHER DATA ON OLIVE SUSCEPTIBILITY/RESISTANCE TO *XYLELLA FASTIDIOSA*

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The recent global re-emergence of *Xylella fastidiosa (Xf)* has been associated to infections on several new host species and in particular to severe infections on olive trees, reported both in Europe and southern America. Although, initial report of *Xf* infections on olives, caused by strains of the subsp. *multiplex*, from California were associated to erratic appearance of olive leaf scorching or branch desiccation, severe phenomena of decline and extensive desiccations have been repeatedly reported associated to strains of the subspecies *pauca* in Italy, Brazil and Argentina. Furthermore, biological and molecular studies on olives belonging to different cultivars and infected with isolates of the subsp. *pauca* identified cultivars with differential susceptibility and traits of resistance. In this work, we used the strain CO33 taxonomically related to subsp. *sandyi*, to infect olive plants of the cultivar Cellina di Nardò and Leccino, previously characterized as susceptible and resistant, respectively. Twelve months after inoculation, *Xf* CO33 induced twig desiccations limitedly to the inoculated plants of the cv Cellina di Nardò. Indeed, the symptomless plants of the cv Leccino harbored lower bacterial populations than cv Cellina di Nardò. Transcriptome profiling of the xylem tissues showed that *Xf* is sensed by plants of both cultivars with high numbers of differentially expressed genes. Among transcripts showing the highest up regulation in cv Leccino are Leucine Rich Receptor like kinases, already identified in the transcriptomes of trees infected with *Xf* subsp. *pauca*. Conversely, tissues from symptomatic plants of Cellina di Nardò display a typical defense reaction with pathogenesis related proteins among major up regulated genes. Besides extending the data on the susceptibility of olives to *Xf* strains of a different subspecies, the data confirm the resistance of the cv Leccino to this pathogen, supporting evidence that different mechanisms of host-pathogen interactions exist in susceptible and resistant olive cultivars.

# SCREENING OLIVE CULTIVARS FOR RESISTANCE TO *XYLELLA FASTIDIOSA*

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Since 2016 field experiments and greenhouse tests have been started to expose plants of different olive cultivars and selections to high pressure of bacterial inoculum, either by vector-mediated transmission or by needle inoculation of *Xylella fastidiosa* subsp. *pauca* ST53. The majority of the cultivars/selection under evaluation were from propagations obtained from the World Olive Germplasm Bank (CAP-UCO-IFAPA). All the accessions under evaluation have been identified by both morphological and molecular markers. The results collected from the first field experiment set in 2016 in the infected area with 19 cultivars, showed that after two seasons of exposure to the population of infected vectors, natural infections have occurred for all cultivars, although with significantly different incidences.The data collected in this experimental parcel confirmed the evidence recovered in the past years in the commercial orchards: Leccino showed the lowest incidence of infections (8.3%), whereas infections in Cellina di Nardò and Ogliarola reached 35% and 37.5%, respectively. Among the remaining 16 cultivars: (i) Toscanina, Maiatica and Simone showed infection rate below 10% (i.e. close to that recorded for Leccino); (ii) Pendolino, Oliastro, Bella di Spagna, Itrana showed the highest infection rates comprised between 30% and 45%. Intermediate values, between 15% and 30%, were recorded for the remaining cultivars. Similarly, for the second group of 60 accessions from IFAPA whose experiments started in 2017, results from field evaluations and needle inoculations allowed to differentiate them in three categories according to the infection rates. Indeed, initial symptoms of shoot dieback were recorded on some accessions in the greenhouse experiments. However, especially for the field experiments affected by the weather and climatic conditions, observations and quantitative assays need to be prolonged in order to acquire conclusive data from multi-year surveys.

# LIPIDS INVESTIGATION IN *XYLELLA FASTIDIOSA* SUBSP. *PAUCA* STRAIN DE DONNO LIFESTYLE

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Bacteria organize their lipid membrane composition to survive under different environmental conditions. The lipid signals are messengers to modulate motility, biofilm formation and virulence in phytopathogenic bacteria as well as represent a class of signals exchanged during the perception, and regulation of defence mechanism in the host-pathogen interaction. LC-TOF and LC-MS/MS were adopted to assess the lipid composition of *X. fastidiosa* subsp. *pauca* strain De Donno and polar, non-polar lipids, free fatty acids and oxylipins were characterized and quantified in bacterial cells and cultural filtrate. The lipid profile of *X. fastidiosa* subsp. *pauca* strain De Donno during the interaction with the model plant *Nicotiana tabacum* Petit Havana SR1 was exploited. This study highlighted specific class of lipids (e.g. ornitholipids and oxylipins) accumulate differently in infected plant tissues compared to uninfected ones. The *in vitro* and *in vivo* studies spotlight the following scenario: the lipid profile of *X. fastidiosa* contributes to shape its lifestyle *in vitro* during change from twitching motility to biofilm formation and during its relation with the host. The results suggest the presence of other DSFs than the already described ones (i.e. XfDSF1 and 2), and lipid entities, such as OL1, TAG 52:2, oleic acid and 7,10-diHOME, may constitute an arsenal of molecules that actively contribute to plant-pathogen cross-talk. Moreover, naturally infected and healthy olive samples (cv. Ogliarola salentina) were collected in an affected olive orchard (Lecce and Taranto provinces, Apulia region) to investigate their lipidomic profile. It emerges a scenario in which the *X. fastidiosa*-infected olive samples presented at least bacterial specific lipid and, other host-pathogen shared lipid entities.

# IDENTIFYING *XYLELLA FASTIDIOSA* HOST ADAPTATION CANDIDATE GENES: THE CASE OF *X. FASTIDIOSA* SUBSP. *PAUCA* ISOLATES AND OLIVE TREES IN ITALY

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# SYNTHESIS OF CIS-2-UNSATURATED FATTY ACIDS FOR BIOCHEMICAL CONTROL OF BIOFILM PRODUCTION IN *XYLELLA FASTIDIOSA*

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Different chemical species are utilized from bacteria as signals for cell-to-cell communication during Quorum Sensing. A widely investigated family of chemical signals from plant pathogenic bacteria are the diffusible signal factors (DSFs). In *Xylella fastidiosa* and related *Xanthomonas* species, these DSFs are produced by a synthase (RpfF) and interact with a cognate sensor (RpfC), resulting in the activation of a response regulator (RpfG) able of coordinating genes involved in the expression of virulence, spreading and biofilm formation. By recent investigations, *X. fastidiosa* exploits cis-2-unsaturated fatty acids as its own DSFs, and an imbalance in their concentration in bacterial cells seem to provoke blockage of xylem infection, by altering biofilm formation and architecture in a strategy called "pathogen confusion". Here we report the synthesis of *cis*-2-unsaturated fatty acids with a chemical structure related to DSFs family, in order to test the *in vitro* alteration of biofilm production in *X. fastidiosa* 'De Donno' strain, the etiological agent of Olive Quick Decline Syndrome in Apulia region.

A modified olefination stereoselective reaction of Horner-Emmons, called Still-Gennari, allowed for the synthesis of $\alpha-\beta$ unsaturated fatty acids in cis (Z) conformation starting from commercial aldehydes. A preliminary investigation is carried out testing different commercial cis-2-unsaturated fatty acids (cis-2-decenoic acid, cis-2-dodecenoic acid, cis-2-(11)-methyl-dodecenoic acid) known as DSFs in *Xanthomonas, Burkholderia and Pseudomonas* genera, for their effect on *X. fastidiosa* ability to form a biofilm ring adhering to the surface of glass tubes or microtiter plates. These *in vitro* assays will be used to test the synthesized $\alpha-\beta$ unsaturated fatty acids.

# DEVELOPMENT OF XYLAPP$_{EU}$_2.1.2 FOR THE HARMONIZATION OF MONITORING DATA PROCESS OF XYLELLA FASTIDIOSA FROM FIELD TO LABORATORY

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# SESSION 4

# *XYLELLA FASTIDIOSA*: VECTORS AND CONTROL STRATEGIES

# VIBRATIONAL COMMUNICATION AND MATING BEHAVIOR OF *PHILAENUS SPUMARIUS* (HEMIPTERA: APHROPHORIDAE)

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# CAN FUNGAL ENDOPHYTES PRESENT IN *COLEOSTEPHUS MYCONIS* BE USED TO MANIPULATE THE DISTRIBUTION OF *PHILAENUS SPUMARIUS*?

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*Coleostephus myconis* is an herbaceous plant belonging to the Asteraceae family, which is normally present in olive groves soils in the Trás-os-Montes region, Portugal. It was observed that in the same olive grove and time period, *C. myconis* displays different levels of *Philaenus spumarius* infestation. Thus, *P. spumarius* nymph appears to selectively choose plants on which to feed or developed. The causes of the insect preference by a specific plant host are unknown. This work aims to assess the correlation between the fungal endophytic community of *C. myconis* with the feeding and nymph development preference of *P. spumarius*. Fungal endophytes where isolated from the stems, leaves and inflorescences of plants with three levels of *P. spumarius* infestation (no infestation, moderate and high infestation). Fungal isolates where molecularly identified by sequencing the ITS region of rDNA and the partial *tef1*. A total of 138 fungal OTUs, belonging to 37 different genera, were isolated, being *Alternaria*, *Valsa*, *Cladosporium* and *Chalastospora* the most abundant. Some genera were found exclusively in the non-infected plants and others in infected plants. In general, the abundance and diversity of fungal endophytes increased across infestation levels. This pattern was particularly observed within stems, in which the nymphs in their foam typically appear. This organ also displayed the greatest variation on fungal composition among the three levels of plant infestation. Overall, the results indicate that these changes in endophytic fungal composition may have a role in the plant choosing by *P. spumarius* for the nymph development. Our study provides potential fungal candidates to be further analyse, to understand their mechanisms leading to the repellence/attraction of the *P. spumarius* nymphs. These could be a sustainable approach to manipulate the distribution of the vector *P. spumarius*.

# PRELIMINARY RESULTS ON THE TRANSMISSION CHARACTERISTICS OF *XYLELLA FASTIDIOSA* SUBSP. *PAUCA* (ST53) BY *PHILAENUS SPUMARIUS* AND *CICADELLA VIRIDIS*.

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There is little information available on the biology of the transmission of *Xylella fastidiosa* by *Philaenus spumarius* and the most widespread European sharpshooter, Cicadella *viridis*. Although the role of the meadow spittlebug, *P. spumarius,* in the transmission of *X. fastidiosa* subsp. pauca ST53 to olive, periwinkle, oleander and *Polygala myrtifolia* has been proven, the description of vectors' acquisition, persistence and transmission characteristics has not been described so far. A deep knowledge of transmission parameters is of key importance to shed light on the epidemiology of this invasive bacterium and emerging associate diseases in the Old World. For this reason, two sets of experiments have been performed, in order to describ (i) the kinetics of the bacterial multiplication and persistence in the spittlebug *P. spumarius* and in the sharpshooter *C. viridis;* (ii) the influence of environmental factors (temperature, season) and insect age on simulated epidemics progression on olive plants under controlled and natural climatic conditions (indoor-outdoor). Both experiments have repeated in 2017 and 2018, twice a year (June-July and September-October). Insects used for acquisition were collected in *Xf*-free areas, whereas naturally infected olives and needle- inoculated periwinkle were used as source of inoculum.

For the kinetics experiments, after an acquisition access period (AAP) of 72h, insects were transferred in groups of five, at different times post-acquisition on olive or periwinkle test plants for an inoculation access period of 72h.

In the second sets of experiments, after the AAP groups of insects were isolated in cages with 16 olive seedlings for 3, 7, 14 and 21 days' inoculation periods.

Acquisition and transmission rates were assessed by testing individual insects recovered after the IAP and by testing all recipient plants 6 and 10 months after the inoculations.

Although the results from 2018 have to be collected yet, preliminary results from 2017 indicate for *P. spumarius*: a) an higher acquisition efficiency in September as compared to July; b) a lower acquisition efficiency when periwinkle was used as source plant compared to olives, conversely transmission was higher on periwinkle plants compared to olives. Very low acquisition efficiency and no transmission ability were recovered for *C. viridis*.

Experiments are still ongoing, and preliminary results need to be confirmed by 2018 assays. Because of the negative results obtained so far with *C. viridis*, in the last set of experiments (conducted on September-October 2018) we applied an *in vitro* acquisition system for *X. fastidiosa* ST53 for the sharpshooter. The results will be available soon.

# ATTEMPTS TO REDUCE THE SPREAD OF *XYLELLA FASTIDIOSA* IN OLIVES USING KAOLIN APPLICATIONS

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Different synthetic and organic insecticides have been tested in the recent past years against *Philaenus spumarius*, the principal vector of *Xylella fastidiosa*, in olive orchards in the Apulia region (southern Italy). The results showed the need of additional research on new formulations and new testing to pursue a more sustainable and effective vector control.

In this work, we tested the effects of kaolin on vector transmission and spread of the infections in olives. Experiments were carried out in an olive plot planted in April 2016 in the demarcated infected area, and consisting of 3-year-old olive plants of the susceptible cv Cellina di Nardò. The experimental controls included the non-treated plants and plants treated with imidacloprid on a calendar basis. Applications were performed for three consecutive years (2016, 2017 and 2018) starting in April or first decade of May until October. Treatments were repeated every 10-15 days or after major rain events.

Visual inspections were periodically performed and, once a year, plants were sampled and tested for the presence of *X. fastidiosa* by real time PCR. Visually inspections recorded the presence of shoot dieback on the untreated plants since the first year after planting, and reached a percentage of 20% during the third year. Conversely, none of the plants treated with kaolin or imidacloprid have so far showed symptoms. Diagnostic tests carried out after three years, identified bacterial infections in approx. 40% of the untreated and kaolin-treated plants. This percentage decreased to ca. 18% in the control plants threated with imidacloprid, used in ours trials as positive control, but whose use practically is strictly in European level, due to the acute and chronic effects on honey bees.

The overall results confirm the difficulties to achieve an effective reduction of the spread of the infections through the control of the adult spittlebugs, even when a relevant number of treatments per year (12-15) is applied. Strategies for reducing the juvenile populations combined with applications targeting the new emerged adults before they acquire and transmit the bacterium on olive trees should be emphasised.

# EVALUATION OF STICKY TRAPS FOR MONITORING THE POPULATION OF SPITTLEBUGS IN SOUTHERN ITALY

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Estimating the relative abundance and seasonal patterns of adults of *Philaenus spumarius*, the predominant European vector of *Xylella fastidiosa*, is important for implementation of surveillance and control strategies for the diseases caused by the bacterium and currently emerging in Europe. Sweep net or coloured sticky trap are a quick and simple sampling method for monitoring the relative abundance of one or more insect species over a large region. In this work we evaluated sweep net and different coloured sticky traps placed in olive and almond orchards as methods for sampling spittlebugs (i.e. the two vectors *P. spumarius* and *Neophilaenus campestris*) and other xylem sap feeders occurring in the two crops. In a first field experiment, six different coloured traps (white, red, blue, yellow, yellow with circle-pattern and yellow with line-pattern) (Russel IPM) were screened. Forty and 22 traps/color were placed in olive and almond orchards, respectively, on the southwest side of the trees and left for 1 week. The results of the inspections of these traps indicated that yellow colored traps are more attractive for spittlebugs than the other traps. Among the yellow traps, those with line and circle pattern captured more spittlebugs as well as more planthoppers (i.e. less selective than the plain yellow traps). Subsequently in the same orchards, yellow sticky traps were placed and surveys performed at 2-weeks interval were performed from June to September 2018. Fifty traps were randomly replaced every two weeks and concomitantly the same orchards were monitored by sweep net (20 random sample units/orchard, with 10 sweep net per each sampling unit). The data so far collected indicate that yellow traps are more efficient in capturing of *P. spumarius*. This was particularly evident in the almond orchard, where a peak of 50 specimens/trap/week were recorded, versus peaks of maximum 8 individuals collected by sweep net.

In the site used for our study, characterized by abundant population of spittlebugs, yellow sticky traps resulted the most attractive coloured traps, and surveys using sticky traps were more efficient than sweep net. These indications may be useful to support further studies for dispersal dynamics or evaluation of the efficacy of insecticides applications.

# SOIL MANAGEMENT TECHNIQUES FOR THE CONTROL OF JUVENILE POPULATIONS OF SPITTLEBUGS IN OLIVE GROVES

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In the olive groves, the main factor regulating spittlebugs population level is the abundances of vegetables ground cover and its species composition.

Control of the juvenile's vector population by soil tilling to reduce the emergence of adults represents one of the most efficient and environmentally sustainable approach toward the control of the spread of *Xylella fastidiosa* in the infected area, or to reduce the risk of its establishment in the buffer zones or free areas. In order to identify alternative strategies for soil management in olive groves, for two consecutive years (2017 – 2018) we compared five different soil management techniques: (i) natural and undisturbed ground vegetation; (ii) soil tilling performed twice (in early winter and in spring when the majority of the nymphs were at the IV instar); (iii) soil tilling performed only in winter; (iv) sowing *Lolium* spp; (v) sowing *Hordeum vulgare*.

Two surveys per year were carried out. In each thesis, of approx. 3,000m$^2$, 30 subplots of 0.25 m$^2$ randomly distributed were surveyed by counting the number of spittle/plants and number of specimens (*P. spumarius* and *N. campestris*)/spittle. Soil tilling performed in winter and spring, effectively reduced almost to zero the presence of both spittlebug species, with an Abbott's index equal to 99.6%. Conversely, when soil tilling was performed only in winter, it proved to be effective for reducing *N. campestris* population (Abbott's index ranged from 50% to 60%) but not for *P. spumarius*. Sowing in winter *Lolium* spp. and *Hordeum vulgare* reduced *P. spumarius* juvenile populations, with an Abbott's index of 60% and 40%, respectively. Whereas, results were inconsistent for *N. campestris*: in 2017 on both *Poaceae* treatment, the juvenile populations were lower than on the undisturbed natural ground cover, with an Abbott index equal to 58% (*Lolium*) and 86% *(Hordeum vulgare),* while the population resulting mostly abundant during survey carried out in 2018. These data further support the effectiveness of mechanical interventions for reducing incidence of juvenile of spittlebugs, and that using poaceae species to replace the ground vegetation may contribute to lowering *P. spumarius* populations.

# CONTINUOUS INDOOR REARING OF *PHILAENUS SPUMARIUS*, THE MAIN EUROPEAN VECTOR OF *XYLELLA FASTIDIOSA*

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The phytosanitary emergence triggered by *Xylella fastidiosa* introduction in Europe urgently calls for research on its main vector, the meadow spittlebug, *Philaenus spumarius*. Effective containment of bacterial spread strongly relies on vector management, and, therefore, on a detailed knowledge of the vector itself. Nevertheless, biological, ecological, and behavioural studies about *P. spumarius* are currently scattered and continuous research efforts are urgently needed. *P. spumarius* is a univoltine species that undergoes to obligate separate ovarian and overwintering diapause. Thus, a major limitation for conducting research on *P. spumarius* is the difficulty in continuously rearing the spittlebug under controlled conditions. Here we propose a methodology to rear *P. spumarius* in the laboratory providing a supply of nymphs and adults before they become available in the field. This new method will overcome the seasonality barrier and reduce time and efforts currently required for experimentation on the meadow spittlebug. The proposed methodology would allow producing the data urgently required to fill the knowledge gap and finally set up an effective and environmentally friendly control strategy of *P. spumarius*.

# FLIGHT BEHAVIOUR AND PATTERNS OF DIRECTIONAL MOVEMENT ON *PHILAENUS SPUMARIUS*

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The recent emergence of *Xyllela fastidiosa* in Europe is a major threat for key crops such as olive, almond or grapevines. *Philaenus spumarius* was identified as the predominant vector involved in the spread of *X. fastidiosa* in southern Italy. This meadow spittlebug is distributed among most of the Palearctic and Nearctic regions. Understanding vector movement is critical to develop effective control measures and limit the spread of the diseases caused by *X. fastidiosa*. Flight mills, as well as capture-mark-recapture studies, can provide information on the dispersal potential. Other tools such as directional Malaise traps and horizontal-net traps can provide information of the migratory patterns of *P. spumarius* and other potential vectors of *X. fastidiosa*. Our goal is to determine the duration and speed of flight of *P. spumarius* under laboratory conditions using a modified commercial flight mill. We measured how long do insects flight and counted the number of spins that an insect makes when attached to a flight mill under controlled conditions. Experiments are being conducted with three different light intensities: 0, 1 and 4.8 µmol $m^{-2}s^{-1}$. Our preliminary flight mill results have shown that the dispersal potential of *P.spumarius* is much higher than it was thought. Weaver & King (1954) observed that this insect species is able to travel more than 30 m in a single flight and up to 100 m in 24 hours. However, our data show that both, male and female of *P. spumarius* are able to fly at least 1000 m in less than a single 1-hour continuous flight. The timing of the migratory behaviour from olive groves to oversummering hosts such as pine or oak trees and the immigration of adults back to olive groves in the fall is being studied. During late spring (early June), we observed that potential vectors tend to move from surrounding ground cover to olive fields. Horizontal-net traps seem to be more useful than the malaise directional traps according to our preliminary data. In the near future, we plan to study the migratory behaviour and dispersal potential by marking the vectors with fluorescent powders.

# UNDERSTANDING *PHILAENUS SPUMARIUS* BEHAVIOR AND HOSTS PREFERENCE FOR PREDICTING *XYLELLA FASTIDIOSA* SPREAD UNDER CENTRAL-EUROPEAN CONDITIONS

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Within Europe, the meadow spittlebug *Philaenus spumarius* is the only epidemiologically relevant vector of *Xylella fastidiosa* identified so far, playing a key role in bacterial transmission to olive in South Italy. However, considering its large host range and wide distribution, the spittlebug could be a relevant vector across all the outbreaks detected in Europe by now. In 2016, a single oleander plant infected with *X. fastidiosa* ssp. '*fastidiosa*' was spotted in Germany; the plant was destroyed and the outbreak is nowadays considered eradicated. Nevertheless, the finding prompted a survey aimed at characterizing candidate vectors presence, biology and ecology.

A previous study in Southwest Germany already confirmed the occurrence of *P. spumarius, Neophilaenus campestris* and 12 other potential vector species belonging to the families Aphrophoridae (5 species), Cercopidae (3 species) and Cicadellidae: Cicadellinae (4 species). The survey focused especially on the xylem-feeder community in areas like gardens/parks. the most likely pathway of the bacterium into Germany, wherefrom it could be spread to adjected areas like vineyards and cherry orchards. Indeed, *X. fastidiosa* could be introduced through ornamental plants, such as rosemary and oleander, being subsequently transmitted to cultivated plants, like grapevine and cherry, by polyphagous vectors as *P. spumarius*.

Therefore, in order to infer possible pathways of spread of *X. fastidiosa* in case of a large-scale introduction of the bacterium in Germany, we carried out several experiments to gather epidemiologically essential data on *P. spumarius* feeding behaviour and host plant choice/suitability. The probing and feeding activities of adult *P. spumarius* on rosemary, oleander, cherry and grapevine were analyzed by EPG (Electrical Penetration Graph). Furthermore, we conducted observations on host plant acceptance and settlement behavior through choice and no-choice tests under controlled conditions. Additionally, the phenology and host plant shifting under field conditions were observed in vineyards and cherry orchards within two winegrowing-regions in Germany ('Mosel' and 'Palatinate'). Overall, the data of this multidisciplinary project furnish important and urgently required indications on the potential risk posed by *P. spumarius* for the establishment and spread of *X. fastidiosa* under Central-European conditions.

# PRESENCE, SEASONAL ABUNDANCE AND MOLECULAR STUDIES ON GENETIC AND BACTERIAL DIVERSITY OF INSECT VECTORS OF *XYLELLA FASTIDIOSA* IN OLIVE ORCHARDS FROM GREECE

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# EVALUATION OF DETECTION METHODS ON INSECT VECTOR *PHILAENUS SPUMARIUS* BY INTRALABORATORY AND INTERLABORATORY COMPARISON TESTS.

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Recently detected in Italy, France, Germany and Spain, the bacterium *Xylella fastidiosa* (Xf) attacks many wild and cultivated plant species. It is transmitted and carried by xylem-sap feeder insects. Only a few groups of Hemipteran, all belonging to the suborder Auchenorrhyncha, are known to be effective vectors of the disease. They are mainly leafhoppers, spittlebugs, aphrophorids and cicadas. To date, only *Philaenus spumarius* has been identified as the main efficient vector insect in Italy. In France it has been detected positive for the presence of Xf when collected in outbreaks areas of Corsica and French Riviera. In the context of the emergence of Xf in France and the epidemiological surveillance of this bacterium, work targeting insects has been conducted by ANSES Plant Health Laboratory since 2016. A method of detection by real time PCR has been optimized and validated on individual *Philaenus spumarius*. Performance criteria of the method (analytical sensitivity, specificity, repeatability, reproducibility) were evaluated on spike insect crushing's. An interlaboratory test performance study proposed in parallel by ANSES in the framework of the H2020 POnTE and EUPHRESCO PROMODE projects permitted to evaluate available and practiced methods within the network of European partners (20 participant laboratories) on naturally positive insects and spiked insect crushing's. Moreover the performance of the method on groups of insects is currently evaluated. The updated results will be presented.

# APHROPHORIDAE DYNAMIC AND FEEDING PREFERENCE FOR PLANTS IN NATURAL GROUND COVER IN OLIVE GROVES FROM TRÁS-OS-MONTES

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# POTENTIAL NATURAL BIOCONTROL AGENTS OF APHROPHORIDAE EGGS

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# POTENTIAL VECTORS OF *XYLELLA FASTIDIOSA* IN PORTUGAL VINEYARDS

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# A *ZELUS RENARDII* ITALIAN JOURNEY

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Any available control action shall compose the frame of a possible IPM strategy devoted to the control of *Philaenus spumarius* Linnaeus, 1758 (Ps), *P. italosignus* Drosopoulos & Remane, 2000, and *Neophilaenus campestris* (Fallén, 1805): these Hemiptera Aphrophoridae were ascertained as vectors (HAv) of *Xylella fastidiosa pauca* ST53, responsible for the Olive Quick Decline Syndrome (OQDS) disease now decimating olives in Apulia, South Italy. The knowledge gathered until now may also help to sketch alternative control options in HAv-IPM to preview future unpleasant invasions of different *X. fastidiosa* entities and/or different vectors assemblage. The evidence of HAv population, as the number of individuals per ha living in the OQDS outbreak areas, proves the inadequacy of the vector's Natural Enemies Complex (NEC). In the studies we exploit the *Zelus renardii* Kolenati 1856 (Hemiptera Reduviidae, Zr) potential for HAv biocontrol, based on direct observations and experimental evidence on a total of 1249 individuals. Namely: 487 specimens were collected in nature to study in the laboratory the Zr bionomics and the general predatory behavior; 8, also picked up from nature, to evaluate Zr attitude to prey 1600 offered Ps adults; 754 to study the Zr attitude to mass-breed on living preys or artificial diets in the laboratory. *Zelus renardii* is a stenophagous predator that prefers hemipteran preys of manageable size, also in relation to its size during ontogeny, that includes HAv. Zr if starved and confined in a breeding box can attack and kill honeybees, extra-sized cockroaches, and feed on *Rhynchophorus ferrugineus* eggs, but it can also survive on hemipteran honeydew. However, the latter diet does not permit Zr to lay egg batches, while feeding on hemipterans does. In 320 experimental repetitions, we offered 5 Ps per Zr every 24 hours, and the predator caught and killed all of the offered preys in 308 repetitions, leaving one alive in 12 repetitions. Each killing and feeding sequence needed a mean time of 73 min, and the five per day a total mean time of about 6 hours. Zr also re-fed on Ps carcasses. Mass-breeding experiences were carried out on a diets based on living preys (Dm) [*Drosophila melanogaster* (Meigen, 1830), wild type], or artificial diets such as: oligidic (D0, beef liver plus egg yolk homogenate); meridic (D1, D2 & D3, marketed neutral Meritene® or marketed Nidina® 2 OPTIPRO®); and holidic (D4, D0 + D1). The diets were more or less successful: D4 gave the best performance (18,8% of adults) followed by D3 (14.3%) and D0 (= 10.9%). Zr also probes and accepts the diets capsulated in alginate beads, but fails to uptake an appropriate food quantity, partly because of the multi-locular beads structure and because of the stiff consistency of the shell of the beads. Evidence suggests that Zr is an intriguing biocontrol option for the control of Ps adult vectors, being potentially a good candidate for inoculative release because of its 1) prey range and preference, 2) impact on beneficial, 3) breeding attitude and mass breeding opportunities, and 4) olive secondary pest control attitude. Consequently, we shall consider the use of this biological control option in HAv-IPM.

# MAIN APHROPHORIDAE (HEMIPTERA) VECTORS OF *XYLELLA FASTIDIOSA PAUCA* ST53 JUVENILE QUANTITATIVE SAMPLING

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Collecting quantitative population data of *Xylella fastidiosa pauca* ST53 vectors is the primary step in planning control strategies to manage the pathogen invasion in olive orchards. The species, number, and phenology of vectors are crucial to identify, choose, and integrate effective control actions in key-points. Here, we focus on a juvenile quantitative sampling method needed to quantify the control intensity of second key-point of vectors management: the preimaginal vector IPM. In a first approach we collected Aphrophoridae juveniles living on olive orchards herbs framed in a 0,25 m x 1 m transects (Sub-Sampling Unit - SSU - 1/4 $m^2$: 2.500 $cm^2$). Later the herbs were scored in the laboratory by stereomicroscopes and juveniles stored in EtOH 75%for subsequent study. One-year long dataset scrutiny revealed truncated population data at the beginning and the end of the sampling season. Consequently, we elaborated an innovative sequential rinsing sampling technique called AquaSamPling (ASP) by a biomimetic approach to gather data of better quality and to suggest a sampling modality available for the field technician and stakeholders. ASP consists of washing the collected plants several times with a saline solution (0,9% sodium chloride in tap water) to dissolve the insects-froth and force the insects to float in the liquid. The rinsing solution is filtered through a non-woven piece of fabric and used for re-rinse the same herbs batch until filters collect any further juvenile. The rinsing is gentle enough to keep the insects alive, intact and ready to be breed or preserved in EtOH 75%, for counting and identifying. One-year long ASP gathered dataset revealed expected population increase and decreased at the beginning and the end of the sampling season, also allowing to collect eggs in full winter, and to scrutiny eggs development and hatching later in January and February, giving us the ability to forecast new-born presence in the field. ASP has a feasible and essential approach and bypasses the need of a laboratory, making stakeholder able to detail timely and accurately the vector population size and phenology at a fraction of efforts due with a conventional technique.

We further experimented ASP resolving power by subdividing the SSU in six SSSU (Sub-Sub-Sampling Units: 5 x 400 $cm^2$ + 1 x 500 =2.500 $cm^2$) to wash them separately and then rejoined in a single 2.500 $cm^2$ SSU. Counting the total of collected juveniles revealed that five 20 x 20 (400 $cm^2$: 2.000 $cm^2$) SSSU work betters than a single 2.500 $cm^2$ SSU. Overall field ASP for vector control suggests sampling the densest and aged herbs belonging preferably to Asteraceae, Apiaceae, Rubiaceae and Primulaceae in the orchard, to get the highest vectors to count and the most advanced phenology of vectors.

This "worst case" sampling helps to better trigger the juvenile vector control actions intensity in correspondence with the observed population field peak at the pass from last naiad to first nymph.

# A LATTICE MODEL TO SHAPE *PHILAENUS SPUMARIUS* VECTOR AND *XYLELLA FASTIDIOSA PAUCA* ST53 TRANSMISSION MANAGEMENT STRATEGIES

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*Philaenus spumarius* (Ps) is the main xylem-sap feeder vector of *Xylella fastidiosa pauca* ST53 (XF), the bacterium responsible for the OQDS symptoms, a devastating plant disease now invading the olive orchards of Apulia. Vector control is expected to be the main action to manage insect-borne pathogens and to contain the following diseases.

We suggest tuning the actual IPM (Integrated Pest Management) strategy to manage the transmission events instead of the vector only. Achievements in Aphrophoridae sampling techniques permit to add a physical (mechanical) control action against vectors eggs in IPM as hitherto sketched. A series of subsequent control actions imposed into a restricted time interval decimate juveniles. Only adults acquire XF feeding on infected plants, to infect (primarily), re-infect and super-infect (secondarily) without latency the olives they encounter until the recipient plants hard too much to be accepted. We consider vector IPM during pre-imaginal life for overall population control and adult control in the way we suggest both crucial to prevent and protect the plants from the infections.

The lattice model we present can help to shape IPM by forecasting the effectiveness of adult control actions through the analysis of the way in which the transmission depends on 1) spacing; 2) number, frequency, intensity, and kind of control actions, and 3) vector mobility in open field or greenhouse-like environments.

# EFFECTIVENESS OF FIELD CHEMICAL CONTROL ACTIONS AGAINST *PHILAENUS SPUMARIUS* AND *NEOPHILAENUS CAMPESTRIS* (HEMIPTERA APHROPHORIDAE) ADULT VECTORS OF *XYLELLA FASTIDIOSA PAUCA* ST53

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The need of controlling the adults of *Philaenus spumarius* (L., 1758) and *Neophilaenus campestris* (F., 1805) increased suddenly with the evidence of their attitude to transmit the phytopathogenic bacterium *Xylella fastidiosa pauca* ST53 that is responsible for Olive Quick Decline Syndrome (OQDS). From the introduction of the quarantine bacterium, several experiences targeted the knowledge of most effective active ingredients, formulations and distribution techniques for the control of adult vector. This control action is essential to avoid *X. fastidiosa* invasion and OQDS containment through the use of preventive and protective products and appropriate IPM strategies. Here we report the first results about the effectiveness of four formulated products based on different active ingredients (Sulfoxaflor, Imidacloprid, Acetamiprid, Dimethoate) belonging to 1B, 4A and 4C IRAC groups, distributed, at label rate, both by atomiser and tree injected BITE (**B**lade for **I**nfusion in **T**r**E**es) technique. The injection method and the device allow driving all the amount recommended on the label into the plants, to get the maximum possible mortality also to reduce environmental chemical pollution and undesirable side effects. We executed the trials in experimental plots located in *X. fastidiosa*-free areas and included in an olive orchard of 10-15 years planted with *FS17 Favolosa* clone. On each plot, two opposite branches per plant were isolated by an insect-proof cage of proper mesh size to avoid *N. campestris* escaping. From 20 to 30 adult of both vectors were introduced in each net (for a total of 80-120 insect per plot) after insecticidal application, then the mortality of the two species was evaluated separately during each assessment. The formulate persistence resulted from the weekly re-introduction of the two pests species. Death counting shows that foliar application of Imidacloprid provided the highest mortality with more than 90%. Tree injection, for the same insecticide, was less effective killing 62-67% of the vectors. The other treatments applied by tree injection, gave a control from 52% to 75%, no statistically different. The insecticides distributed with the two techniques shown the persistence of over 30 days.

# SESSION 5

# *XYLELLA FASTIDIOSA*: SOCIO-ECONOMIC IMPACTS AND MODELING FOR DISEASE SPREAD AND SURVEILLANCE

# SPREAD MODELS APPLIED TO *XYLELLA FASTIDIOSA* IN OLIVES IN EUROPE

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In the last years, diseases caused by the bacterium *Xylella fastidiosa* in several plant species in Europe are generating a considerable ecological, economic and social concerns. In this work, we focus on the potential spread of *X. fastidiosa* in olives in Europe, making an approximation of pathogen dispersal over time. With this objective, different spread scenarios were simulated using the georeferenced layer of olive trees in Europe gathered from the Corine Land Cover inventory. The potentially suitable area for *X. fastidiosa* establishment and spread was defined using an ad hoc Maxent model based on monthly climatic variables obtained from the WorldClim database (Hijmans *et al*., 2005). Models of radial expansion and logistic growth described by Robinet *et al*. (2012) were projected for a 50-year period. Parameters in both spread models were estimated using the available data of presence and absence of *X. fastidiosa* from the official surveillance program in Apulia, Southern Italy, from 2013 to 2018. For the radial expansion model, the parameter of radial rate of range expansion (km $yr^{-1}$) was estimated based on the mean Euclidean distance of disease expansion throughout this period. In the case of the logistic growth model, the parameter of relative rate of increase of the invaded area ($yr^{-1}$) was estimated by nonlinear regression (Ritz and Streibig, 2008) with the Gauss-Newton algorithm. The logistic growth model was implemented using three different spread scenarios; i) grid cells in Europe are invaded randomly, ii) grid cells with olives are invaded first, and iii) grid cells without olives are invaded first. Simulations with the radial expansion model resulted in 53.3% of the cells with olives invaded after 50 years. In the case of the logistic growth model, considering the three spread scenarios, all grid cells with olives in Europe were invaded after 50 years. Nevertheless, models predictions showed to be highly sensitive to parameter values, which were solely based on the observed spread of the disease in Apulia. Furthermore, the assumption that disease dynamics will behave similarly in other European regions may be not equivalent. Therefore, improved methods for parameter estimation are needed, for instance using expert knowledge elicitation.

# SPATIAL BAYESIAN MODELLING APPLIED TO THE SURVEYS OF *XYLELLA FASTIDIOSA* IN THE DEMARCATED AREA IN ALICANTE (SPAIN)

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The bacterium *Xylella fastidiosa* is characterized by genetic diversity, pathogenic plasticity and wide host range. Climate is known to play an important role limiting the geographic range of *Xylella* diseases. In North America, risk levels for *X. fastidiosa* subsp. *fastidiosa* were described based on minimum winter temperature thresholds. However, it is unknown whether these temperature thresholds can be extrapolated to other *X. fastidiosa* subspecies or other geographic regions. An outbreak of almond leaf scorch, caused by *X. fastidiosa* subsp. *multiplex*, was detected in 2017 in Alicante province, mainland Spain. Here, the effect of climatic and spatial factors in the distribution of the pathogen in the demarcated area was studied. Data on the prevalence of the pathogen were gathered from official surveys, conducted according to Decision (EU) 2015/789. Monthly average climate data from 1950 to 2000 were obtained from the WorldClim database. Data were analyzed using a Bayesian spatial hierarchical model in which the spatial component was incorporated via a conditional autoregressive structure (iCAR). Posterior distributions of the model parameters were approximated using the Integrated Nested Laplace Approximation method. Due to the limited study area, the climatic covariates had little variability and they were not very influential in the models compared with the spatial component. Nevertheless, the pathogen was detected in almond within all minimum winter temperature thresholds, from <1.1°C to >4.5°C, further confirming the climatic adaptability of *X. fastidiosa* subsp. *multiplex*. Results indicated a strong effect of the spatial component in the models, indicating that spatial structure has a profound impact on disease spread dynamics.

# ESTIMATING THE DISPERSAL AND SPREAD OF *XYLELLA FASTIDIOSA* IN PUGLIA

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Understanding the dispersal of *Xylella fastidiosa* is essential for effective containment of the disease. In Puglia, Italy, surveillance is focused on buffer and containment zones, which have been established at the edge of the infected region with the aim of stopping further spread. The success of this strategy will strongly depend on whether these zones are wide enough to form a barrier to long distance dispersal of the bacterium. In this presentation, I will describe our progress towards estimating the dispersal range of *Xylella* in Puglia using a spatial epidemiological model. The model simulates the spread of the disease across a landscape depending on the location and timing of introduction, the distribution of host plants, the rate of infection growth in infected olive groves and both short and long distance dispersal. The long distance dispersal seems to be a crucial feature of the *Xylella* epidemic, causing rapid spread of the disease over large areas but in an unpredictable manner. To estimate long distance dispersal, we are attempting to calibrate the epidemiological model using observed detections in surveillance monitoring data from 2013 to 2018. To ensure the calibration is as rigorous as possible, we have introduced to the *Xylella* spread model a new submodel that simulates the surveillance programme and diagnostic testing. This allows us to generate simulated monitoring data from the epidemiological model and to therefore calibrate the model parameters for long distance dispersal using the real monitoring data. I will present preliminary results from these attempts to estimate the long distance dispersal and outline planned future developments of the modelling work.

# STAKEHOLDER PERCEPTION OF *XYLELLA FASTIDIOSA* (*XF*) DISEASE RISK ASSESSMENT: FIRST RESULTS FROM PUGLIA (IT), CHANIA (GR), VALENCIA AND ANDALUCIA (ES)

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In the frame of XF-ACTORS-Task 8.1, analysis of the environmental, socio-economic and governance impact and vulnerability to *Xylella fastidiosa* has been carried out in selected case study areas. The progress made in in Italy, Greece and Spain is presented.

To reach this goal, a comprehensive stakeholder survey was conducted with the support of a comprehensive questionnaire. A total of 263 questionnaires were compiled (150 in Puglia, 30 in Crete, 30 in Valencia and 53 in Andalusia). Questionnaires were completed through face-to-face interviews to local farmers, decision makers, extension experts and practitioners. Answers were collected and processed with the support of computer-assisted software.

The survey aimed to: 1) collect the point of view and perception of people about Xf disease and the containment plans; 2) identify possible gaps in communication, understand possible weak points in the communication strategy that could hamper the application and the effectiveness of containment measures; 3) understand the network of relationships existing among stakeholders in territorial contexts; and 4) collect suggestions from local people with a view to improving the management of information related to the disease.

The questionnaire comprises 13 different sections and 63 structured and non-structured questions aimed to collect data, but also opinions and informal information, which are necessary to assess the perception of local people in the pilot areas.

Stakeholders were identified on the base of a methodology resembling the "snowball methodology". This approach also allowed a map of the existing connections among the actors to be elucidated by highlighting the relationships between them and measuring the intensity of such connections. Questions were grouped under different main variables or criteria: Knowledge, Perception, Practices, Involvement, Effectiveness, Responsibility. A scoring procedure assigned to each question a value in order to assess the corresponding indexes: DKI-Disease Knowledge Index, DPI-Disease Perception Index, FPI-Farm Practices Index, INV-Involvement Index, EFF-Effectiveness Index, RES-Responsibility Index. The overall values of RISK (DKI/DPI/FPI) and GOVERNANCE (INV/EFF/RES) were then obtained.

The correlation analysis shows how indices are highly correlated in the RISK and GOVERNANCE domains as well as between these two domains.

The proposed methodology proves to be useful to describe attitudes of respondents when facing the epidemic, as well as how they appreciate and tackle disease management.

The work allows practical suggestions to be made to improve the knowledge-perception relationship that directly influences willingness to adopt preventive/control measures against the pathogen, and consequently will increase the efficiency of Xf disease management.

The work in now being extended to other case studies and sensitive crops.

# IDENTIFYING LOOKOUTS FOR EPIDEMIO-SURVEILLANCE: APPLICATION TO THE EMERGENCE OF *XYLELLA FASTIDIOSA* IN FRANCE

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Recent detections of *Xylella fastidiosa* in Corsica island (France) has raised concerns on its possible spread to mainland France and the rest of the Mediterranean basin. Early detection of infected plants is paramount to prevent the spread of the bacteria, but little is known about this pathosystem in European environments, hence standard surveillance strategies may be ineffective. We hence present a new methodological approach for the design of risk-based surveillance strategies, adapted to the emerging risk caused by *Xylella fastidiosa*. Our proposal is based on a combination of machine learning techniques and network analysis that aims at understanding the main abiotic drivers of the infection, produce risk maps and identify lookouts for the design of future surveillance plans. The identified drivers coincide with known results in laboratory studies about the correlation between environmental variables, such as water stress and temperature, and the presence of the bacterium in plants. Furthermore, the produced risk maps overlap nicely with detected foci of infection, while they also highlight other susceptible regions where *Xylella fastidiosa* has not been found yet. We conclude the paper presenting a list of recommended regions for an optimized risk-based surveillance campaign based on the predicted spread and probability of detection of the disease.

# A 3-YEAR STUDY ON SPATIAL AND TEMPORAL DYNAMICS OF OQDS AT PLOT LEVEL IN PUGLIA

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# OPTIMISING SURVEILLANCE FOR EARLY DETECTION OF NEW *XYLELLA FASTIDIOSA* OUTBREAKS IN APULIA PROVICE, ITALY

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Early detection surveillance is key to minimising the risk of spread of *Xylella fastidiosa* CoDiRO from the "infected zone" in the south of the region of Apulia in Italy into the more northerly "uninfected zone". Whilst it is important that this surveillance strategy is informed by the ecological and epidemiological characteristics of *X. fastidiosa* CoDiRO in this setting, we currently have limited insight into the nature of long-distance pathogen spread (which is largely associated with the movement of infected insect vectors). Our previous work, in which we linked a spatially explicit model of pathogen spread with a statistical model of a sampling process, has demonstrated that the characteristics of this spread has considerable impacts on the optimal arrangement of surveillance resources required to maximise the probability of detecting new incursions. In particular, we found that focussing surveillance efforts adjacent to the buffer zone is the optimal strategy when long-distance spread is restrained, but when spread is unrestrained, surveillance resources are better placed throughout the uninfected zone, especially in regions of high olive density. In the current report, we focus on how well these different surveillance strategies (and other more generic strategies) are able to detect new incursions, in the face of the different long-distance spread patterns described above. This is valuable since the nature of long-distance spread is often relatively unknown when planning surveillance activities. We find that for a range of different sample sizes, the probability of detection when sampling at 'optimised' locations (even when optimised to the wrong spread model) was higher than that for locations selected by ranking either the infection risk (from a simulation model) or the olive tree density, regardless of the true nature of long-distance spread. We also find that sampling at the 'optimised' locations generally results in a comparable detection probability to random sampling when the incorrect spread model is assumed, If either restricted or unrestricted spread is equally likely, the average detection probability is highest when optimisation is performed using the assumption of restricted spread. The distribution of sampling sites under this strategy broadly corresponds with current surveillance policy, being focussed in areas of higher olive tree density close to the border of the infected zone.

# ASSESSING THE POTENTIAL ECONOMIC IMPACT OF *XYLELLA FASTIDIOSA* IN EUROPEAN OLIVES: A SCENARIO-BASED DYNAMIC MODELING APPROACH

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The content of this work is currently only available to the registered members of POnTE and XF-ACTORS research consortia.

# SESSION 6

# *XYLELLA FASTIDIOSA*: CAPACITY BUILDING AND DISSEMINATION PLANS

# XF-ACTORS DIGITAL RESEARCH OBJECT PORTAL (DROP)

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In order to capitalize existing information and new results from the research activities developed within the XF-ACTORS consortium, an online publicly accessible portal has been built and it is intended to be made available for public consultation, use and re-use by the project's stakeholders and more generally by anyone interested in finding information and developing solutions against the bacterium and its vector. The portal functionalities will be presented, and discussion will be held on the best way for XF-ACTORS partners to feed the infrastructure with any data or document they intend to open. A project deliverable will be submitted to the European Commission by 2018-10-31.

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